Nephrol Dial Transplant (1999) 14: 930–935

Nephrology Dialysis Transplantation

Original Article

Donor age and delayed graft function as predictors of renal allograft survival in rejection-free patients

Francesc Moreso[1], Daniel Serón[1], Salvador Gil-Vernet[1], Lluís Riera[2], Xavier Fulladosa[1], Rosa Ramos[1], Jeroni Alsina[1] and Josep Maria Grinyó[1]

[1]Nephrology and [2]Urology Departments, Ciutat Sanitària i Universitària de Bellvitge, Barcelona, Spain

Abstract

Background. Transplant recipients of kidneys harvested from old donors have a high incidence of delayed graft function (DGF) and a poor graft outcome. This result is partly explained by the increased incidence of acute rejection in patients suffering from DGF. However, the long-term impact of donor age and DGF in rejection free renal transplants is not well established. The aim of the present work is to evaluate the impact of donor age and DGF on long-term outcome in renal transplants with or without acute rejection.

Patients. We review all cadaveric kidney transplants performed in our centre between April 1984 and December 1995 treated with a cyclosporin-based immunosuppression.

Results. Five hundred and ninety-five patients were included. The overall incidence of DGF was 29.1%, and this event was associated with an increased donor age and cold ischaemia time. Univariate and multivariate analysis showed that graft loss was associated with acute rejection (relative risk (RR) 2.24, 95% confidence interval (CI) 1.62–3.01); DGF (RR 1.83, 95% CI 1.32–2.54); donors >50 years (RR 1.65, 95% CI 1.13–2.38); and retransplantation (RR 1.52, 95% CI 1.01–2.31). In rejection-free patients there were two independent predictors of graft failure: donor >50 years (RR 2.40, 95% CI 1.45–4.01); and DGF (RR 2.42, 95% CI 1.53–3.84).

Conclusions. Regardless of the presence of acute rejection, delayed graft function amplifies the detrimental effect of advanced donor age on long-term graft outcome.

Key words: acute rejection; delayed function; donor age; long-term graft outcome; renal transplant

Introduction

In renal transplantation the absence of immediate allograft function is known as delayed graft function (DGF), and it is commonly defined as the need for dialysis during the first week after transplantation. In kidney cadaveric transplantation, DGF usually ranges between 10 and 50%, makes the management of patients more complex, the diagnosis of rejection more difficult, prolongs hospital stay, increases the financial cost of transplantation, and reduces graft survival rates [1,2]. During recent years it has been shown that DGF associated with acute rejection implies a poor long-term graft outcome [3,4]. However, the influence of DGF on long-term graft survival in rejection-free patients has not been well established. While some authors support that DGF only has a negative impact on long-term graft survival in patients who presented acute rejection [4], others maintain that the negative effect of DGF is mediated through mechanisms not involving acute rejection [5].

The persistent shortage of donors has stimulated different strategies in order to increase the donor pool. For example, aged or non-heart-beating donors are frequently considered for cadaveric kidney transplantation. However, advanced donor age is clearly associated with an increased risk of DGF and a rather poor graft outcome [6].

The aim of the present retrospective study is to determine the effect of donor age and post-transplant DGF on allograft outcome in patients with or without acute rejection. Since we evaluate long-term results, subgroups with immediate graft loss after transplantation have been eliminated from the study.

Subjects and methods

Patients

Between April 1984 and December 1995 we performed 625 renal transplants obtained from cadaveric heart-beating donors. Since the aim of the present study was to investigate the influence of donor age and delayed graft function on long-term results, early graft losses were not considered. Thus, never-functioning kidneys ($n=9$), technical failures ($n=9$), hyperacute rejection ($n=2$), recurrence of haemolytic–uraemic syndrome ($n=3$), and patient's death during the first week after renal transplantation ($n=7$) were not

Correspondence and offprint requests to: Josep M. Grinyó MD, Nephrology Department, Hospital de Bellvitge, c/ Feixa Llarga s/n, E-08907 L'Hospitalet, Barcelona, Spain.

evaluated. Finally, 595 patients were considered in this study. The follow-up ranged from 1 to 12 years.

Organ preservation and transplant procedure

All organs were procured according to the *in block* technique, most of them locally, and preserved by using simple hypothermic storage. The graft was placed into the right or left iliac fossa extraperitoneally, and ureteral anastomosis was performed according to the Leadbetter–Politano technique. Mannitol and frusemide were administered before the renal artery was unclamped. Frusemide was continued intravenously after surgery if urine output decreased to less than 100 ml/h, or central venous pressure was higher than 12 cm H_2O. Intravenous crystalin solutions were given to maintain central venous pressure above 7 cm H_2O. Intravenous dopamine at β doses was given for 48 h after transplantation.

Variables

The following variables were evaluated in each patient at the time of surgery: donor age and sex, cold ischaemia time (CIT), last panel-reactive antibodies (PRA), HLA mismatches, recipient age and sex, number of renal transplant and immunosuppressive treatment. After surgery, the following variables were recorded: delayed graft function, acute rejection, graft failure and the reason of graft failure, as well as serum creatinine levels at 1 and 3 months, and thereafter yearly.

The cause of graft failure was recorded as one of the following four categories: acute rejection, chronic transplant nephropathy, patient's death with a functioning graft, and others.

Immunosuppression

All patients were treated with cyclosporin (CsA) based immunosuppressive regimens. In all patients CsA was given from the day of transplantation. One hundred and forty-eight patients received a dual therapy consisting of CsA at an initial dose of 14 mg/kg/day and prednisone as previously described [7]. The remaining 447 patients were treated with lower doses of CsA (8 mg/kg/day) and steroids in conjunction with a concomittant induction therapy with polyclonal or monoclonal antibodies as previously described [8]. Since post-transplant evolution in our patients treated with polyclonal or monoclonal antibodies was very similar [8] we classify our population into two immunosuppressive groups: dual and induction.

Clinical definitions

DGF was defined as the need for dialysis during the first week after transplantation once hyperacute rejection and vascular or urinary-tract complications were ruled out.

The diagnosis of acute rejection was based on classical clinical data and in most instances histological criteria. For this analysis, any antirejection treatment was considered as a rejection episode.

Chronic transplant nephropathy was clinically defined as a progressive and sustained decline in renal function leading to return to dialysis, usually in conjunction with proteinuria and hypertension. Suspected urinary-tract obstruction, chronic urinary-tract infection, or renal transplant artery stenosis were ruled out by standard clinical procedures. Suspected recurrent or *de novo* glomerulonephritis were confirmed or discarded by histological studies.

Statistics

Values are expressed as mean $\pm$SD. In order to examine differences between patients who presented DGF and patients who did not we employed the chi-square test with the Yates' continuity correction and Student's t test for categorical and quantitative data respectively. To further analyse independent predictors of DGF a logistic-regression model was applied.

Kaplan–Meier analysis was used to calculate actuarial graft survival and the log-rank test was employed to compare survival between groups. Risk factors for graft loss were first examined using univariate Cox's proportional hazard analysis. For factors with only two categories, Cox's proportional hazard model calculates the relative risk estimate. For continuous factors, Cox's analysis estimates the relative risk per unit of measurement. Thus we studied the best cut-off of those continuous variables associated with graft survival in the univariate analysis. Covariates that tended to correlate with survival on univariate analysis ($P<0.10$) were also examined using multivariate Cox's analysis.

All P values were two-tailed and a P value <0.05 was considered significant.

Results

Delayed graft function

The incidence of DGF was 29.1% (173 of 595 patients). The occurrence of DGF was associated with an increased donor age and cold ischaemia time, as well as with the use of a dual immunosuppressive therapy (Table 1). In order to further characterize the relative contribution of these variables to the occurrence of DGF, we analysed data with a logistic regression model. In this model, donor age, a dual therapy with CsA and steroids, and cold ischaemia time were predictors of DGF (Table 2).

Table 1. Demographic characteristics and clinical evolution after transplantation in patients who presented DGF and in patients who did not

Variable	No DGF ($n=422$)	DGF ($n=173$)	$P=$
Donor age	31.1 $\pm$ 14.5	36.8 $\pm$ 16.5	<0.0001
Donor sex (m/f)	295/127	120/53	n.s.
Recipient age	41.7 $\pm$ 13.0	42.4 $\pm$ 13.4	n.s.
Recipient sex (m/f)	261/161	118/55	n.s.
PRA (%)	13.3 $\pm$ 24.7	14.9 $\pm$ 26.5	n.s.
Transplant (1/2/3)	372/46/2	145/28/0	n.s.
A mismatches	1.1 $\pm$ 0.7	1.1 $\pm$ 0.7	n.s.
B mismatches	1.2 $\pm$ 0.6	1.2 $\pm$ 0.6	n.s.
DR mismatches	0.59 $\pm$ 0.58	0.67 $\pm$ 0.57	n.s.
CIT (h)	24.1 $\pm$ 6.8	26.1 $\pm$ 7.9	0.0035
Immunosuppression			
Dual	81 (54.7%)	67 (45.3%)	
Induction	341 (76.3%)	106 (23.7%)	<0.0001
Acute rejection (no/yes)	312/110	92/81	<0.0001

DGF, delayed graft function; PRA, panel-reactive antibodies; CIT, cold ischaemia time.

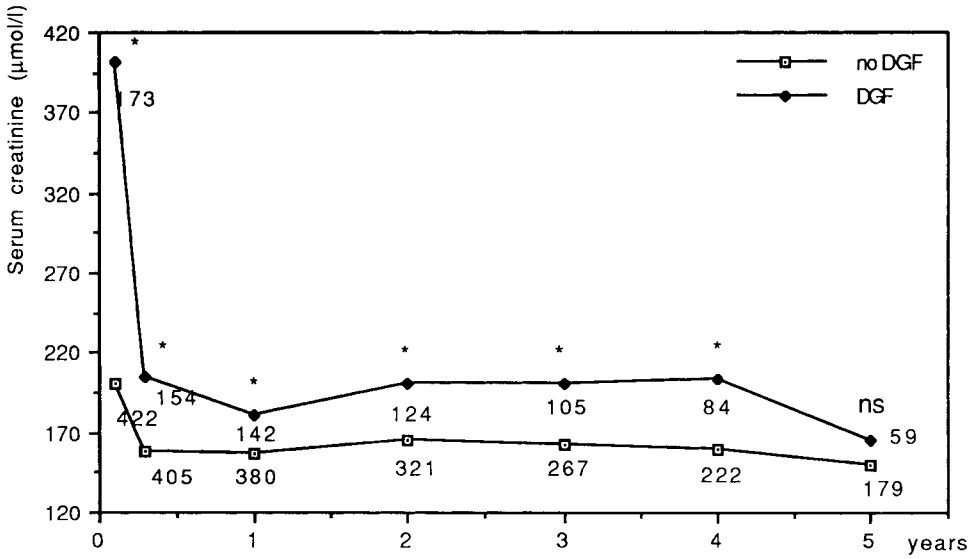

Fig. 1. Evolution of serum creatinine during the first 5 years of follow up in patients with and without DGF. Number of evaluated patients at each time period are indicated. $*P<0.01$.

Patients with DGF showed an increased serum creatinine during the first 4 years after transplantation (Figure 1) and a higher incidence of acute rejection (Table 1). In our set of patients three different combinations between DGF and acute rejection can be considered: (i) acute rejection diagnosed during DGF ($n=35$), (ii) acute rejection diagnosed after recovery of DGF ($n=46$), and (iii) acute rejection in patients who did not suffer from DGF ($n=110$). In the first group a renal biopsy was performed in 23 of 35 patients at 9 ± 4 days after surgery. Histological diagnosis according to the Banff schema were: normal ($n=1$), acute tubular necrosis ($n=4$), borderline changes ($n=2$), acute rejection grade I ($n=6$), grade II ($n=5$) and grade III ($n=5$). In the second group a renal biopsy was performed in 25 of 46 patients. Histological diagnosis were: normal ($n=5$), acute tubular necrosis ($n=2$), borderline changes ($n=2$), acute rejection grade I ($n=7$), grade II ($n=6$) and grade III ($n=3$). In 62 of 110 patients with acute rejection who never experienced DGF, a diagnostic renal biopsy was carried out and histological diagnosis were: normal ($n=3$), borderline changes ($n=20$), acute rejection grade I ($n=21$), grade II ($n=16$), and grade III ($n=2$). There were no statistical differences in the distribution of histological diagnoses in biopsies performed in these three groups of patients.

Table 2. Risk factors for delayed graft function

Variable	$P=$	Odds ratio
Donor age >50 years	0.0021	2.12 (1.30–3.45)
Dual therapy	0.0007	2.14 (1.37–3.36)
CIT (each h)	0.0021	1.04 (1.02–1.07)

Relative risks and (in parentheses) 95% confidence interval for logistic regression model.

Graft survival

During follow up 168 grafts were lost at 4.5 ± 3.2 years (median 30 months, range 2–125 months). The causes of graft loss were: chronic transplant nephropathy in 80 cases (47.6%), acute rejection in 30 cases (17.8%), and patient's death with a functioning graft in 27 cases (16.1%). The remaining 31 failures (18.4%) were for other reasons.

Univariate graft survival analysis showed that donor age, retransplantation, DGF, rejection, and dual therapy with CsA and steroids were associated with a poor prognosis (Table 3). In the case of donor age, we categorized this variable by decades in order to find its best cut-off and this analysis showed that the relative risk for graft failure only increases after the fifth decade (data not shown). Thus we categorized this variable as younger or older than 50 years. Multivariate analysis showed that donor age, retransplantation, DGF, and rejection were the only independent covariates associated with a poorer graft outcome (Table 3). Dual therapy with CsA and steroids was not a significant predictor of graft outcome in the multivariate analysis. This was an expected result since in our population dual therapy was associated with a higher incidence of DGF (45.3 *vs* 23.7%, $P<0.001$) and acute rejection (47.2 *vs* 23.2%, $P<0.001$). Donor and recipient sex, cold ischaemia time, PRA, histocompatibility, and recipient age did not have a significant influence on graft survival (data not shown).

Recipients from kidneys harvested from old donors had a higher incidence of DGF. Because of this we analysed whether the association of these two events, donor age and DGF, may amplify their detrimental effect on graft survival. For this reason we grouped our patients into four categories according to the presence or absence of DGF and donor age older or younger than 50 years (Figure 2). Cox's analysis

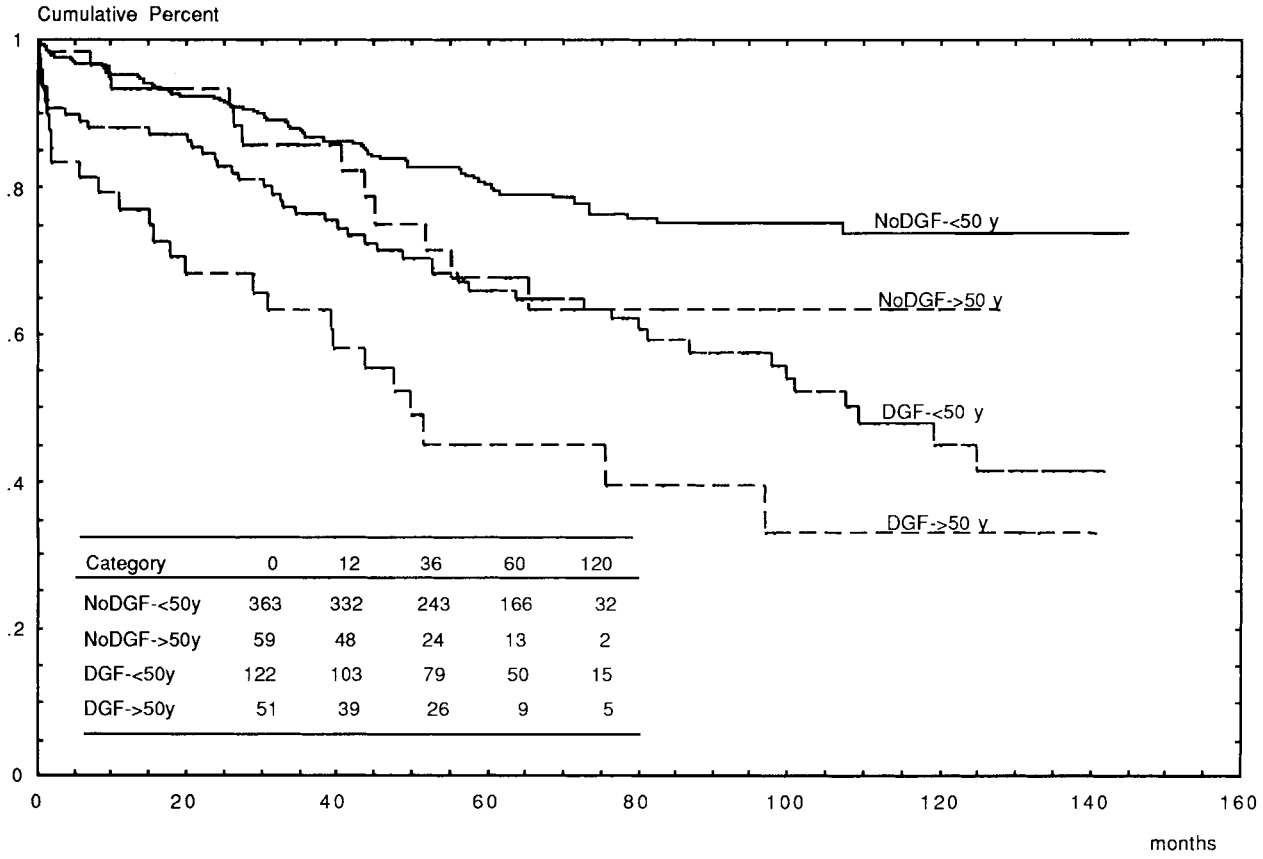

Fig. 2. Graft survival according to donor age (older or younger than 50 years) and the presence or absence of delayed graft function ($P < 0.0001$). The Table summarizes the number of patients at risk at 0, 12, 36, 60 and 120 months.

showed that patients who received a kidney from a donor older than 50 years and developed DGF had the highest risk of late graft loss (relative risk and 95% confidence interval *vs* patients with delayed function who received a kidney from a donor younger than 50 years: 1.73, 1.07–2.80, *vs* patients with early function who received a kidney from a donor older than 50 years: 2.50, 1.26–4.76, and *vs* patients with early function who received a kidney from a donor younger than 50 years: 3.70, 2.38–5.88).

To evaluate whether the detrimental effect of donor age and DGF on graft survival could be partially explained by an increased incidence of acute rejection, we reanalysed our data by means of multivariate Cox's analysis in rejection-free patients. This analysis confirms that only donor age and DGF are independent predictors of graft survival (Table 4). Moreover, in an attempt to establish a relationship between donor age and DGF on the development of chronic transplant nephropathy, we further analysed our data censoring patients who died with a functioning graft and excluding grafts lost due to any other reason than chronic transplant nephropathy. In this analysis of 348 patients and 48 events, donor age and DGF were once again the only independent predictors of graft loss due to chronic transplant nephropathy (Table 5).

Discussion

In this retrospective analysis of adult kidney cadaveric transplants treated with cyclosporin DGF occurred, as

Table 3. Risk factors for graft failure

Variable	Univariate analysis	$P=$	Multivariate analysis	$P=$
Acute rejection	2.63 (1.94–3.56)	0.0001	2.24 (1.62–3.01)	0.0001
DGF	2.43 (1.79–3.29)	0.0001	1.83 (1.32–2.54)	0.0003
Donor age >50 years	1.96 (1.35–2.85)	0.0004	1.65 (1.13–2.38)	0.0082
Second transplants	1.64 (1.09–2.44)	0.0189	1.52 (1.01–2.31)	0.0476
Dual therapy	1.55 (1.12–2.15)	0.0075	1.10 (0.78–1.56)	0.5753

Relative risks and (in parentheses) 95% confidence interval for univariate and multivariate Cox's proportional hazard models.

Table 4. Risk factors for graft failure in rejection-free patients

Variable	$P=$	Multivariate analysis
DGF	0.0002	2.42 (1.53–3.84)
Donor age >50 years	0.0007	2.40 (1.45–4.01)
Second transplant	0.1308	1.61 (0.86–2.98)
Dual therapy	0.6346	1.13 (0.68–1.88)

Relative risks and (in parentheses) 95% confidence interval for multivariate Cox's proportional hazard model.

previously described, almost in one-third of the cases [1–4]. The identified risk factors for DGF were advanced donor age, prolonged cold ischaemia time, and the use of a dual therapy consisting of CsA and prednisone. Despite finding a strong association between DGF and acute rejection, we show that DGF is an independent predictor of late graft outcome. Moreover, in rejection-free patients, we observed that there are two major risk factors for graft failure: donor age and DGF.

Age-related donor renal damage, measured either with a semi-quantitative scale or with a quantitative paramenter such as the percentage of sclerosed glomeruli or the expansion of interstitial space, correlates with the incidence of post-transplant DGF [9–11]. On the other hand, prolonged cold ischaemia has been classically recognized as a risk factor for DGF [12]. However, while donor age is an independent predictor of graft survival, the influence of cold ischaemia on graft outcome has not been well characterized. Nevertheless, a link between cold ischaemia time and chronic rejection has been established in experimental transplantation [13]. The other identified detrimental factor for DGF in the present study was the lack of antilymphocyte antibodies as induction therapy. This observation has been attributed in part to the avoidance of CsA nephrotoxicity in the immediate post-transplant period [14], although it should be taken into consideration that antilymphocyte antibodies may improve renal function through other mechanisms, i.e. attenuating the immunological reactions involved in the ischaemia–reperfusion injury [15].

Although there is a strong association between DGF and acute rejection [3,4], the mechanism leading to an increased incidence of acute rejection in patients suffering from DGF remains to be elucidated. It has been suggested that reperfusion injury increases the immunogenicity of the graft, thus favouring the allograft reaction [16]. Further support for this hypothesis comes from the observation that scavengers of oxygen free radicals reduce the incidence of acute rejection and increase long-term graft survival in clinical renal transplantation [17]. However, the immune activation induced by DGF might trigger an immune reaction that could favour the development of chronic transplant nephropathy even in the absence of acute rejection. Despite the postulation that DGF in the absence of rejection has no long-term impact [3,4], we observed that donor age and DGF are the only independent predictors of long-term graft survival in rejection free patients. Furthermore, in our study we show that donor age and DGF predict late graft loss due to chronic transplant nephropathy. Recently Lethonen *et al.* [18] showed that the combination of DGF and rejection only have a deleterious effect on retransplanted patients, but not in primary transplants. However, when all causes of graft loss other than chronic rejection were censored, they also observed that DGF and rejection were independent predictors of graft outcome. Moreover, the results of Feldman *et al.* [5] suggest that the effect of DGF on graft survival is mediated in part through mechanisms not involving acute rejection. In this regard, Cosio *et al.* [19] have shown that long-term graft survival depends on early allograft function and acute rejection.

In the present study we not only found that the presence of DGF implies a poor outcome, but we also observed that patients receiving a kidney from an old donor who suffer from DGF will have a very poor outcome in comparison to patients receiving a kidney from an old donor who had immediate renal function, suggesting that the deletereous effect of donor-dependent damage may be amplified by the ischaemia–reperfusion injury. In this regard, it has been shown in an experimental model that isografts develop chronic renal changes that mimic those of chronic allograft rejection, and that these lesions are modulated by the severity of the ischaemic injury [20] and the amount of nephron mass [21]. In the clinical setting, there is a growing body of evidence on the negative effect of advanced donor age on renal function and graft survival [22]. Taking into consideration that ageing implies a reduction in the nephron number [23], the above-mentioned results suggest that the senescent kidney might be more vulnerable to the ischaemia–reperfusion injury which may further reduce the nephron mass. Then a permanent functional impairment unable to supply the recipient's metabolic demand [24], could trigger hyperfiltration-mediated damage [25].

Table 5. Risk factors for chronic transplant nephropathy in rejection-free patients

Variable	Univariate analysis	P	Multivariate analysis	$P=$
DGF	3.67 (2.08–6.49)	<0.001	3.01 (1.64–5.52)	0.0004
Donor age >50 years	3.06 (1.58–5.92)	=0.0009	2.17 (1.09–4.33)	0.0270
Dual therapy	1.64 (0.87–3.01)	=0.1239	1.21 (0.66–2.33)	0.5712
Second transplants	1.37 (0.58–3.24)	=0.4752	1.40 (0.59–3.33)	0.4399

Relative risks and (in parentheses) 95% confidence interval for univariate and multivariate Cox's proportional hazard models.

In summary, we show that patients receiving a kidney from an old donor who suffer from DGF have a very poor long-term graft survival even in rejection-free patients. These data indirectly support the suggestion that the deleterious effect of DGF on late graft outcome is amplified in patients receiving a kidney harvested from an old donor. Consequently it seems reasonable to control those factors associated with DGF such as cold ischaemia time or cyclosporin nephrotoxicity in order to improve long-term results when organs from elderly donors are accepted for transplantation.

Acknowledgements. This study was supported by Fondo de Investigación Sanitaria (FIS) grant 96/0865.

References

1. Sanfilippo F, Vaughn WK, Spees EK, Lucas BA. The detrimental effects of delayed graft fuction in cadaver donor renal transplantation. *Transplantation* 1984; 38: 643–648
2. Rosenthal JT, Danovitch GM, Wilkinson A, Ettenger RB, The high cost of delayed graft function in cadaveric renal transplantation. *Transplantation* 1991; 51: 1115–1118
3. Troppmann C, Gillinham KJ, Benedetti E *et al.* Delayed graft function, acute rejection, and outcome after cadaver renal transplantation. A multivariate analysis. *Transplantation* 1995; 59: 962–968
4. Troppmann C, Gillingham KJ, Gruessner RWG *et al.* Delayed graft function in the absence of rejection has no long-term impact. *Transplantation* 1996; 61: 1331–1337
5. Feldman HI, Gayner R, Berlin JA *et al.* Delayed function reduces renal allograft survival independent of acute rejection. *Nephrol Dial Transplant* 1996; 11: 1306–1313
6. Cosio FG, Qiu W, Henry ML *et al.* Factors related to the donor are major determinants of renal allograft function and survival. *Transplantation* 1996; 62: 1571–1576
7. Griñó JM, Alsina J, Sabater R *et al.* Antilymphoblast globulin, cyclosporine and steroids in cadaveric renal transplantation. *Transplantation* 1990; 49: 1114–1117
8. Griñó JM, Castelao AM, Serón D *et al.* Antilymphocyte globulin *vs* OKT3 induction therapy in cadaveric kidney transplantation. A prospective randomized trial. *Am J Kidney Dis* 1992; 20: 603–610
9. Gaber LW, Moore LW, Alloway RR, Amiri MH, Vera SR, Gaber AO. Glomerulosclerosis as a determinant of posttransplant function of older donor renal allografts. *Transplantation* 1995; 60: 334–339
10. Leunissen KL, Bosman FT, Nieman FHM *et al.* Amplification of the nephrotoxic effect of cyclosporine by preexistent chronic histological lesions in the kidney. *Transplantation* 1989; 48: 590–593
11. Serón D, Carrera M, Grinyó JM *et al.* Relationship between donor renal interstitial surface and post-transplant renal function. *Nephrol Dial Transplant* 1993; 8: 539–543
12. Peters TG, Shaver TR, Ames JE, Santiago-Delpin EA, Jones KW, Blanton JV. Cold ischemia and outcome in 17,937 cadaveric kidney transplants. *Transplantation* 1995; 59: 191–196
13. Yilmaz S, Paavonen T, Häyry P. Chronic rejection of rat renal allografts. II. The impact of prolonged ischemia time on transplant histology. *Transplantation* 1992; 53: 823–827
14. Halloran P, Aprile M, Farewell V for The Ontario Renal Transplant Research Group. Factors influencing early renal function in cadaver kidney transplants. A case-control study. *Transplantation* 1988; 45: 122–127
15. Weight SC, Bell PRF, Nicholson ML. Renal ischaemia-reperfusion injury. *Br J Surg* 1996; 83: 162–170
16. Shoskes DA, Parfrey NA, Halloran P. Increased major histocompatibility complex antigen expression in unilateral ischemic acute tubular necrosis in the mouse. *Transplantation* 1990; 49: 201–207
17. Land W, Schneeberger H, Schleibner S *et al.* The beneficial effect of human recombinant superoxide dismutase on acute and chronic rejection events in recipients of cadaveric renal transplants. *Transplantation*. 1994; 57: 211–217
18. Lethonen SRK, Isoniemi HM, Salmela KT, Taskinen EI, von Willebrand EO, Ahonen JP. Long-term graft outcome is not necessarily affected by delayed onset of graft function and early acute rejection. *Transplantation* 1997; 64: 103–107
19. Cosio FG, Pelletier RP, Falkenhain ME *et al.* Impact of acute rejection and early allograft function on renal allograft survival. *Transplantation* 1997; 63: 1611–1615
20. Tulius SG, Heemann U, Hancock WW, Azuma H, Tilney NL. Long-term kidney isografts develop functional changes that mimic those of chronic allograft rejection. *Ann Surg* 1994; 220: 425–435
21. Mackenzie HS, Tullius SG, Heeman U *et al.* Nephron supply as a major determinant of long-term renal allograft outcome in rats. *J Clin Invest* 1994; 94: 2148–2152
22. Chertow GM, Brenner BM, Mackenzie HS, Mildford EL. Non-immunologic predictors of chronic renal allograft failure: data from the United Network of Organ Sharing. *Kidney Int [Suppl]* 1995; S48–51
23. Nyengaard JR, Bendtsen TF. Glomerular number and size in relation to age, kidney weight, and body surface in normal man. *Anat Rec* 1992; 232: 194–201
24. Moreso F, Serón D, Anunciada AI *et al.* Recipient body surface area as a predictor of posttransplant renal allograft evolution. *Transplantation* 1998; 65: 671–676
25. Brenner BM, Mildford EL. Nephron underdosing: a programmed cause of chronic renal allograft failure. *Am J Kidney Dis* 1993; 21: 66–72

Received for publication: 6.7.98
Accepted in revised form: 11.12.98