ORIGINAL ARTICLE

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Effect of cold ischaemia time reduction on the graft function of marginal donors

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ABSTRACT

Background: Transplantation has improved survival of end-stage renal disease patients. There are more patients on waiting lists than there are available grafts. An increasing number of donors considered marginal are currently sources for transplantation.

A cold ischaemia time over 24h in marginal kidneys has a negative influence on immediate renal function and graft survival.

Our aim was to evaluate whether a decrease in cold ischaemia time influences the outcome in grafts from marginal donors.

Patients and Methods: We studied the patients transplanted in our centre from January 1st 2002 to December 31st 2005 who received a kidney from a deceased marginal donor.

Results: Cold ischaemia time over 18 hours has a significantly greater incidence of slow graft function (73.3% *versus* 65.8%) and delayed graft function (31.3% *versus* 18.9%). A trend was observed between the cold ischaemia time and the creatinine at five years (p=0.07). Neither the number of HLA mismatching, donor´s age or cause of donor death were significantly related to slow graft function.

No relationship was observed between cold ischaemia time and the incidence of acute rejection or the serum creatinine values at 3, 6 and 12 months post-transplantation.

Conclusions: Efforts to shorten cold ischaemia time below an 18-hour limit have been rewarded with a decrease in the incidence of slow graft function and beneficial effects on renal function.

Key-Words:
Cold ischaemia time; delayed graft function; marginal donor; renal transplantation.

INTRODUCTION

Renal transplantation has been proven to improve survival of end-stage renal disease (ESRD) patients[1,2]. However, there are more patients on waiting lists than there are available grafts. An increasing number of donors considered marginal for acceptance are currently sources for renal transplantation. The grafts from these donors (expanded donor pool) have a lower survival than those from referent donors. As an alternative, they have been proposed for use in patients with lower life expectancy[3]. In fact, these donors improve the survival of these older patients compared with those remaining on dialysis[4]. In sum, the use of marginal donors helps to decrease the shortage of available organs, with the old-for-old policy.

Multiple variables have been reported to influence graft survival. Among them, cold ischaemia time (CIT)

stands out, and is one potentially modifiable factor[5,6]. It is known that a CIT over 36 hours has a negative influence on the outcome of renal allografts[7]. Moreover, a cold ischaemia time of over 24 hours in marginal kidneys has a negative influence both on immediate renal function and graft survival[8,9]. It is not known whether reducing CIT to less than 24 hours in marginal donors improves immediate and long-term graft outcome. Only one study has suggested that the effort to achieve shorter CIT is worthwhile in this type of patient, even below a 12-hour period[5].

The aim of our study was to evaluate whether a significant decrease in CIT influences the immediate outcome in grafts from marginal donors, with the hypothesis that these grafts are more susceptible to the negative effect of ischaemia time.

PATIENTS AND METHODS

Definitions

A reference (ideal) donor is a person under 60 years of age with encephalic death and heartbeat[10].

A marginal donor is a person over 60 years of age or between 50-59 years of age with at least one of the following risk factors: previous history of hypertension or diabetes mellitus without proteinuria, cerebrovascular event as cause of death or a current serum creatinine over 1.5 mg/dl.

Slow graft function (SGF) occurs when the serum creatinine is greater than 3 mg/dl without need of a dialysis session in the first week after transplantation.

Delayed graft function (DGF) is defined as the requirement of dialysis in the first weed after transplantation[11]. Number of SGF days is the time until the creatinine level begins to decrease.

Acute rejection is defined as those episodes of renal function deterioration with high clinical suspicion, which have been treated with immunosuppressive therapy. Whenever the patient's clinical situation allowed, a renal biopsy was performed (85% of cases).

Cold ischaemia time is the period during transplantation beginning when the kidney is cooled with a cold perfusion solution after the replacement surgery and ending when the tissue reaches physiological temperature during the implantation procedure.

Warm ischaemia time is defined as the time passed between starting venous anastomosis and the end of arterial anastomosis during graft implantation.

Patients

Patients transplanted in our centre from January 1st 2002 to December 31st 2005 who received a kidney from a deceased marginal donor were studied. This group was chosen because the loss of grafts for each 100 patient/years in this age group has been demonstrated to be high[12]. The period selected corresponds to the implementation in our centre of a policy addressed to specifically reduce CIT. Fifty-four patients received a graft defined as marginal during the period in question; 35% of the total number of transplants. The mean follow-up was 5 years.

The decision to implant these organs was based on minimal clinical requirements of the donor (serum creatinine less than 1.5 mg/dl, age less than 70 years, controlled arterial hypertension, diabetes with no proteinuria) and on a normal macroscopic appearance of the kidneys. No renal biopsy biopsies were performed. Final selection of recipients was based on blood group and HLA compatibility, the macroscopic exam of the kidneys by a transplant surgeon, and a matching of ages and heights of donor and recipient.

The induction immunosuppression protocol consisted of baxilisimab, tacrolimus (0.1mg/kg/d) or cyclosporine neoral (5 mg/kg/d), mycofenolate mofetil and steroids. This immunosuppressive regimen was occasionally modified according to the immunogenic risk of the patient. Six patients (11.1%) were included in a clinical protocol consisting of basilixiimab, tacrolimus (0.05 mg/dl/d) and sirolimus.

The following variables were included:

Donor: Blood group and HLA, cause of death, age, gender, weight and height, hypertension and diabetes mellitus.

Recipient: Age, gender, weight and height, hypertension, renal disease, time and type of dialysis, diabetes mellitus, number of transplants, blood group, HLA mismatching, percentage of anti-panel antibodies, cold and warm ischaemia times, immunosuppression, acute rejection incidence, delay of graft function (with and without need for dialysis), incidence of surgical complications, mortality, and creatinine at 3, 6 and 12 months.

Ethical aspects

All patients signed an informed consent form for the transplant procedure, in which the possibility of using their data for scientific purposes was included. The ethical committee of the hospital approved this consent form.

Statistical analysis

Groups were formed according to their ischaemia times and compared using the chi-square or Kruskal-Wallis tests for the nominal variables. Mann-Whitney and Anova tests were used to analyse the quantitative variables.

Multiple linear regression and bivaried correlation were used to identify those factors with a significant influence on the graft function at 6 and 12 months and on mortality. The analysis of survival was carried out using the Kaplan Meier method. All analyses were carried out using the SPSS program version 15.0.

RESULTS

During the four years of the study, we carried out 54 transplants from marginal or expanded criteria donors. This donor type grew in availability over this period (41% of the total in 2005).

The demographic characteristics of the recipients are detailed in Table I.

Table I.

Demographic characteristics of recipients and donors

Group	General
n	54
Recipient	
Age (years)	55.3±12
Sex (%)	
Male	63
Female	37
Renal disease (%)	
Diabetes Mellitus	5.6
ADPKD	25.9
Vascular	9.3
Other	44.4
Unknown	14.8
Mode of dialysis (%)	
Haemodialysis	51.9
Peritoneal dialysis	44.4
Time in dialysis (months)	30.4±23.9
Weight (Kg)	70.9±11.8
Height (cm.)	166±10
Actual/max antibodies (›20)	0
2º Transplant (%)	7.4
CIT (hours)	13.6±5.5
CIT (with DGF)	15.2±6.1
CIT (without DGF)	12.1±4.6
Warm ischaemia time	50±12
Donor	
Age (years)	60.6±5.1
Sex (%)	
Male	61.1
Female	38.9
Cause of death	
Traumatic	14.8
Nontraumatic	85.2
Blood group	
O	55.6
A	33.3
B	9.3
AB	1.9
Weight (Kg)	73.7±11.3
Height (cm.)	164.6±8
HLA incompatibilities (%)	
›4	50.1
Immunosuppressant drugs	
Induction	
Anti-CD25(%)	79.6
OKT 3 (%)	13
Steroids (%)	100
Calcineurin inhibitors	29.6
CyA (%)	53.7
FK (%)	11.1
Sirolimus to discharge (%)	

Graft short-term outcomes

The principal characteristics of the graft outcome are shown in Table II. In order to review the immediate graft function, we performed a univariate analysis with all factors that could influence SGF appearance, and we observed that CIT is statistically significant ($p<0.05$). Receiver operative curves (ROC) demonstrated that a significantly greater incidence of SGF

Table II.

Graft outcome according to groups with different cold ischaemia time

Groups	CIT<18	CIT≥18
Patients (number)	37	**16**
Hospital stay (days)	19.8±20.9	18.9 ± 14.3
CIT (hours)	10.8±3.7	20.4±2.3
Warm ischaemia time (min)	47.1±13.2	56.8±18.4
SGF (%) *		
Yes	65.8	73.3
No	34.2	26.7
DGF(%)		
Yes	18.9	31.3
No	81.1	68.7
Day of efficacious diuresis	4.6±7.4	7.5 ± 8.5
Acute rejection (%)	22.2 (8)	25 (4)
Creatinine at 1 month (mg/dl)	2.7±1.9	3.35 ± 2.7
Creatinine at 6 months (mg/dl)	1.8±0.6	1.9 ± 0.7
Creatinine at 1 year (mg/dl)	1.8±0.6	1.9 ± 0.7
Creatinine at 5 years (mg/dl)	2.1±1.2	2.1±1.4
Surgical complications (%)	18.9 (7)	12.5 (2)
Graft loss at 1 year (%)	12.9 (4)	6.3 (1)
Graft loss at 5 years (%)	11.4 (4)	6.7 (1)
Survival 1 year (%)	94.7 (36)	87.5 (14)
Survival at 5 years (%)	88.9	81.3

* p<0.05

was associated with CIT longer than 18 hours (73.3% *versus* 65.8%).

Further, when we analysed the 27 patients with a CIT less than 12 hours, the incidence of SGF decreases to 33.3%.

Another variable that proved to have a statistically significant influence on SGF was warm ischaemia time.

Neither the number of HLA mismatching, donor´s age (all of them marginal ones), the fact that the patient had a higher immunological risk (including in this group the recipients of a second transplant and the hyperimmunised ones), nor the cause of donor death, were significantly related to SGF in our study.

No relationship was observed between CIT and the incidence of acute rejection.

Graft outcomes

We used serum creatinine level at 6 months, 1 and 5 years from the transplant as a marker of medium and long-term graft survival as it has been generally recognised as representative[13].

No relationship was observed between CIT and serum creatinine values at 3, 6 and 12 months after transplantation, although a trend was observed between CIT and the creatinine at 5 years (p=0.07).

When we analysed the variables that show a significant association with creatinine level at 5 years, only warm ischaemia time and creatinine level at 6 and 12 months had a statistically significant relationship. However, only warm ischaemia time maintained this significance in the multiple linear regression.

Neither the antecedent of acute rejection nor DGF proved to influence the outcome of renal function. When we analysed the influence of the immunosuppression scheme over the graft outcome at 5 years, there was no statistically significant difference between them.

Warm ischaemia time

We found a significantly statistical association between this time and the incidence of DGF, serum creatinine levels at 6 months, 1 and 5 years, and number of hospital stay days.

Patient and Graft survival

With an average monitoring time of 76 months (range 52-97 months), patient survival has been higher than 85%. Of the seven deceased patients during this period, five perished from cardiovascular causes and the other two due to infectious complications. Three deaths occurred during the first six months after transplantation. We did not observe a statistically significant relationship between mortality and any of the variables studied.

Regarding graft survival, 90% of patients had a functioning graft at five years (as death with functioning graft was censored). Slow graft function, DGF and surgical complications in the first six months, as well as serum creatinine at the first year after transplantation were related to the risk of graft loss at five years.

DISCUSSION

Over the last decade, due to the combined action of various factors such as improvement in immunosuppressant drugs and the decrease of the incidence of acute rejection, priorities in transplant patient handling have moved towards study of those factors that may influence the outcome and survival of the renal graft at medium and long-term.

The waiting list has continuously grown due to the inclusion of elderly people, who have been increasingly included since patients' life expectancy has been shown to be better with transplant than with dialysis[4]. At the same time, there has been a plateau in the number of donations from traumatic deaths among young people. Both conditions have led to greater use of suboptimal donor grafts.

Although there are some doubts about the negative influence of cold ischaemia on SGF/DGF and its effect on medium and long-term kidney graft outcome, few studies have been performed to clarify until what point the efforts to lessen CIT are rewarded with better patient outcomes. Diminishing CIT is worthwhile but costly both in terms of personnel requirements and strict intervention protocols. Those involved in kidney transplantation do not always recognise the importance of their efforts to reduce CIT, because they do not perceive the resulting improvement.

The decrease of CIT in our centre has been the consequence of several factors. One is the scarce use of kidney biopsy pretransplantation, which is controversial because selection is preferably based on clinical criteria[14]. Another is the broad awareness-raising effort among staff, expediting the selection process and accelerating candidates' preparation. As a result we have achieved a CIT of around 13 hours, much lower than that suggested by clinical international guides. Only Vacher-Coponat *et al*.[15] have shown the beneficial effects of an effective reduction of CIT to less than 13 hours, and they achieved a statistically significant reduction of DGF incidence from 34.7-20.7% over a period of two years. All other positive benefits on renal graft outcome from this practice were partially recorded at medium term. These data are in agreement with our results in SGF and DGF incidence.

When we compared this data with our own data from the kidney transplants from marginal donors performed in our centre 1998-2001, we observed CIT had decreased from 21.2±5.8 hours to 13.6±5.5, and SGF incidence from 57.9 to 45.3%. We did not implement any additional measure to improve donor management during this period which might account for the reduction in the SGF incidence.

The latest papers published on CIT influences[5,8,10,15,16] have proven that CIT is an independent risk factor for poor medium and long-term outcomes of renal grafts, although CIT was greater than 18-20 hours in the majority of these studies.

It has been agreed that the best results obtained in living donor transplantation, relative to immediate function and long-term survival despite immunogenic barriers, may be related to the CIT effects that differentiate these grafts from deceased donors. Nevertheless, Roodnat *et al*.[17] demonstrated that, despite the important influence CIT has on these differences, they are not the only acting factor. To take into account other factors influencing outcome, we examined the effect of warm ischaemia time, which was the stronger marker for graft outcome in our study.

Warm ischaemia time constitutes a numeric representation of the intraoperatory difficulties for correct vascular anastomosis in the graft, in such a way that elevated values would constitute an indicator of graft vascular damage with a potential increased risk of mortality and graft loss at medium-term. Warm ischaemia was related to subsequent mortality in our series. However the figure, less than 10%, was related to infectious factors or previous cardiovascular morbidity in recipients.

The principal limitations of our study were the relatively scarce number of patients studied, as well as the lack of a control group.

In conclusion, the experience of our centre with the use of marginal donors has been positive, with a more than acceptable medium and long-term graft outcome in marginal donors. The efforts to shorten CIT below a 15-hour limit have been rewarded with a decrease in the incidence of SGF and DGF and beneficial effects on renal function and patient survival.

Conflict of interest statement. None declared.

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