The benefits of hypothermic machine preservation and short cold ischemia times in deceased donor kidneys

Kox JJHFM, MD[1], Moers C, MD, PhD[2], Monbaliu D, MD, PhD[3], Strelniece A, MSc[4],

Treckmann J, MD[5], Jochmans I, MD, PhD[3], Leuvenink HGD, PhD[2],

Van Heurn LWE, MD, PhD[1], Pirenne J, MD, PhD[3], Paul A, MD, PhD[5], Ploeg RJ, MD, PhD[2,6]

[1] Department of Surgery, Maastricht University Medical Center, Maastricht, Netherlands

[2] Department of Surgery - Organ Donation and Transplantation, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

[3] Abdominal Transplantation, Department of Microbiology and Immunology, KU Leuven, Leuven; Abdominal Transplant Surgery, University Hospitals Leuven, Leuven, Belgium

[4] Eurotransplant International Foundation, Leiden, Netherlands

[5] Department of General, Visceral and Transplantation Surgery, University Hospital Essen, Essen, Germany

[6] Oxford Transplant Centre, Oxford, United Kingdom

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Correspondence Information

Address: Maastricht University Medical Center

             Jasper Kox, 2.G1.027

             P.O. Box 5800, 6202 AZ Maastricht

             The Netherlands

E-mail: j.kox@maastrichtuniversity.nl

Authorship

Authorship statement

Kox JJHFM: Writing of the paper, participated in data analysis

Moers C: Writing of the paper, participated in data analysis, participated in research design, participated in performance of the research

Monbaliu D: Participated in research design, participated in performance of the research, participated in paper revision

Strelniece A: Participated in data analysis, participated in paper revision

Treckmann J: Participated in research design, participated in performance of the research, participated in paper revision

Jochmans I: Participated in research design, participated in performance of the research, participated in paper revision

Leuvenink HGD: Participated in research design, participated in performance of the research, participated in paper revision

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Van Heurn LWE: Participated in research design, participated in performance of the research, participated in paper revision

Pirenne J: Participated in research design, participated in performance of the research, participated in paper revision

Paul A: Participated in research design, participated in performance of the research, participated in paper revision

Ploeg RJ: Participated in research design, participated in performance of the research, participated in paper revision

Abbreviations

CIT, cold ischemia time

CS, cold storage

DBD, donation after brain death

DCD, donation after circulatory death

DGF, delayed graft function

ECD, expanded criteria donor

HLA, human leukocyte antigen

HMP, hypothermic machine preservation

HR, hazard ratio

HSP, heat-shock protein

HTK, histidine-tryptophan-ketoglutarate

KLF2, Kruppel-like factor type 2

OR, odds ratio

SCD, standard criteria donor

SD, standard deviation

SRTR, Scientific Registration of Transplant Recipients

UW, University of Wisconsin

Abstract

BACKGROUND

Hypothermic machine perfusion (HMP) of deceased donor kidneys is associated with better outcome when compared to static cold storage (CS). Nevertheless, there is little evidence whether kidneys with short cold ischemia time (CIT) also benefit from HMP and whether HMP can safely extend CIT.

METHODS

We analysed prospectively collected data from the Machine Preservation Trial, an international randomized controlled trial. 752 Consecutive renal transplants were included: 1 kidney of each of the 376 donors was preserved by HMP, the contralateral organ was preserved by CS.

RESULTS

The mean CIT was 15:05 hours (SD 4:58 hours). A subgroup analysis was performed, groups were based on CIT duration: 0-10 hours, 10-15 hours, 15-20 hours, or 20 hours and more. Delayed graft function (DGF) incidence in the subgroup with up to 10 hours CIT was 6.0% (N=3/50) in the HMP arm and 28.1% (N=18/64) in the CS arm (univariable p=0.002, multivariable OR 0.02, p=0.007). CIT remained an independent risk factor for DGF for machine perfused kidneys recovered from DBD donors OR 1.06, 95% CI 1.017-1.117, p=0.008), DCD donors (OR 1.13, 95% CI 1.035-1.233, p=0.006) and ECD donors (OR 1.14, 95% CI 1.057-1.236, p=0.001).

CONCLUSION

In conclusion, HMP resulted in remarkably lower rates of DGF in renal grafts that were transplanted after a short CIT. Also, CIT remained an independent risk factor for DGF in HMP-preserved kidneys.

Introduction

Kidney transplantation is the preferred treatment for end-stage renal disease. Transplantation numbers are limited by a shortage of organ donors. To utilise all available deceased donor organs, optimal preservation of allografts between procurement and transplantation is paramount. In kidney transplantation, preservation of deceased donor kidneys by hypothermic machine perfusion (HMP) has been associated with better posttransplant outcome than static cold storage (CS).[1-6] Other benefits of HMP include the opportunity to study renovascular and perfusate parameters.[7-10]

Hypothermically preserved grafts still sustain cold ischemic injury. During cold ischemia, there is a build-up of toxic substances, lysosomal instability and cellular oedema, through inactivation of Na+/K+ ATPase pumps, resulting in cold ischemic damage.[11,12] Longer CIT in renal transplantation is associated with the occurrence of DGF, PNF and decreased graft survival.[13,14] Especially marginal kidneys, kidneys from ECD and DCD donors, have shown to be more sensitive to cold ischemia.[13,15] These processes are responsible for an increased risk of delayed graft function of the transplanted kidney and have a negative effect on the long-term graft and patient survival, particularly if the cold ischemia time is prolonged.[16]

It is generally presumed that HMP only benefits marginal kidneys or kidneys that sustain significant ischemic injury and that kidneys that are transplanted after a short cold ischemic period do not benefit from this preservation technique.[2,4,17] Another general clinical presumption is that cold ischemia time only has a small effect on outcome when a kidney is preserved by HMP, ie that a kidney is "safer" on the machine than it is in cold storage. The aim of this study was to test these 2 hypotheses.

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Materials and Methods

MACHINE PRESERVATION TRIAL

We performed a post hoc data analysis of the Machine Preservation Trial data.[4,5] This international multi-center randomized controlled trial was conducted in the Netherlands, Belgium and in the North Rhine-Westphalia federal state of Germany and investigated the effects of machine perfusion on delayed graft function (DGF), graft survival and other relevant end points. In the study, all consecutive donation after circulatory death (DCD) category III or IV and donation after brain death (DBD) kidney pairs were randomized in such a way that 1 kidney of each pair underwent HMP and the contralateral organ was cold stored.

STUDY POPULATION AND INCLUSION CRITERIA

376 DBD or DCD kidney donors aged 16 years or older were included. DCD donors were in Maastricht category III (awaiting circulatory death after withdrawal of treatment).[18] Kidney pairs were only included if both organs were transplanted into 2 different recipients. Exclusion criteria were multi-organ transplantation in 1 or both recipients and death of a recipient within 1 week after transplantation (follow-up of 7 days was required for the primary end point: DGF).

PRESERVATION

All included kidneys underwent in situ vascular flush with either cold storage University of Wisconsin (CS-UW) or histidine-tryptophan-ketoglutarate (HTK) solution, based on local retrieval team practices. Kidney pairs were flushed out with the same solution. The HMP-assigned kidneys were connected to the perfusion machine (LifePort Kidney Transporter, Organ Recovery Systems, Itasca, IL, USA) for the whole duration of the preservation period.

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HMP was done with machine preservation solution (Kidney Preservation Solution-1, KPS-1, Organ Recovery Systems) at 1-8 degrees Celsius and a set perfusion pressure of 30mmHg.

STATISTICAL ANALYSIS

The patient cohort was divided into 4 different groups, based on the duration of the cold ischemia time. The empirically chosen groups are 0-10 hours, 10-15 hours, 15-20 hours or greater than 20 hours.

Continuous variables are presented as mean ± standard deviation (SD) if there is a normal distribution and as median and range otherwise.

Differences in baseline characteristics between donor types were tested with independent-samples t-tests for normally distributed continuous variables, Mann-Whitney U-tests for non-normally distributed continuous variables and χ^2-tests for categorical variables.

DGF was defined as any dialysis requirement in the first week after transplantation. Death-censored graft survival was calculated with the Kaplan-Meier survival analysis method. Multivariable survival analysis was done with the Cox proportional hazards analysis, using the same covariates as in previous analyses with these data. The choice of these covariates was based on relevant literature. The risk of DGF was modelled with multivariable logistic regression analysis.[1,4-6,19] Variables used in the Cox proportional hazards analysis and multivariable logistic regression analysis were: HMP or CS, donor and recipient age, time spent on the waiting list, panel-reactive antibody percentage, cold ischemia time, expanded or standard criteria donation, total number of HLA mismatches and the number of previous transplants. To further explore the impact of the preservation method on the effect that the

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CIT had on the risk of DGF, an interaction term (preservation method x CIT) was added to the multivariable logistic regression model for DGF.

All analyses were performed with SPSS (Statistical Package for the Social Sciences) version 22.0. A p value of ≤0.05 was considered to indicate statistical significance.

EXPANDED CRITERIA DONATION

Expanded criteria donation was defined as a donor age over 60 years or a donor age between 50 and 60 years with at least 2 of the following: a cerebrovascular cause of death, a serum creatinine level greater than 132 μmol/L (1.5mg/dL), or a history of hypertension.[5,20]

ETHICAL APPROVAL

Approval for this international trial was obtained from ethics review boards in each trial region and from the Eurotransplant Ethical Advisory Committee and the Eurotransplant Kidney Advisory Committee.

Results

Between November 1, 2005 and September 12, 2007, a total of 376 consecutive kidney donors were included and a total of 752 kidneys were randomly assigned to either HMP or CS.

As a result of the study's strictly paired design, donor characteristics were exactly the same for both treatment arms. Median donor age was 49.6 years (range 16-81). There were 294 DBD donors and 82 DCD category III donors. 105 Were expanded criteria donors, the other 271 were standard criteria donors.

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Table 1 shows the previously reported overall baseline characteristics.[5] For the HMP group, median recipient age was 53 years (range 11-79) and for the CS group, this was 52 (range 2-79). Cold ischemia times were 15 hours and 10 minutes (range 3-47 hours) and 15 hours and 5 minutes (range 3-30 hours), respectively. Table 2 shows baseline characteristics for each CIT subgroup.

THE EFFECT OF HMP ON DGF ACROSS DIFFERENT COLD ISCHEMIA TIMES

The effect of HMP on the incidence of DGF is shown in Table 3. We have performed a subgroup analysis after empirically dividing the dataset into 4 CIT groups (less than 10 hours, 10-15 hours, 15-20 hours and more than 20 hours). The impact of HMP in this dataset was largest in the subgroup that had sustained the least amount of cold ischemic injury, a CIT less than 10 hours. In this subgroup, the incidence of DGF after transplantation was 6.0% (3 out of 50) for kidneys preserved by HMP, whereas after CS this incidence was 28.1% (18 out of 64). This difference was statistically significant in both a univariable analysis (p=0.002) and a multivariable analysis (odds ratio (OR) = 0.015, p=0.007). In the group with the longest cold ischemic period, there was also a difference in DGF occurrence between the 2 preservation methods. Although this difference did not reach statistical significance, there was a definite trend, especially in the multivariable analysis. In this subgroup with the longest CIT, the OR for the risk of developing DGF was 0.335 (95% CI 0.11-1.015), p=0.053. In the other subgroups, that sustained 10-15 and 15-20 hours of cold ischemia, the differences in DGF incidence between CS and HMP preserved grafts were small. Full data are provided in Table S1 (<10 hrs), Table S2 (10-15 hrs), Table S3 (15-20 hrs) and Table S4 (>20 hrs) (SDC, http://links.lww.com/TP/B552).

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THE EFFECT OF HMP ON 3 YEAR GRAFT SURVIVAL

Kaplan-Meier and multivariable Cox proportional hazards analysis was performed to investigate whether the observed differences in DGF incidence would lead to a long-term effect of HMP versus CS on graft survival in each of the 4 CIT groups. For HMP and CS respectively, the graft survival rates were 88.0% vs 81.3% for 0-10 hours of CIT, 91.9% vs 90.1% for 10-15 hours, 93.8% vs 88.5% for 15-20 hours and 84.9% vs 83.7% for more than 20 hours of cold ischemia. There was no statistically significant 3 year graft survival benefit for HMP preserved kidneys in any of the 4 subgroups. Kaplan-Meier graphs are shown in Figure 1.

THE EFFECT OF CIT ON THE RISK OF DGF DURING HMP VERSUS CS

In a multivariable logistic regression analysis modelling the risk of DGF, the OR of each additional hour of CIT during CS overall was 1.08 (95% confidence interval (CI) 1.03-1.14, p=0.004). The multivariable OR of each hour of CIT during HMP overall was also 1.08 (95% CI 1.04-1.14, p=0.003). Multivariable interaction analysis showed that HMP vs CS did not change the effect that the CIT had on the risk of DGF (nonsignificant interaction with no evidence of effect modification, p=0.797). Next, multivariable analyses were performed for various donor type subgroups: The multivariable OR of an additional hour of CIT during HMP was 1.06 (95% CI 1.017- 1.117, p=0.008) for DBD kidneys and 1.13 (95% CI 1.035-1.233, p=0.006) for DCD grafts. For ECD organs, the OR was 1.14 (95% CI 1.057-1.236, p=0.001).

THE EFFECT OF PERFUSION SOLUTION

Kidney pairs were flushed out with either CS-UW (n=226), HTK (n=143) or another perfusion solution (n=7). To rule out effects of the preservation solution in our analyses, we

compared groups for the prevalence of HTK and CS-UW use and performed both univariate and multivariate analyses.

For 0-10 hours of CIT, 55% of the kidney pairs were flushed with CS-UW. For 10-15, 15-20 and >20hrs, the percentage of kidney pairs flushed with CS-UW was 62%, 59% and 55%, respectively. There was no significant difference in the number of CS-UW flushed kidney pairs throughout the 4 CIT groups (χ^2, p=0.48).

In cold storage preserved kidneys, there was no significant effect of the preservation solution for either DBD (p=0.760) or DCD kidneys (p=0.829). The multivariate OR for DGF when comparing CS-UW to HTK as a preservation solution during cold storage was 1.481 (0.797-2.752, p=0.214).

Discussion

Optimal preservation of deceased donor organs is paramount and sustains organ viability in an ex vivo setting.[21] Hypothermic machine perfusion has been associated with lower rates of delayed graft function and better long term graft survival than its more widely employed counterpart, static cold storage.[4,5,22-24]

Interestingly, in current clinical practice, there are some general assumptions about the benefits and limitations of hypothermic machine perfusion, based on the evidence that supports HMP as a more beneficial preservation method.[1-6,19,25] This may have a paradoxical effect: the underestimation of some of the most well-known risk factors for delayed graft function, especially cold ischemia time, during HMP.

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Our data suggest that these general assumptions may be a misconception about the protective effect of HMP. It is thought by many that the duration of cold ischemia time can be relatively safely extended if kidneys are machine perfused. We have shown that, quite surprisingly, HMP actually yields the largest benefit in kidneys which are preserved for a relatively short period, less than 10 hours of CIT. There was also a trend towards a relevant beneficial effect of HMP in kidneys that are stored for more than 20 hours. However, in this subgroup the differences in DGF incidence between HMP and CS were not significant, which is likely due to the relatively small number of organs in the prolonged CIT subgroup. In the other 2 subgroups, the relative effect of HMP vs CS on the risk of DGF was less pronounced and, with limited numbers in each group, not statistically significant. Irrespective of a potential benefit of HMP, the present study shows that in HMP preserved kidneys it is also essential to limit CIT as much as possible. Previous studies have shown that CIT is an independent risk factor for DGF during CS.[13,16,26] In a multivariable data analysis, we have shown that the detrimental effect of CIT during HMP has a similar magnitude as during CS: Regardless of whether the kidney is on the pump or in a bag, every additional hour of cold ischemia increases the odds for developing delayed graft function with 8%.

In current clinical practice, HMP is used most often to preserve kidneys that are expected to undergo a long period of cold ischemia. The resulting lack of data on the beneficial effect of HMP versus CS for short preservation times may have led to the common presumption that there is no additional value of HMP in kidneys that are transplanted quickly. Gill et al have reported data from the Scientific Registration of Transplant Recipients (SRTR)[27] which showed a significant beneficial effect of HMP in kidneys recovered from SCD, ECD and DCD donors, with various CIT. In contrast with our findings, data from these 94,709 transplant recipients, showed no significant benefit in a subgroup of kidneys from ECD or

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DCD donors, transplanted after a short CIT. There are several differences in study design which may explain this discrepancy. First, ours was a prospectively randomized study, with no selection of which kidneys were pumped and which were cold stored. Second, in our study kidneys were all machine perfused during the entire preservation period, whereas most renal grafts in the SRTR region were only on HMP for a few hours just prior to transplantation, preceded by a longer period of CS. Third, our dataset was not large enough to also create meaningful subgroups of ECD and DCD kidneys out of the subgroup of kidneys with a short CIT.

The present analysis of prospectively collected data shows that there is a clear cut and quite remarkable benefit of HMP for kidneys that undergo no more than 10 hours of cold ischemia. In this subgroup, the incidence of DGF in kidneys preserved by HMP was much lower than for cold stored organs. Such a relatively large benefit of HMP over CS in terms of DGF reduction has not been reported before. The underlying mechanisms are unknown and different causes can only be hypothesized. The beneficial effect could perhaps be attributed to vasodilatation and a lower vascular resistance after HMP. De Vries et al have shown that the renal resistance of a recently procured and cooled kidney usually decreases in the first 30-60 minutes of machine perfusion; after this period renal resistance hardly decreases any further.[8] Vasodilatation may lead to improved reperfusion after transplantation with potentially beneficial effects on renal cortical perfusion, oxygen and nutrient delivery after transplantation. When CIT is long, renal oedema inhibits capillary flow in both HMP and CS and the benefits of the initial vasodilatation may be cancelled out.

In addition, HMP may in theory help to better wash out vasoactive and inflammatory molecules and limit inflammatory reactions inside the graft after transplantation.[28,29] A

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relatively short period of HMP is likely to provide an already quite extensive wash out, with longer HMP times perhaps not eliminating much more of these components. During HMP, there is a more continuous exchange of electrolytes and other molecules between the preservation solution and the intracellular space, compared to static CS. Because of this, accumulation of pro-inflammatory cytokines such as interleukin-1, interleukin-6, tumour necrosis factor-α and tumour growth factor-β, heat-shock proteins (mostly HSP-60 and HSP-70) and complement factor 3a may be partially prevented.[7,28,30-32] Inhibition of the innate immune system through toll-like receptors and the complement system could be 1 of the underlying mechanisms of action of HMP.[28,31]

In addition, Kruppel-like factor 2 may play a pivotal role in protection of the vascular endothelium during HMP.[33] KLF2 is a transcription factor that plays a role in the development and activation of the vascular endothelium. It also attenuates induction of pro-inflammatory molecules like E-selectin and vascular cell adhesion molecule 1 (VCAM-1), regulates gene expression of thrombomodulin and activates of endothelial nitric oxide synthase (eNOS) potently. [34,35] KLF2 is upregulated by pulsatile endothelial shear stress.[35,36] This may explain part of the protective effect of HMP and this particular mechanism of benefit is not likely to be stronger after longer periods of machine perfusion. The provision of pulsatile flow upregulates KLF2 to protect the vascular endothelium of an organ during the preservation period.[33]

HMP may also have potential deleterious effects on the preserved graft. Shear stress can cause vascular injury, particularly endothelial injury, as a result of pressure driven flow of cold perfusate. Oedema may occur as a result of ischemic injury, resulting in higher vascular resistance.[37,38] This may further intensify perfusion injury. These deleterious effects seem to

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be most pronounced during prolonged perfusion and in kidneys that are most vulnerable to cold ischemia time. Gill et al have shown that in DCD donors, HMP and CS have comparable results after 36 hours of CIT.[27] This may be in part be explained by the hypothesis that beneficial effects of HMP diminish over time and eventually even are outweighed by the method's deleterious effects.

In conclusion, the overall superiority of HMP over CS as a preservation method for renal grafts is evident and has been shown before by many studies. In the present analysis of data obtained in a large randomized controlled trial, we have shown that HMP is beneficial when cold ischemia times are short. Also, we have found that cold ischemia time remains an independent and equally relevant risk factor for DGF when kidneys are preserved by HMP instead of CS.

LIMITATIONS

We have performed a post hoc data analysis of a randomized controlled trial. Hence, common limitations of post hoc analyses also apply to the present study. One of the most important issues is that due to the division of original data into 4 subgroups, statistical power diminishes. As a result of relatively small numbers per subgroup, we may for example not have picked up differences in graft survival between HMP and CS preserved kidneys, which were evident in the overall study group that had a larger sample size.

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Figure legends

Figure 1: Kaplan-Meier analysis with up to 3 year follow-up data for different cold ischemia times. Multivariable p values derived from Cox models are displayed in each graph.

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Figure 1
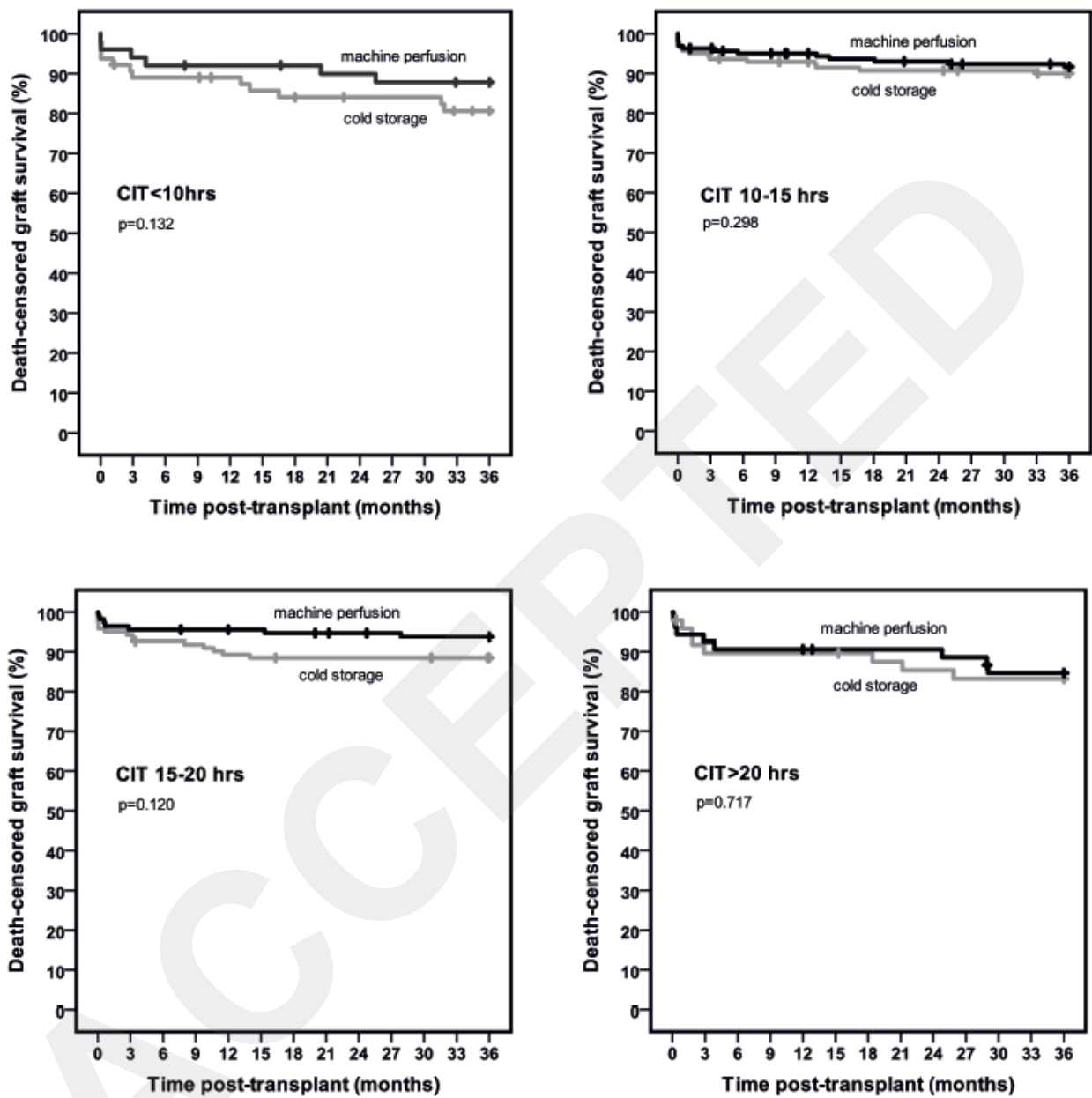
Table 1: Overall baseline study characteristics. Data are presented as median (range) or as number of patients.

		HMP	CS
Donor characteristics			
Age (yrs)	Median (range)	49.6 (16-81)	49.6 (16-81)
Donor type			
DBD		294	294
DCD category III		82	82
Standard criteria		271	271
Expanded criteria		105	105
Recipient characteristics			
Age (yrs)	Median (range)	53 (11-79)	52 (2-79)
Cold ischemia time (hrs:min)		15:10 (3-47)	15:05 (3-30)

Table 2: Baseline characteristics split by cold ischemia time. Data are presented as number of cases (percentage) or median (range).

CIT group		0-10 hours		10-15 hours		15-20 hours		20+ hours	
		HMP	CS	HMP	CS	HMP	CS	HMP	CS
Cohort size (no.)		50	64	160	141	113	122	53	49
Donor age (yrs)	Median (range)	61 (18-81)	57 (20-77)	48.5 (16-78)	49 (17-81)	48 (17-68)	49 (17-78)	50 (21-77)	48 (16-71)
Donor sex	Male	31 (62.0%)	37 (47.8%)	85 (53.1%)	80 (56.7%)	74 (65.5%)	72 (59.0%)	30 (56.6%)	31 (63.3%)
	Female	19 (38.0%	27 (42.2%)	75 (46.9%)	61 (43.3%)	39 (34.5%)	50 (41.0%)	23 (43.4%)	18 (36.7%)
Recipient age (yrs)	Median (range)	64 (26-75)	62,5 (26-75)	52 (13-72)	52 (7-79)	54 (11-79)	49.5 (2-77)	49 (24-76)	50 (13-68)
CIT (hrs:min)	Median (range)	7:31 (4-10)	8:02 (3-10)	14:10 (10-15)	13:30 (10-15)	16:59 (15-20)	16:58 (15-20)	22:39 (20-30)	22:33 (20-47)
Renal arteries (no.)	1	27 (54.0%)	38 (59.4%)	95 (59.4%)	70 (49.6%)	48 (42.5%)	58 (47.5%)	21 (39.6%)	26 (52.1%)
	2	21 (42.0%)	22 (34.4%)	52 (32.5%)	64 (45.4%)	48 (42.5%)	47 (38.5%)	24 (45.3%)	11 (22.4%)
	?	2 (4.0%)	4 (6.3%)	13 (8.1%)	7 (5.0%)	17 (15.0%)	17 (13.9%)	8 (15.1%)	12 (24.5%)
Donor type	DBD	47 (94.0%)	60 (93.8%)	131 (81.9%)	118 (83.7%)	80 (70.8%)	85 (69.7%)	36 (67.9%)	31 (63.3%)
	DCD	3 (6.0%)	4 (6.3%)	29 (18.1%)	23 (16.3%)	33 (29.2%)	37 (30.3%)	17 (32.1%)	18 (36.7%)
ECD donor	Yes	27 (54.0%)	31 (48.4%)	44 (27.5%)	34 (24.1%)	24 (21.2%)	29 (23.8%)	10 (18.9%)	11 (77.6%)
	No	23 (46.0%)	33 (51.6%)	116 (72.5%)	107 (75.9%)	89 (78.8%)	93 (76.2%)	43 (81.1%)	38 (22.4%)
HLA mismatches (no.)	0	5 (10.0%)	2 (3.1%)	23 (14.4%)	30 (21.3%)	18 (15.9%)	17 (13.9%)	9 (17.0%)	5 (10.2%)
	1	1 (2.0%)	4 (6.3%)	14 (8.8%)	11 (7.8%)	6 (5.3%)	8 (6.6%)	10 (18.9%)	5 (10.2%)
	2	13 (26.0%)	17 (26.6%)	43 (16.9%)	31 (22.0%)	31 (27.4%)	41 (33.6%)	11 (20.8%)	13 (26.5%)
	3	13 (26.0%)	18 (28.1%)	48 (30.0%)	45 (31.9%)	42 (37.2%)	40 (32.8%)	16 (30.0%)	21 (42.9%)
	4	9 (18.0%)	15 (23.4%)	24 (15.0%)	19 (13.5%)	14 (12.4%)	12 (9.8%)	4 (7.5%)	5 (10.2%)
	5	4 (8.0%)	4 (6.3%)	3 (1.9%)	2 (1.4%)	2 (1.8%)	2 (1.6%)	2 (3.8%)	0 (0.0%)
	6	5 (10.0%)	4 (6.3%)	5 (3.1%)	3 (2.1%)	0 (0.0%)	2 (1.6%)	1 (1.9%)	0 (0.0%)
Time spent on waiting list (yrs)	Median (range)	5,5 (1-8)	6 (1-8)	5 (1-8)	5 (1-8)	5 (1-8)	4 (1-8)	5 (1-8)	5 (2-8)
Previous transplants (no.)	0	40 (80.0%)	51 (79.7%)	110 (68.8%)	112 (79.4%)	76 (67.3%)	78 (63.9%)	34 (64.2%)	26 (53.1%)
	1	10 (20.0%)	10 (15.6%)	42 (26.3%)	24 (17.0%)	33 (29.2%)	41 (33.6%)	13 (24.5%)	19 (38.8%)
	2	0 (0.0%)	3 (4.7%)	7 (4.4%)	5 (3.5%)	3 (2.7%)	3 (2.5%)	4 (7.5%)	4 (8.2%)

	3	0 (0.0%)	0(0.0%)	1 (0.6%)	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (1.9%)	0 (0.0%)
	4	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.9%)	0 (0.0%)
PRA	<5%	48 (96.0%)	60 (93.8%)	137 (85.6%)	127 (90.0%)	112 (91.8%)	112 (91.8%)	47 (88.7%)	42 (85.7%)
	5-25%	2 (4%)	3 (4.8%)	14 (8.8%)	9 (6.3%)	5 (4.1%)	5 (4.1%)	0 (0.0%)	3 (6.1%)
	25-50%	0 (0%)	1 (1.6%)	6 (3.8%)	3 (2.1%)	2 (1.6%)	2 (1.6%)	2 (3.8%)	1 (2.0%)
	50-85%	0 (0%)	0 (0%)	3 (1.8%)	2 (1.4%)	1 (0.8%)	1 (0.8%)	2 (3.8%)	3 (6.1%)
	>85%	0 (0%)	0 (0%)	0 (0.0%)	0 (0.0%)	2 (1.6%)	2 (1.6%)	2 (3.8%)	0 (0.0%)

Table 3: The incidence of DGF in the subgroup analysis. Data presented as number of cases and percentage of group total. Full multivariable models are provided in Table S1 (<10 hrs), Table S2 (10-15 hrs), Table S3 (15-20 hrs) and Table S4 (>20 hrs) (SDC, http://links.lww.com/TP/B552).

CIT	Delayed graft function					
	HMP		CS		Univariable	Multivariable OR (95% CI)
	N	%	N	%		
<10 hrs	3	6.0	18	28.1	p=0.002	0.015 (0.001-0.317, p=0.007)
10-15 hrs	39	24.4	32	22.7	p=0.593	0.893 (0.485-1.647, p=0.718)
15-20 hrs	31	27.4	42	34.4	p=0.689	0.663 (0.355-1.240, p=0.199)
>20 hrs	19	35.8	26	53.1	p=0.08	0.335 (0.110-1.015, p=0.053)
Total	92	24.5	118	31.4		

Variable	Hazard Ratio (95% Confidence interval)	P value
Donor age (yrs)	1.093 (0.997-1.199)	0.057
Recipient age (yrs)	0.991 (0.926-1.062)	0.804
Machine perfusion vs cold storage	0.460 (0.168-1.264)	0.132
Cold ischemia time (hrs)	1.036 (0.761-1.412)	0.821
DCD donor vs DBD donor	0.000	0.984
Expanded criteria vs standard criteria	0.122 (0.008-1.812)	0.126
HLA mismatches (no.)	1.905 (1.162-3.121)	0.011
Panel-reactive antibody level (%)	0.945 (0.763-1.171)	0.607
Duration of pretransplant dialysis (yrs)	0.875 (0.669-1.144)	0.329
Retransplantation vs first transplantation	2.086 (0.730-5.963)	0.170

Table S1: Multivariate Cox risk analysis for delayed graft function. Cold ischemia time <10 hours.

Variable	Hazard Ratio (95% Confidence interval)	P value
Donor age (yrs)	1.022 (0.972-1.075)	0.387
Recipient age (yrs)	0.998 (0.967-1.030)	0.916
Machine perfusion vs cold storage	0.659 (0.301-1.446)	0.298
Cold ischemia time (hrs)	1.187 (0.886-1.590)	0.252
DCD donor vs DBD donor	1.274 (0.395-4.111)	0.686
Expanded criteria vs standard criteria	2.758 (0.818-9.297)	0.102
HLA mismatches (no.)	1.023 (0.785-1.334)	0.866
Panel-reactive antibody level (%)	0.987 (0.944-1.033)	0.585
Duration of pretransplant dialysis (yrs)	0.962 (0.806-1.148)	0.666
Retransplantation vs first transplantation	1.635 (0.927-2.883)	0.089

Table S2: Multivariate Cox risk analysis for delayed graft function. Cold ischemia time between 10 and 15 hours.

Variable	Hazard Ratio (95% Confidence interval)	P value
Donor age (yrs)	1.085 (1.015-1.160)	0.0168
Recipient age (yrs)	0.981 (0.948-1.016)	0.2791
Machine perfusion vs cold storage	0.469 (0.180-1.220)	0.1204
Cold ischemia time (hrs)	1.018 (0.754-1.376)	0.9052
DCD donor vs DBD donor	0.922 (0.315-2.701)	0.8826
Expanded criteria vs standard criteria	0.354 (0.085-1.476)	0.1540
HLA mismatches (no.)	0.942 (0.667-1.331)	0.7349
Panel-reactive antibody level (%)	1.016 (0.996-1.035)	0.1142
Duration of pretransplant dialysis (yrs)	1.109 (0.890-1.381)	0.3564
Retransplantation vs first transplantation	1.423 (0.724-2.798)	0.3065

Table S3: Multivariate Cox risk analysis for delayed graft function. Cold ischemia time between 15 and 20 hours.

Variable	Hazard Ratio (95% Confidence interval)	P value
Donor age (yrs)	0.996 (0.941-1.055)	0.900
Recipient age (yrs)	0.958 (0.915-1.004)	0.074
Machine perfusion vs cold storage	0.824 (0.290-2.342)	0.717
Cold ischemia time (hrs)	0.922 (0.782-1.087)	0.333
DCD donor vs DBD donor	1.290 (0.369-4.506)	0.690
Expanded criteria vs standard criteria	3.471 (0.744-16.198)	0.113
HLA mismatches (no.)	1.484 (0.957-2.300)	0.078
Panel-reactive antibody level (%)	1.030 (1.000-1.061)	0.050
Duration of pretransplant dialysis (yrs)	0.985 (0.753-1.288)	0.911
Retransplantation vs first transplantation	0.588 (0.203-1.700)	0.327

Table S4: Multivariate Cox risk analysis for delayed graft function. Cold ischemia time longer than 20 hours.