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ORIGINAL ARTICLE

Impact of cold ischemia time on renal allograft outcome using kidneys from young donors

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Summary

Prolonged cold ischemia time (CIT) is associated with delayed graft function and worse kidney transplant (KT) outcome, but the effect of CIT on long-term allograft survival in KT from younger donors has not been well established. We investigated the predictive value of CIT exposure on long-term death-censored graft loss in 829 KT recipients from younger donors (<50 years) that were performed in our center between 1991 and 2005. Overall death-censored graft failure rate was significantly higher in CIT≥19 h group versus CIT<19 h group (26 vs. 16.5%; $P = 0.002$). Significant differences were also observed when patients with primary nonfunctioning graft were excluded (21 vs. 14%; $P = 0.020$) and in patients who received tacrolimus plus mycophenolate mofetil (12 vs. 4%; $P = 0.05$). By multivariate Cox analysis, CIT was found to be independently associated with death-censored graft loss with a 20% increase for every 5 h of CIT [relative risk (RR) 1.04; 95% Confidence Interval (CI): 1.01–1.1; $P = 0.021$]. Likewise, graft loss risk significantly increased in CIT≥19 h group versus CIT<19 h group (RR 1.5; 95%CI: 1.1–2.1; $P = 0.023$). Prolonged CIT is an independent predictor of graft survival in KT from younger donors. Efforts at minimizing CIT (<19 h) should improve transplant outcome significantly in this population.

Introduction

Although there has been substantial improvement in the early survival of kidney transplantation (KT), the chronic allograft loss, particularly those from deceased donors, continues to occur at an unacceptably high rate [1]. Prolonged cold ischemia time (CIT) is a strong risk factor for delayed graft function (DGF) in KT recipients. Both risk factors (CIT and DGF) have been associated with longer hospitalizations, acute rejection, and impaired graft function, contributing significantly to chronic allograft loss in the long term [2]. Accordingly, ischemic/reperfusion injury makes the allograft more immunogenic by up-regulating molecules involved in the immune response [3].

Although evidence that kidneys from elderly donors are more vulnerable to ischemic-reperfusion injury induced by prolonged CIT, it is less known whether a longer CIT may have deleterious effect on graft outcomes in KT from younger donors. Previous studies have reported that longer CIT is associated with detrimental graft outcome regardless of human leukocyte antigen (HLA) matching and donor age [4–10], but some important risk factors for transplant outcome such as acute rejection or modern immunosuppressants, were not assessed in most of them. Thus, a more detailed analysis in KT from only younger donors has not been extensively documented. Such analysis may contribute in estimating both the size of the effects and the potential benefit of efforts directed to

decreasing CIT in recipients from young donors. We hypothesized that cold ischemia-induced renal injury may contribute to graft loss in the long term independently of immunologic dysfunction and DGF, even when optimal clinical conditions (i.e. donor age<50 years) are present.

Thus, the aim of this retrospective cohort study was to assess the impact of prolonged CIT on long-term allograft outcome in adult recipients in KT using kidneys from deceased younger donors.

Patients and methods

Design

This is a retrospective cohort study in consecutive adult Caucasian patients who received a deceased KT from younger donors to evaluate the association between the duration of CIT and graft outcome.

Study population

Between January 1991 and December 2005, 1395 deceased KT were carried out in the regional transplant center of the University Hospital of the Canary Islands (Tenerife, Spain). The seven Canary Islands are an Atlantic overseas region of Spain, and most donor kidneys are shipped from the five reference hospitals located in four major islands, to the University Hospital in Tenerife. All grafts were procured from heart-beating deceased donors. According to the study objective, only KT from donors <50 years were included in this study. The same cutoff age has been previously established using the Organ Procurement and Transplantation Network (OPTN)/Scientific Registry of Transplant Recipients (SRTR) database to identify expanded donor kidneys [11]. In addition, all recipients who had no reported CIT values ($n = 42$, 4.8%) were excluded from further analysis. Thus, a total of 829 KT recipients met the inclusion criteria for this study.

Table 1. Donor and recipient characteristics.

No.	829
Donor factors	
Mean age (years)	32 ± 12
Males (%)	61
Cardiovascular death (%)	39
Mean serum creatinine (mg/dl)	0.9 ± 0.4
Preservation solution, Euro-Collins:UW:others (%)	3.5:77:19.5
Recipients factors	
Mean age (years)	41.5 ± 12
Males (%)	65.6
Primary cause of renal disease (%)	
Diabetes	21.5
Glomerulonephritis	26
Polycystic disease	10
Nephrosclerosis	8
Interstitial nephritis	10
Unknown	13
Others	11.5
Mean time on dialysis (months)	26 ± 27
Modality dialysis (HD:PD)	705:124
Mean peak PRA	9.5 ± 22
Mean total HLA mismatches	3.3 ± 1
Mean cold ischemia time (h)	19.4 ± 5
Retransplant (%)	11.6
Pretransplant cardiovascular disease (%)	16
Initial immunosupression (%)	
Antibody induction	76
Cyclosporine	77
Tacrolimus	20
Mycophenolate mofetil	38
Azathioprine	42
Rapamycin	2.5
Delayed graft function (%)	24
Primary nonfunctioning graft (%)	4.2
Acute rejection (%)	15
Mean hospitalization days	22 ± 18
Death-censored graft loss (%)	22
Mortality (%)	14
Mean follow-up (months)	75 ± 52

UW, University of Wisconsin; HD, hemodialysis; PD, peritoneal dialysis; PRA, panel reactive antibodies.
Pretransplant cardiovascular disease includes: ischemic heart disease, stroke and peripheral vascular disease.

Data collection

Transplantation-related variables regarding donor, recipient and the transplant process were gathered from our regional transplant database, which is periodically updated (Table 1). Cold storage was the only method of preservation during the entire study period. DGF was defined as need for dialysis within the first week following transplantation. Those patients with prolonged DGF beyond the first post-transplant week underwent biopsy to confirm the diagnosis. In patients who had DGF, the dose of calcineurin inhibitors was reduced until recovery of graft function had been achieved (SCr ≤3 mg/dl) according to standard clinical practice. Primary nonfunctioning graft was defined as early and permanent dialysis treatment requirement of nonimmunological origin. Clinical rejections were confirmed by biopsy within 48 h of initiation of anti-rejection therapy. Biopsy-proven rejection was classified according to the Banff criteria [12]. Prior to the introduction of the Banff classification, rejection was diagnosed by a histopathologist on qualitative basis.

Review of medical records was performed according to Spanish law with reference to clinical data confidentiality protection (Spanish Official Bulletin, BOE No 298, 14-12-1999, pp. 43088–43099). This cohort study was approved

by the Ethics Committee of the University Hospital of the Canary Islands and was conducted according to the provisions of the Declaration of Helsinki.

Immunosuppression

Immunosuppression consisted of anti thymocyte globulin (ATGAM Upjohn, Kalamazoo, USA until 1995 and thereafter Thymoglobulin IMTIX-SANGSTAT, Lyon, France) or basiliximab (Simulect, Novartis, Basel) for induction and prednisone, cyclosporine (Neoral, Novartis, Basel) or tacrolimus (Prograf, Fujisawa, Japan), and mycophenolate mofetil (Cellcept, Roche, Basel) or azathioprine for maintenance. Rapamycin (Rapamune, Wyeth, Radnor, PA, USA) was administered instead of a calcineurin inhibitor after May 2000 in participating patients of selected randomized clinical trials ($n = 20$). Episodes of acute rejection were initially treated with three boluses of 500 mg intravenous methyl prednisolone. A 7–10 days course of OKT3 (5 mg/day) (Muromonab CD3; Ortho Pharmaceutical, Raritan, NJ, USA) was used to treat steroid-resistant rejection.

Between 1991 and 1998, patients received high-dose oral acyclovir (800–3200 mg/day, adjusted according to renal function) for the first 12 weeks post-transplant as anti-CMV prophylaxis. Between 1998 and 2004, the recipients received i.v. ganciclovir (5 mg/kg/day with the dose adjusted for renal function) for the first week post-transplant followed by daily oral acyclovir (800–3200 mg/day adjusted for renal function) for 12 weeks. After 2004, daily oral valganciclovir for 12 weeks (900 mg/day adjusted for renal allograft function) after i.v. ganciclovir was used in patients who received a seropositive donor.

Outcome

Death-censored graft survival was considered the primary outcome in the present study. Survival was measured in months from the date of renal transplantation to the date of graft loss. Graft survival data were evaluated in December 2006 and were available in respect of all the patients.

Statistical analysis

Renal transplants performed in our regional center during the entire study period had a median CIT of 19 h (interquartile range 16–23 h). Thus, we compared clinical data of patients with a CIT exposure shorter or longer than 19 h under routine clinical practice, as previously reported [6,10]. Comparison between the two groups was performed by means of Chi-squared test for categorical variables and unpaired *t* test for continuous data. Death-censored functional graft survival rate was estimated by the Kaplan-Meier method and comparisons between groups were performed by log rank test. Cox regression model was used to determine the relative impact of known risk factors, including CIT, on death-censored graft loss. Thus, in the multivariate model the following known predictors of graft survival were included: donor age, recipient age, HLA mismatches, peak panel-reactive antibodies, time on dialysis, type of dialysis, DGF, preservation fluid (Euro-Collins, University of Wisconsin and others), acute rejection, renal function at discharge expressed as serum creatinine, transplant era (1991–1995, 1996–2000, and 2001–2005), and immunosuppression at discharge and CIT. Effect of CIT on the death-censored graft failure risk was determined by introducing CIT as continuous variable. Analysis was repeated by introducing CIT as categorical variable (lesser or longer than 19 h). This analysis was performed with a backward elimination procedure of variables with *P* greater than 0.05, using the likelihood-ratio test to confirm that each removed factor did not contribute significantly to the multivariable model [13]. The final Cox model was built observing the rule that no more than one covariate per 10 events should be used in multivariate models. For each of the major covariates included in the Cox proportional hazards analysis, we tested the assumption of proportionality by visually inspecting the hazard functions and by using time-dependent covariates in bivariate models. Covariates included in the Cox proportional hazard analysis did not violate the proportionality assumption. In addition, interactions between CIT and the other independent variables were investigated. Quantitative variables are expressed as mean ± standard deviation or median and interquartile range as appropriate. Statistical analysis was carried out with SPSS software version 13.0 (SPSS, Inc., Chicago, IL, USA). Two-tailed *P*-values less than 0.05 were considered significant.

Results

Median follow up at the time of this analysis (December 31, 2006) was 69 months (interquartile range, 30–115 months). Table 1 summarizes demographic and clinical characteristics of donors and KT recipients. According to our local immunosuppressive protocol, most patients received antibodies induction and cyclosporine or tacrolimus plus azathioprine or mycophenolate mofetil (MMF) for maintenance. Likewise, the preservation solution more frequently employed in our institution was UW (University of Wisconsin) and the mean CIT was 19.4 ± 5 h. The overall incidence of DGF and acute rejection was 24% and 15%, respectively. Thirty-five patients had a primary nonfunctioning graft, and overall death-censored graft loss rate during the

study period was 22%. Clinical and demographic characteristics of excluded patients (n = 42, 4.8%) were comparable to study group (data not shown).

Table 2 shows demographic and clinical data by CIT groups. As expected, a greater DGF and acute rejection rates were observed in the group with CIT≥19 h when compared with the lower CIT group. As a consequence, the proportion of patients with primary nonfunctioning graft was significantly higher in the group with CIT≥19 h versus CIT<19 h group, as well as length of hospitalization. With the exception of donor age, which was significantly higher in the CIT≥19 h group, other population characteristics including immunosuppression, HLA mismatches, preservation solution and renal function at discharge, did not differ between the two CIT groups.

Kaplan–Meier curve revealed that death-censored graft survival was significantly different between both CIT groups (Fig. 1 and Table 2). As an example, the overall 5-year death-censored graft survival was 91% for patients with CIT<19 h and 81% for those with CIT≥19 h (log-rank-test; P = 0.004; Fig. 1). Significant differences between both groups for graft survival were also observed when patients with primary nonfunctioning (n = 35) were excluded (log-rank-test; P = 0.042). Similarly, a subanalysis of patients who received optimal immunosuppression with tacrolimus plus MMF (n = 167) showed a lower death-censored graft survival in the higher CIT group (long-rank-test; P = 0.048; Fig. 2). In particular, the 5-year death-censored graft survival in this subpopulation was 96% and 84% for patients with CIT<19 h and CIT≥19 h, respectively.

Table 3 summarizes risk factors associated with death-censored graft loss in the multivariate Cox regression analysis. When analyzed as a continuous variable, CIT

Table 2. Characteristics of kidney transplant recipients classified by cold ischemia time exposure groups (<19 vs. ≥19 h).

	CIT <19 h (n = 334)	CIT ≥19 h (n = 495)	P value
Donor factors			
Age (years)	31 ± 12	33 ± 12	0.020
Males (%)	61	62	0.763
Cardiovascular death (%)	36	41	0.109
Preservation solution, Euro-Collins:UW:others (%)	2:80:18	4:76:20	0.226
Recipients factors			
Age (years)	41 ± 13	42 ± 12	0.318
Males (%)	65	66	0.636
Pretransplant diabetes mellitus (%)	24	20	0.200
Mean time on dialysis (months)	26 ± 25	27 ± 28	0.539
Retransplant (%)	11	12	0.703
Type of dialysis: Hemodialysis (%)	85	86	0.614
Pretransplant cardiovascular disease (%)	17.2	17.3	0.996
Mean peak PRA	8 ± 20	10 ± 24	0.302
Mean HLA mismatches	3.3 ± 1.2	3.3 ± 1.1	0.849
Acute rejection (%)	10.5	18	0.003
Delayed graft function (%)	21	28	0.018
Mean serum creatinine at discharge (md/dl)	1.6 ± 0.8	1.6 ± 0.9	0.879
Primary nonfunctioning graft (%)	2.1	5.7	0.012
Mean hospitalization days	20 ± 14	23 ± 20	0.041
Immunosuppression at discharge (%)			
Antibodies induction	79	75	0.164
Cyclosporine	75	78	0.253
Tacrolimus	22.5	19	0.173
Mycophenolate mofetil	40	38	0.461
Azathioprine	41	43	0.729
Rapamycin	2.3	2.4	0.979
Transplant era (%)			
1991–1995	30.5	35	0.248
1996–2000	35	30	
2001–2005	34.5	35	
Death-censored graft loss (%)	16.5	26	0.002
Mortality (%)	13.5	15	0.552

CIT, cold ischemia time; UW, University of Wisconsin; PRA, panel reactive antibodies.

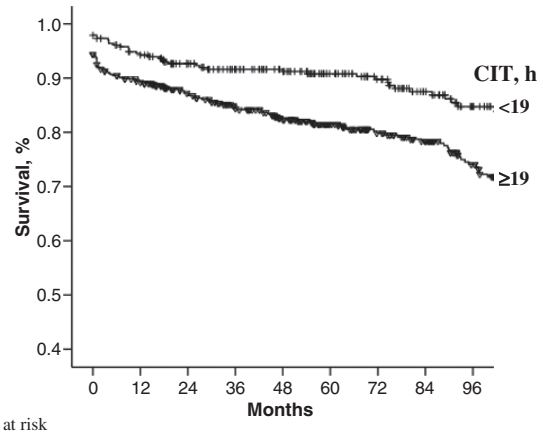

Figure 1 Kaplan–Meier analysis of death-censored graft survival stratified by duration of cold ischemia time (log-rank analysis: $P = 0.004$). Allograft survival was estimated in each cold ischemia time group. CIT, cold ischemia time.

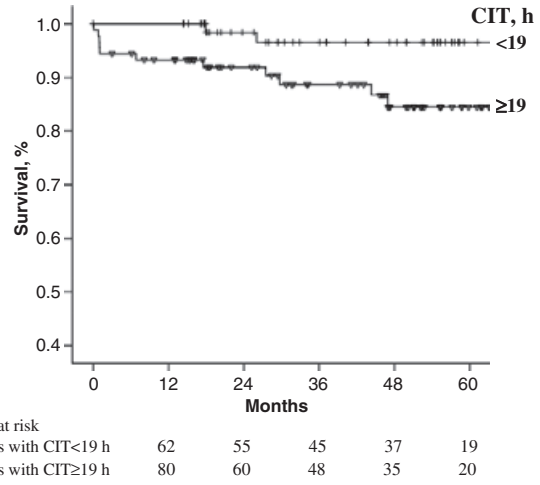

Figure 2 Effect of cold ischemia time (<19 h vs. ≥19 h) on death-censored graft survival in patients who received tacrolimus plus mycophenolate mofetil ($n = 167$; log-rank analysis: $P = 0.048$). CIT, cold ischemia time.

was strongly associated with graft failure independently of DGF and acute rejection so that the relative risk of death-censored graft loss was 20% for every 5-h increase in CIT [Relative risk (RR) = 1.04; 95% Confidence interval [CI] 1.01–1.1; $P = 0.021$). When CIT was introduced in the analysis as a categorical variable (lesser or longer than 19 h), a CIT≥19 h independently increased the risk of death-censored graft failure versus CIT<19 h (RR = 1.5; 95% CI, 1.1–2.1; $P = 0.023$).

Table 3. Risk factors for death-censored graft failure in 829 kidney transplant recipients*.

Variable	RR†	95% CI	*P* value
DGF	2.3	1.7–3.2	<0.001
Acute rejection	1.9	1.3–2.7	<0.001
Cold ischemia time (h)	1.04	1.01–1.1	0.021
Donor age	1.003	0.98–1.02	0.690

DGF, delayed graft function.
*Cox proportional-hazard model. Shown are relative risks (RR) and 95% confidence interval (95% CI).
†Adjusted for all risk factors listed in the table. A relative risk greater or less than 1.00 indicates a higher or lower risk for death-censored graft failure, respectively. Included in the model, but not in the table were, donor and recipient age, donor and recipient gender, transplant number, preservation solution, cause of renal disease, cause of donor death, modality of dialysis, transplant era (1991–1995 vs. 1996–2000 vs. 2001–2005), peak panel-reactive antibodies, HLA mismatches, serum creatinine donor, time on dialysis, renal function at discharge, pretransplant cardiovascular disease, and immunosuppression at discharge (each with $P > 0.05$).

Discussion

The most important finding of this cohort study was that a longer CIT is associated with a poorer long-term graft survival for the recipients in KT using kidneys from deceased younger donors. Additionally, this effect was independent of other important risk factors for graft outcome such as acute rejection and DGF. In order to better understand the effect of prolonged CIT on graft survival, we only included in this analysis younger donors (<50 years) from our KT database. The same age cutoff value has been previously determined using the data from OPTN/SRTR to identify expanded kidney pool [11]. Although immunsuppressive regimens changed during entire study period, combinations of newer and more potent immunosuppresants were analyzed in the present study. These results may stimulate the implementation of strategies to shorten CIT and, consequently, to reduce the persistent high rate of long-term deceased renal allograft loss.

Classically, cold ischemia injury has been considered as a strong risk factor for graft survival, but this effect has not been studied extensively in KT involving kidneys from deceased younger donors. Indeed, previous cohort studies have explored the negative impact of longer CIT on graft survival. However, most of them did not analyze in detail KT involving kidneys from deceased younger donors under modern immunosupressants [5–9]. A more recent study using the Collaborative Transplant Study database demonstrated that a CIT>18 h was associated with decreased graft survival independently of HLA matching and donor age, but an important risk factor

such as DGF was not analyzed thoroughly [10]. From multiple risk factors taken into account in the Cox analysis in the present study, CIT emerged as a variable strongly and independently associated to death-censored graft failure. In particular, this association was independent of other important risk factors such as DGF, acute rejection. Moreover, graft survival was not significantly influenced by donor age. In fact, when CIT was analyzed as a continuous variable there was a progressive increase in the failure rate with each increment in cold storage time. Likewise, appreciable increase in graft loss rate was also observed when CIT exceeded 19 h. Therefore, our findings are consistent with the general observation of previous reports [6,10], suggesting that cold injury still remains a potentially modifiable risk factor for the long-term graft failure. In this study we confirm that this finding also apply to optimal clinical conditions, such as renal transplants with young donors.

Mechanisms behind the deleterious effect of CIT are not totally known, but there are some matters to account for the putative negative impact of CIT on graft survival. CIT has been shown to be the principal factor leading to DGF. Both DGF and CIT may contribute to chronic allograft loss through an increased rate of immunological dysfunction and tubulo-interstitial structural damage [14]. In fact, ischemia-reperfusion injury increases the kidney expression of many molecules that are involved in the immune response [3]. In addition, CIT has been identified as an independent predictor of increased HLA class I antibody production after KT, which may predispose to the development of chronic rejection [15]. In this way, severe ischemic injury induces persistent renal immune changes, particularly CD4+ infiltration, leading to progression of renal disease in transplanted and native kidneys, as observed in animal models [16]. Accordingly, our patients with longer CIT had a higher acute rejection rate as well as a greater DGF and graft loss rate. Based on these observations, we hypothesize that a synergistic adverse effect of CIT, DGF and acute rejection could have contributed to a lower graft survival in patients with longer CIT, and this may be even more evident in younger donors. In agreement with these arguments, a higher incidence of silent acute rejection has been observed during prolonged DGF leading to reduced kidney allograft survival [17]. Although renal allograft function at discharge was similar between CIT groups, we cannot rule out the presence of sub clinical immunological dysfunction during the follow up in the longer CIT group. Whether future prospective studies, using protocol biopsies, may elucidate this issue remains undetermined.

Overall, transplant results have improved with the combination of new immunosuppressants such as tacrolimus plus MMF, which provides better transplant outcome in terms of renal function and immunologic dysfunction [18–20]. Thus, a beneficial effect could be expected in patients under this particular immunosuppression even when unfavorable conditions such as a longer CIT are present. However, the subanalysis of our patients who received tacrolimus plus MMF showed a lower graft survival in CIT≥19 h group compared with shorter CIT group. This finding suggests that the negative effect of CIT could surpass the protective role of more optimal immunosuppression in KT from young donors. Obviously, long-term prospective studies under combination of newer immunosuppressants are needed to clarify this concern.

While certain previous cohort studies have demonstrated that HLA mismatches may influence the outcome of KT s independently of CIT [21], others have not [22]. We did not find an interaction between HLA mismatches and longer CIT, but a more detailed analysis of the influence of HLA matching on graft survival was not performed in this study. HLA matching is only one of the many factors that influence the transplant outcome and deceased young donor kidneys have an excellent outcome when implanted within the first 20 h [6], as observed in our study. Thus, in presence of shorter CIT, a superior graft survival could be expected when favorable factors such as, donor age <50 years and optimal HLA matching, concur.

The use of UW preservation solution has been associated with significantly better transplant outcome, mainly when ischemia exceeds 24 h [10]. Different cold storage solutions were used during the study period, but most of our KT were performed using UW solution. In any case, death-censored graft survival rate was not significantly different among preservation solutions used in the present study, including those with longer CIT (data not shown). Although particular components of UW solution may be responsible of its biological benefits on allograft, it is conceivable that avoiding both longer CIT and DGF may overcome the effects of preservation solution, especially in KT using kidneys from deceased young donors.

This study has several potential limitations. First, this is a retrospective cohort study and emergent risk factors for graft survival were not recorded during the follow up. However, this was consistent with the study aim, that is, to assess the relationship between the duration of CIT and the long-term graft survival in incident KT recipients. Second, we included patients who underwent transplantation during a large period (1991–2005). This is an unavoidable limitation when assessing graft outcome in the long term. Nevertheless, when both transplant periods and immunosuppressants were considered as a covariate in the multivariable analysis, the results did not change.

Finally, we did not perform protocol biopsies to know whether cold ischemia injury led to progressive chronic allograft nephropathy. This is beyond the immediate scope of this analysis and, thus, a different study design would be required to achieve such objective.

In conclusion, CIT is strongly associated with graft survival in KT recipients from younger donors (<50 years), independently of acute rejection and DGF. This finding may be very useful in daily clinical practice and efforts to minimize CIT (<19 h) should improve significantly transplant outcome in this population.

Authorship

DH, JMG-P, AT: designed the study; DH, SE, GP, DL, PD, AR, DM EP: performed the study; SE, GP, DL, PD, AR, DM, EP: collected data; DH, MR, JMG-P: analyzed data; DH, MR, AT: wrote the paper.

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