Original Article

Major Effects of Delayed Graft Function and Cold Ischemia Time on Renal Allograft Survival

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Abstract

Background: There is mounting evidence from experimental and clinical studies that the quality of organs from cadaver donors may be influenced by events occurring around the time of brain death, and that these may affect transplant outcome. The aim of this study is to investigate the influence of donor factors on renal allograft outcome in a homogeneous cohort of 518 patients transplanted in a single center over a 9-year period.

Methods: End points of the study were delayed graft function (DGF), acute rejection (AR), 1-year graft survival, and long-term survival of those grafts that reached 1 year. Multivariate analysis was performed to determine factors that may have influenced the graft outcome indicators.

Results: DGF was the major predictor of graft failure overall with cold ischemia time (CIT) as an important independent factor. The level of histocompatibility did not influence graft survival. DGF was the major factor affecting 1-year graft survival ($P < 0.0005$) with effects persisting beyond 1 year. DGF was significantly influenced by CIT, donor age, female kidney into male recipient, and donor creatinine ($P < 0.05$). Other donor factors and factors associated with donor management were not risk factors for DGF, rejection episodes, or graft survival. The risk factors for a number of AR episodes were HLA–DR mismatch and DGF ($P < 0.005$). When grafts surviving for 1 year were considered, only CIT, recipient age, and creatinine at 1 year ($P < 0.05$) were found to affect graft survival significantly.

Conclusions: The results of this analysis of well-matched transplant recipients show that CIT and DGF are the most important predictors of poor short and long-term graft survival. Therefore, to improve the long-term survival of renal allografts efforts should focus on limiting CIT and the damage that occurs during this period and on improving our understanding of DGF.

Key words: Cold ischemia time, Delayed graft function, Donor factors, Outcome, Renal transplantation

INTRODUCTION

There is mounting evidence from experimental and clinical studies that the level of injury to organs from cadaver donors may be influenced by events occurring in the Intensive Care Unit (ICU)[1] and around the time of brain death,[2] and that these may affect subsequent transplant outcome.

Despite evidence that the quality of organs from cadaver donors is inferior to organs from living donors, it remains controversial whether this is associated with events in the management of the donor in ICU. For example, recent data suggest that the use of inotropes reduces the incidence of acute rejection (AR) and leads to superior long-term survival of the graft. While, in contrast, other studies have found a significant increase in delayed graft function (DGF) and reduced 1 year survival and renal function if the donor had required inotropes before death.[1]

Having been exposed to factors related to the dying process other influences will be added to the donor organ which will impact on the final outcome of transplantation. These will be related to the retrieval process itself and the subsequent period of cold ischemia before reperfusion. Finally, recipient factors will become active on reperfusion and for the lifetime of the graft. It is this blending of multiple donor and recipient factors that generate the final outcome of the transplant process.

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The aim of this study was to analyze a comprehensive database of a large, homogeneous cohort of patients transplanted in a single center over a 9-year period to investigate the influence of donor and recipient factors on renal allograft function and survival.

SUBJECTS AND METHODS

Patients

Between 2009 and 2017, 541 cadaveric renal transplants were performed at the Oxford Transplant Centre. Data relating to donors and the retrieval process were obtained from the UK Transplant National Database Core Donor Data Form (Form CDD1) and from information kept locally by the transplant coordination team. Full donor data were available for 518 patients, which formed the study population. 23 patients were excluded from the study due to the lack of donor data. All kidneys were retrieved from conventional heart-beating, cadaveric donors diagnosed as brain stem dead. The retrieval technique and preservation fluid were unchanged during the study period. A detailed list of the variables studied is given in Table 1. Donor details and recipient demographics are described in Table 2. Clinical and follow-up data were collected prospectively from the Oxford Transplant Centre Database.

Immunosuppression

The immunosuppressive protocol used during the period studied was uniform and consisted of a triple-therapy regimen of cyclosporine (8mg/kg/day divided in two doses), azathioprine (1.5mg/kg/day), and prednisolone (20mg/day if recipient weight 60kg or above; 15 mg/day if weight was <60kg). Cyclosporine dose was adjusted to maintain serum trough levels between 150–300ng/mL in the first 6 months post-transplant and 75–150ng/mL, thereafter. Prednisolone dose was reduced gradually after 2 months to 5 mg/day at 1 year. Most patients discontinued prednisolone at 18-month post-transplantation. Induction therapy with antilymphocyte globulin (ATG) was used only in highly sensitized patients. There was no change to the immunosuppressive protocol in the presence of DGF. From July 1991, patients were started on aspirin 75mg pre-transplantation and continued until 1 month after the transplant to prevent renal vein thrombosis.

End points

The end points of the study were: DGF, AR, 1-year graft survival, and long-term survival of those grafts that reached 1 year.

DGF was defined as the requirement for dialysis within the 1st week after transplantation. Patients transplanted before needing dialysis (pre-emptive transplantation) were considered to have DGF if the creatinine failed to drop in the 1st week.

AR was diagnosed histologically on core renal biopsies according to the Banff classification. Before the introduction of the Banff classification, rejection was diagnosed by a histopathologist on a qualitative basis. Biopsies were scored using the '93 Banff classification from 1996 to 1999, and the revised '97 Banff classification from April 1999. All patients in our unit had protocol renal transplant biopsies at days 7 and 28 after the transplant irrespective of renal function. In addition, diagnostic biopsies were performed at any time when clinically indicated. Three 500mg doses

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Table 1: List of variables studied

Year of transplant	Donor cause of death	Recipient sex	DGF
Donor sex	Trauma Y/N	Recipient age	AR Y/N
Donor age	Cardiovascular disease Y/N	Recipient blood group	AR day-7 Y/N
Donor age </≥50 years	Donor ventilation time (h)	Recipient rhesus (±)	AR day-14 Y/N
Donor weight	Donor infection Y/N	Recipient blood transfusions Y/N	Methylprednisolone treatments
Donor height	Donor antibiotics	Recipient number of blood transfusions	Antithymoglobulin treatment
Donor SeCr	DDAVP Y/N	Cause of ESRF	SeCr and CrCl[a] day-7
Donor SeCr </≥150 μmol/L	Donor inotropes Y/N	First transplant/regraft	SeCr 3 and 6 months
Donor CrCl[a]	Adrenaline Y/N	Days on transplant waiting list	Weight 3 and 6 months
Donor CrCl[a] </≥60 mL/min	Dobutamine Y/N	Pre-transplant antibodies[e]	CrCl[a] 3 and 6 months
Donor serum sodium	Dopamine Y/N	Highly sensitized[f]	Survival 3 and 6 months
Donor serum potassium	Noradrenaline Y/N	HLA class I MM	SeCr 1–9 years
Donor blood group	Multiorgan/kidneys only	HLA class II MM	Weight 1–9 years
Donor rhesus (±)	Local/imported organ	HLA A MM	CrCl[a] 1–9 years
Donor CMV status (±)	Graft damage[c]	HLA B MM	Survival 1–9 years
Hypotension Y/N[b]	Type of damage	HLA DR MM	Death with functioning graft
Cardiorespiratory arrest	CIT[d]	HLA MM	
Donor blood transfusion	Anastomosis time	Recipient CMV (±)	

[a]Calculated by the Cockcroft–Gault formula [(140−age) × (1.23♂/1.04 ♀) × weight (kg)/serum creatinine (SeCr) (μmol/l)], [b]systolic blood pressure≤80 mmHg for>10 min, [c]any type of injury (vascular, ureteric, or capsular) reported to the national database by the retrieving surgeon or recorded at the recipient center, [d]time from cold perfusion to the time the kidney was taken out of ice to start the anastomosis, [e]HLA lymphocytotoxic antibodies reactive with≥10% of a random panel, [f]HLA lymphocytotoxic antibodies reactive with≥85% of a random panel. CIT: Cold ischemia time, AR: Acute rejection

of methylprednisolone were administered on consecutive days for the treatment of confirmed AR. A 10–14 days course of ATG was used to treat steroid-resistant rejection.

Uncensored 1-year graft survival and long-term survival of those grafts that reached 1 year include those recipients who died with a functioning graft. Results from patients dying with a functioning graft were excluded from analysis as indicated. This was performed to maintain the focus of the study on the donor factors rather than recipient factors such as advanced cardiac disease, on graft survival.

Statistical Analysis

Multivariate statistical tests with the SPSS (v12 for Windows) statistics program were used to analyze the data. Logistic regression was used to calculate the odds ratio for the analysis of DGF, AR, and 1-year graft survival. Models were fitted on the basis of improved fit, as measured by change in Chi-squared statistics. The statistical models were

Table 2: Donor and recipient demographics and retrieval factors

Donor factors	
Sex, M: F	281:237
Age	42±15.5
Cause of death	
Trauma Y/N	145/373
CVD Y/N	335/183
CMV ±	249/246 (N/A 23)[a]
Serum creatinine	107±60 μmol/L
Creatinine clearance	84.7±31.9 mL/min
ICU factors	
Cardiorespiratory arrest Y/N	129/389
Hypotension Y/N	339/179
Ventilation time	55±47 (h)
Infection Y/N	130/380 (N/A 8)[a]
DDAVP Y/N	201/317
Inotropes Y/N	423/95
Noradrenaline Y/N	80/438
Dobutamine Y/N	130/388
Dopamine Y/N	299/219
Adrenaline Y/N	93/425
Retrieval factors	
Local/imported	367/151
Damage	85/433
CIT	23.6±8.6 (median=21 h)
Anastomosis time	44.7±2.1 (min)
Recipient factors	
Sex, M: F	316:202
Age	46.4±12.7
Cause of ESRF	
Diabetes Y/N	55/463
Pre-transplant antibodies Y/N	241/277
Highly sensitized Y/N	38/480
CMV ±	259/222 (N/A 37)[a]
Days on waiting list	338±480 (median=168) (days)
Number of transplant 1:2:3:4	444:64:8:3
HLA mismatches 0:1:2:3:4:5:6	33:73:114:139:122:32:5
HLA DR mismatches 0:1:2	269:219:30

[a]Data not available

validated by the "Hosmer–Lemeshow" goodness of fit test. The "Nagelkerke R^2" was used to evaluate the overall fit of the model.[3]

Cox proportional hazard regression, log-rank analysis, and Kaplan–Meier curves were used for the analysis and illustration of the survival of those grafts that reached 1 year and long-term graft survival.

Continuous variables, such as age and cold ischemia time (CIT), were analyzed as such in Cox and logistic regression. However, to permit the use of Kaplan–Meier curves plots were converted to binary variables, by splitting variables at the median.

The significance of results from logistic and proportional hazards regression were confirmed by examining changes in overall log-likelihood of the model.

Possible interactions were examined by comparing coefficients for one factor across levels of the other and calculating a "normal deviate.[4]"

Linear regression was used to study the factors affecting the 1-year creatinine.

To show further the effect of donor age and CIT, these variables were banded by quintiles.

RESULTS

Overall Study End Points

DGF occurred in 31.1% (161/518) of the patients studied [Table 3]. 54% of recipients had at least one episode of biopsy-proven AR. Of the patients with AR, 35.7% had two or more episodes of rejection. Graft survival of 1 and 5 years after censoring for death with a functioning graft was 90.6% and 79.5%, respectively. The full data relating to the end points of the study are given in Table 3.

Of the total set, 23 patients were excluded from the study due to the lack of donor data. However, the incidence of DGF, AR, and 1-year graft survival in these 23 excluded patients (35, 43.5, and 90.5%, respectively) was comparable to the remainder.

Analysis of the Factors Affecting Overall Graft Survival

Survival analysis methods were used to investigate the factors affecting the overall graft survival. DGF emerged as the dominant predictor of subsequent survival. Figures 1 and 2 show the Kaplan–Meier plots of the effects of DGF and CIT and associated log-rank statistics. For this analysis, CIT was categorized as being either above or below the

median CIT of 21h. It can be seen that CIT influenced the long-term survival of the graft significantly.

Results of the proportional hazards regression are presented in Table 4.

A "time-dependent covariate" was used to test departure from the proportional hazards assumption. The effect was not significant but was consistent with a tendency to earlier graft failure in the DGF group. This was confirmed by carrying out separate analyses for 0–3 months, 3 months

Table 3: End points of the cohort studied

DGF	161/518	31.1%
AR	280/518	54%
Number of episodes of AR 1:2:3:4	180:64:29:7	34.7%:12.4%:5.6%:1.4%
1-year graft survival	445/491	90.6%
5-year graft survival	217/273	79.5%
Mortality with functioning graft in 1st year	27/518	5.2%
Mortality with functioning graft in first 5 years	35/318	11%

Table 4: Risk factors affecting the end points analyzed in this stud

Factors		95% CI	*P*-value
Overall graft survival[a]	HR		
DGF	2.92	1.84–4.63	<0.001
CIT (h)	1.027	1.004–1.049	0.016
AR	1.15	0.73–1.82	0.54
Overall graft survival (no DGF)[a]	HR		
CIT	1.023	0.98–1.07	0.28
AR	2.05	1.03–4.08	0.04
Overall graft survival (DGF)[a]	HR		
CIT	1.029	1.004–1.053	0.021
AR	0.71	0.39–1.3	0.27
1-year graft survival[a]	OR		
Tx year	1.264	1.051–1.519	0.012
DGF	0.152	0.069–0.333	<0.005
Long-term survival of kidneys reaching 1 year[a]	HR		
Recipient age	0.963	0.937–0.989	0.005
CIT	1.035	1.002–1.069	0.039
SeCr 1 year	1.006	1.006–1.0104	<0.005
DGF[b]	OR		
Donor age	1.03	1.015–1.044	<0.005
Donor SeCr	1.004	1.0004–1.008	0.028
CIT	1.066	1.041–1.092	<0.005
FtoM	2.437	1.536–3.869	<0.005
Trauma	0.328	0.154–0.699	0.004
AR Y/N[b]	OR		
Donor age	1.013	1.0006–1.026	0.039
DR MM	1.805	1.316–2.477	<0.005
DGF	1.814	1.196–2.749	0.005

[a]Factors affecting the overall survival, and long-term survival of the grafts that reached 1 year, were obtained by Cox regression. [b]Factors affecting DGF, AR, and 1-year graft survival were obtained by logistic regression. Coefficients for continuous variables are expressed per unit of the variable (e.g. per year), DGF: Delayed graft function
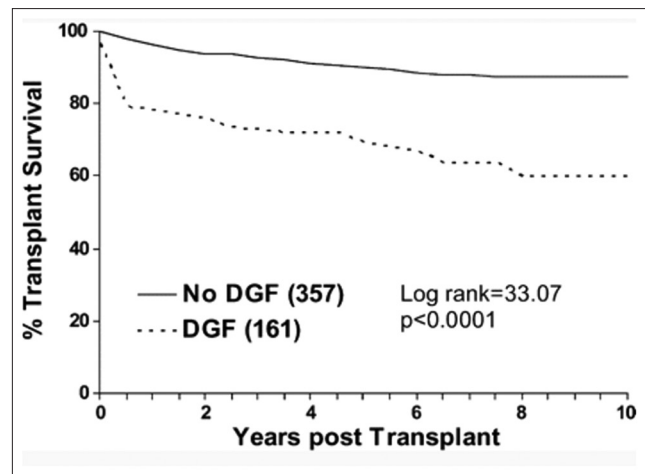

Figure 1: Effect of delayed graft function on renal graft survival. Graft survival is censored for death.
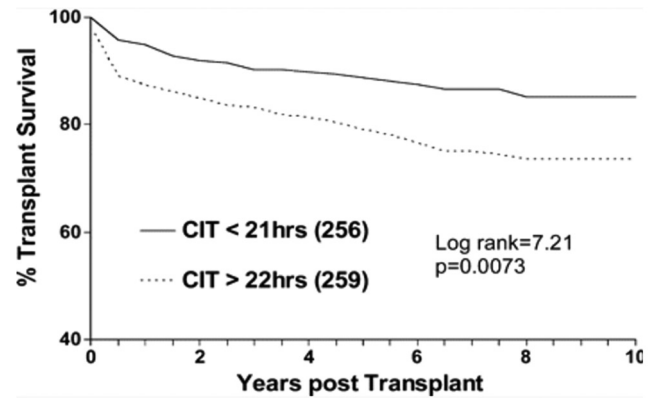

Figure 2: Effect of cold ischemia time on graft survival. Graft survival is censored for death

to 1year, and after 1 year [Table 5] that also showed that the effect of DGF persisted. This post 1-year effect of DGF was not significant after the inclusion of 1-year creatinine, suggesting that this mediated the effect.

Diagnosis of rejection had no overall effect on failure; however, there was a significant interaction with DGF ($z = 2.26$, $P = 0.024$), with rejection significantly increasing failure in the absence of DGF [Table 4].

Factors Affecting 1-year Graft Survival

The 1-year graft survival was studied by logistic regression. In this analysis, survival is treated as the outcome (with 'death with functioning graft' being omitted from the analysis), hence reduction in graft survival is indicated by an OR <1.0. 1-year graft survival was influenced only by DGF and the year of transplant [Table 4]. There were no adverse effects of donor factors or ICU management on 1-year graft survival.

Renal transplants performed in the early years of the study period had a significantly worse 1-year graft survival than those carried out in later years.

Importantly, no other factors were significant, including AR, HLA matching, highly sensitized recipients, regrafts, and donor age. Again, contrasting effects of rejection were found according to the occurrence of DGF, with rejection reducing graft survival in the absence of DGF. Neither result was significant individually; the test for interaction was ($z = 2.0$, $P = 0.046$).

Factors Affecting Long-term Survival of the Grafts that Reached 1 year

Proportional hazards regression analysis was used to investigate factors affecting failure of grafts that survived beyond the 1-year post-transplant. The most important factor identified was serum creatinine at 1 year. CIT was also significant, as were donor and recipient age; older recipients had reduced graft loss [Table 4].

Serum creatinine has stronger predictor value than creatinine. In this analysis, regrafts, highly sensitized recipients and other donor and ICU parameters did not have a significant effect on long-term graft survival after the 1st year.

Risk Factors for DGF

In view of the importance of DGF, possible predictors of this state were investigated.

DGF was found to be significantly influenced by CIT, donor age, and donor creatinine [Table 4]. CIT, donor age, and donor serum creatinine were entered into the statistical model as continuous variables. The statistical analysis indicated that an increase in each of these variables

independently increased the risk of DGF. Hence, an increase in each unit of the variable, each hour of CIT, year of donor age, and micromoles per liter of donor serum creatinine increased the incidence of DGF.

The only other factor that increased significantly the risk of DGF was transplantation of a female kidney into a male recipient ($P < 0.005$), which independently conferred a higher risk of DGF than a female kidney into a female recipient or a male kidney into either sex [Table 4].

No evidence was found for departures from the logistic model. The "Hosmer–Lemeshow" goodness of fit statistic was not significant. Addition of quadratic terms in donor age and CIT did not improve the fit, nor did replacement of CIT by its logarithm. Banded results for donor age and CIT are shown in Table 6.

However, the overall explanatory power of the model was not great, the Nagelkerke R^2 was 0.196. In the "classification table," most of the DGF occurred in those cases where it was not predicted (sensitivity 30%, specificity 93%).

Donor factors, such as death resulting from cardiovascular disease, prolonged donor ventilation time, requirement for inotropes, and other factors associated with ICU management (as in Table 1) were not risk factors for DGF. Recipients who received a kidney from a donor who suffered a traumatic death had a significantly decreased risk of DGF, but this was not significant after inclusion of the "female-to-male" variable.

Risk Factors for AR

The most important predictors of rejection were the number of DR mismatches and the occurrence of DGF. However, there was evidence of an interaction between them with an effect of mismatches only in the absence of DGF [Figure 3], a test for interaction was significant ($z = 2.44$, $P = 0.014$). Donor age was also a significant factor; the relation appeared to be continuous.

Effect of DGF and DR Mismatches on Rejection

Recipients who received kidneys from donors requiring inotropes had a significantly higher risk of early rejection ($P < 0.05$). AR was not more prevalent in highly sensitized patients or patients with pre-transplant HLA antibodies. In addition, the analysis of early AR (within 14 days after the transplant) suggested that DGF and the degree of DR mismatching were still the most significant risk factors ($P < 0.05$ and $P < 0.005$). However, in this early period, unlike in the overall analysis, younger recipients did have an increased risk of developing early rejection ($P < 0.05$). In contrast, in this early time period, donor age was not a statistically significant variable ($P > 0.05$).

Table 5: Effect of DGF on graft failure during different time periods

Time period	Hazard ratio	95% CI
0–3 months	14.1	4.9–40.9
3 months to 1 year	2.9	0.94–9.1
>1 year	1.9	1.02–3.4

Table 6: DGF by bands of CIT and donor age

Factors	Number	% DGF
CIT (h)		
5–17	124	22
18–20	91	21
21–24	106	25.5
25–31	88	35
32–70	91	50
Donor age (years)		
7–27	102	15
28–38	107	22
39–47	98	36
48–56	96	37.5
57–80	97	41

Table 7: Factors affecting 1-year creatinine

Factor	Slope	*P* value
DGF	19.2	0.035
Donor age	1.83	<0.005
Recipient age	−0.89	0.004

Factors Affecting 1-year Creatinine

As creatinine at 1 year was a highly significant predictor of late failure, factors that might predict this were also investigated. Significant predictors were donor and recipient ages and DGF [Table 7]. The overall R^2 was low (0.166), showing again that the model did not explain the variation fully.

DISCUSSION

We have used multivariate analysis to investigate the influence of a number of factors and the relations between them. A single-center study permits the use of more detailed data in a fairly homogeneous set and provides a useful complement to multicenter studies. We selected the decade of the 1990s for this study, because in our unit, this was a period of very uniform immunosuppression and clinical practice. These immunosuppression agents are still of great relevance today as the use of cyclosporine and azathioprine are still part of the UK National Guidelines as laid down by the National Institute of Clinical Excellence. Similar studies published previously have often come from multicenter registry data, where the level of HLA matching is variable, CITs are often longer, and there are large variations in induction and maintenance regimens between centers.[5,6] Very few patients were excluded from this study, only then when there was a lack of donor information. The incidence of DGF, AR, and the 1-year graft survival in these patients showed that this group was no different to the study population, suggesting that loss of these patients was unlikely to have distorted the results of the study.

DGF

The most striking effects found in our study are the strong impact of DGF and CIT, reduced importance of rejection, and the lack of impact of matching. The importance of DGF and rejection for subsequent failure has been a matter of controversy for some time.[7,8]

The lack of effect of matching on graft survival was not surprising. Recently, Su *et al.*[9] have reported a declining effect of matching in the USA during the period covered by our study. It is possible that improved immunosuppression, together with diagnosis and treatment of rejection, mean that in a relatively well-matched population such as ours these factors do not lead to failure (the mean total number of mismatches was 2.7, as against 3.6in a recent multicenter

report[10]).

The incidence of DGF in our series at 31% was consistent with our previous data[11] and comparable to that of other centers. [5,7,8] CIT was the most significant risk factor for the development of DGF and its effect appears to be continuous. This observation is supported by other investigators.[7] Other studies have suggested that there are significant time points after which the risk of DGF accelerates.[5,8,12] The importance of the finding in this study needs to be stressed, as it is attractive to imagine a specific threshold CIT after which the risk of DGF is significantly increased. However, from our findings, it is clear that each hour even at short CIT adds additional risk.

Recent multicenter studies have confirmed the importance of CIT and donor age for graft survival. Su *et al.*[9] show that the effect is significant for times over 37 h compared with baseline. However, they do not test for discontinuity, and overall, their data appear consistent with a continuous effect of CIT. The collaborative transplant study[10] suggests that there is "little effect below 25 h;" however, they do not present any analysis to confirm this, and it is not obvious from their figures. Roodnat *et al.*[13] previously reported effects on graft survival over a wide range of CIT. Our results differ from theirs in that Roodnat *et al.* found the effect to be limited to the short-term, whereas we find evidence of an effect of CIT on late failure.

Despite the large number of variables studied in relation to DGF, the fit of the statistical model is far from complete; indeed one advantage of the model is that it emphasizes this. The "Nagelkerke R2[3]" is a generalization of the standard measure of fit of a multiple linear regression model, but caution should be exercised in interpreting it. However, taken together with the results of the classification table from the logistic regression, it suggests that other unknown factors are having an effect. It is possible that some organs are more sensitive (susceptible) to cold ischemia, reperfusion injury, and the vasoconstrictive effects of calcineurin inhibitors than others and the reason for this need further investigation. We have not examined the effect of time on dialysis, which has been reported as a predictor in several studies.[7] In view of the evidence that DGF is a major predictor of graft failure, efforts should be made to understand better what is involved and how it may be reduced.

AR

The incidence of AR at 54% in this study may be considered to be high by contemporary standards. However, our data correspond to therapy based on cyclosporine, azathioprine, and steroids without routine use of antibody therapy. In our unit, rejection episodes were confirmed by core biopsy and protocol biopsies were carried out at day 7 and 28

post-transplant. Furthermore, fine-needle aspirations were performed routinely to monitor the level of infiltration in the graft in recipients with DGF. We have found as reported by Rush *et al.*[14], that subclinical rejection occurred but was not treated or included in the analysis as AR. The degrees of severity of rejection were not analyzed individually as the study crossed over periods of significant changes in classification. However, ATG was used when severe rejection occurred and the use of ATG is not significant in multifactorial analysis.

DGF is also a significant risk factor for AR. It has been suggested that AR may be masked during DGF, as renal function cannot be used to detect episodes of AR.[8] Hence, treatable episodes of rejection may not be diagnosed, and it is the failure to treat this occult rejection rather than the DGF itself that leads to a reduced long-term outcome. In our study, we believe, this difficulty in analysis has been avoided as all allografts are biopsied routinely and AR is diagnosed irrespective of function.

In the present study, the risk of AR was neither increased in regrafts nor in sensitized patients. Preformed donor-reactive anti-HLA antibodies are detected by modern crossmatching techniques and antibody screening was done to determine if antibody specificity is performed before the transplant. The thorough immunological workup of transplant recipients has prevented the presence of antibodies from being a risk factor for rejection.

1-year Graft Survival
The main determinant of 1-year graft survival in this series, after censoring for death with a functioning graft, was DGF. There were no adverse effects of other donor factors including donor age or ICU management on 1-year graft survival. Importantly, no other factors were significant, including AR, HLA matching, highly sensitized recipients, and regrafts. The lack of an effect of AR on 1-year graft survival is surprising but not unexpected. This phenomenon has been mirrored in many recent publications comparing immunosuppressive regimens. It would suggest that AR in the first year is no longer a good end point for comparative studies.[15] Furthermore, highly sensitized recipients were treated with ATG as induction therapy which could account partly for the good results obtained on these patients as reports suggest that antibody therapy helps to prevent graft loss in these high-risk patients. However, this is not the full story as the variable "ATG on induction" has no independent effect on survival.

The only other significant factor that influenced the 1-year graft survival was the year of transplantation. The immunosuppressive regimen and other factors over this period were unchanged which suggests that other factors not included in the statistical model had an impact on short-term graft survival (Sandimmune vs. Neoral). It is possible that there were medical and technical improvements made over the study period that increased survival. In an observational study, we cannot exclude the possibility of such confounding factors. However, changes over time do not explain the effect of DGF on survival because this is maintained in the proportional hazards analysis including "transplant year," and in log-rank analysis stratified by "transplant year."

Censoring for death is a well-known and common practice in the analysis of transplant outcome, but this might have an unexpected effect on the analysis as it may selectively exclude patients with high serum creatinine. In a recent publication, Meier-Kriesche *et al.*[16] have reported a strong association between renal function at 1 year and the risk of cardiovascular disease and infectious mortality. According to this publication, a serum creatinine level of 1.9–2.1 mg/dL conferred a 50% increased risk of cardiovascular death compared with a serum creatinine level of >1.3mg/dL.

Long-term Graft Survival
The only factors affecting the long-term survival of those grafts that reached 1 year after censoring for death were recipient age and CIT and no apparent effect of AR or HLA matching. Crucially, we have found in our study that CIT affects long-term graft survival independently of the phenomenon of DGF. Other studies have found that DGF is one of the most important factors related to graft loss but have not identified CIT as having an impact in the long-term. In contrast, Ojo *et al.*[5] in a study from American registry data found that prolonged CIT directly and independently of DGF and AR, compromised the long-term graft survival. In this study, we have shown that the effect of CIT on long-term graft survival is linear, and hence, there is no threshold below which CIT is acceptable or a threshold beyond which the deleterious affect of CIT accelerates.

We have not shown an influence of AR on graft survival after 1 year. Even though AR was common (54%), the incidence of severe rejections and steroid-resistant rejections were low. In contrast, it has been demonstrated in less well-matched populations using large retrospective databases that there is an impact of HLA-matching and the more severe forms of rejection on long-term graft survival.

Finally, the serum creatinine at 1 year, rather than the serum creatinine at 3 or 6 months, was found from this analysis to be an excellent predictor of the long-term survival of the graft as was reported by Hariharan *et al.*[6] It was also found to be an important factor in a recent report by He and Johnston.[17]

CONCLUSION

In conclusion, the factors involved in the short-term and long-term outcome and function of the renal transplant graft are multiple and interrelated. Older donors with pre-existing medical conditions and more fragile donor organs will need to be used to bridge the gap between supply and demand for cadaveric organs. Even though this study has not shown a significant impact of donor management, it would still seem appropriate to study ways of minimizing harm to the donor organ during this period. Minimization of the injury during the period of preservation will have increased importance. At a local level, our study lends weight to the need for sympathetic treatment of kidneys at multiorgan retrievals and access to theaters at the earliest possible opportunity. Our study has stimulated research into the cost–benefit ratios of increased use of the more expensive cold preservation solutions. Furthermore, the use of organ perfusion by machine at both cold and warm temperatures is now being revisited.

CIT is clearly identified as an important factor and one that can be controlled. Efforts should, therefore, be made to reduce CIT as much as possible; indeed, it was reduced during the period of this study. It would be unwise to place emphasis on 'cutoff values' that have not been rigorously demonstrated.

HLA matching has been the major focus of national and local sharing schemes to improve outcomes from transplantation. Although sharing could result in an increased in CIT, this has usually been seen as a price worth paying for improved matching. Recent national data show that the CIT of shared kidneys is only slightly longer than that of local kidneys (UK Transplant, Bristol). We do not propose that attention to HLA matching should be reduced but that practices could be reviewed with the objective of reducing CIT while maintaining matching.

REFERENCES

1. Marshall R, Ahsan N, Dhillon S, Holman M, Yang HC. Adverse effect of donor vasopressor support on immediate and one-year kidney allograft function. Surgery 1996;120:663-5.
2. Pratschke J, Wilhelm MJ, Laskowski I, Kusaka M, Beato F, Tullius SG, *et al.* Influence of donor brain death on chronic rejection of renal transplants in rats. J Am Soc Nephrol 2001;12:2474-81.
3. Nagelkerke N. A note on a general definition of the coefficient of determination. Biometrika 1991;78:691-2.
4. Altman DG, Bland JM. Interaction revisited: The difference between two estimates. BMJ 2003;326:219.
5. Ojo AO, Wolfe RA, Held PJ, Port FK, Schmouder RL. Delayed graft function: Risk factors and implications for renal allograft survival. Transplantation 1997;63:968-74.
6. Hariharan S, McBride MA, Cherikh WS, Tolleris CB, Bresnahan BA, Johnson CP, *et al.* Post-transplant renal function in the first year predicts long-term kidney transplant survival. Kidney Int 2002;62:311-8.
7. Kyllönen LE, Salmela KT, Eklund BH, Halme LE, Höckerstedt KA, Isoniemi HM, *et al.* Long-term results of 1047 cadaveric kidney transplantations with special emphasis on initial graft function and rejection. Transpl Int 2000;13:122-8.
8. Troppmann C, Gillingham KJ, Benedetti E, Almond PS, Gruessner RW, Najarian JS, *et al.* Delayed graft function, acute rejection, and outcome after cadaver renal transplantation. The multivariate analysis. Transplantation 1995;59:962-8.
9. Su X, Zenios SA, Chakkera H, Milford EL, Chertow GM. Diminishing significance of HLA matching in kidney transplantation. Am J Transplant 2004;4:1501-8.
10. Opelz G, Döhler B, Ruhenstroth A, Cinca S, Unterrainer C, Stricker L, *et al.* The collaborative transplant study registry. Transplant Rev (Orlando) 2013;27:43-5.
11. McLaren AJ, Jassem W, Gray DW, Fuggle SV, Welsh KI, Morris PJ, *et al.* Delayed graft function: Risk factors and the relative effects of early function and acute rejection on long-term survival in cadaveric renal transplantation. Clin Transplant 1999;13:266-72.
12. Boom H, Mallat MJ, de Fijter JW, Zwinderman AH, Paul LC. Delayed graft function influences renal function, but not survival. Kidney Int 2000;58:859-66.
13. Roodnat JI, Mulder PG, Van Riemsdijk IC, IJzermans JN, van Gelder T, Weimar W, *et al.* Ischemia times and donor serum creatinine in relation to renal graft failure. Transplantation 2003;75:799-804.
14. Rush DN, Karpinski ME, Nickerson P, Dancea S, Birk P, Jeffery JR, *et al.* Does subclinical rejection contribute to chronic rejection in renal transplant patients? Clin Transplant 1999;13:441-6.
15. Paraskevas S, Kandaswamy R, Humar A, Gillingham K, Gruessner RW, Payne WD, *et al.* Predicting long-term kidney graft survival: Can new trials be performed? Transplantation 2003;75:1256-9.
16. Meier-Kriesche HU, Baliga R, Kaplan B. Decreased renal function is a strong risk factor for cardiovascular death after renal transplantation. Transplantation 2003;75:1291-5.
17. He X, Johnston A. Risk factors for allograft failure in united kingdom renal transplant recipients treated with cyclosporine A. Transplantation 2005;79:953-7.