ORIGINAL ARTICLE

Negative impact of prolonged cold storage time before machine perfusion preservation in donation after circulatory death kidney transplantation

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SUMMARY

Kidney grafts are often preserved initially in static cold storage (CS) and subsequently on hypothermic machine perfusion (MP). However, the impact of CS/MP time on transplant outcome remains unclear. We evaluated the effect of prolonged CS/MP time in a single-center retrospective cohort of 59 donation after circulatory death (DCD) and 177 matched donation after brain death (DBD) kidney-alone transplant recipients. With mean overall CS/MP times of 6.0 h/30.0 h, overall incidence of delayed graft function (DGF) was higher in DCD transplants (30.5%) than DBD transplants (7.3%, $P < 0.0001$). In logistic regression, DCD recipient ($P < 0.0001$), longer CS time ($P = 0.0002$), male recipient ($P = 0.02$), and longer MP time ($P = 0.08$) were associated with higher DGF incidence. In evaluating the joint effects of donor type (DBD vs. DCD), CS time (<6 vs. ≥6 h), and MP time (<36 vs. ≥36 h) on DGF incidence, one clearly sees an unfavorable effect of MP time ≥36 h ($P = 0.003$) across each donor type and CS time stratum, whereas the unfavorable effect of CS time ≥6 h ($P = 0.01$) is primarily seen among DCD recipients. Prolonged cold ischemia time had no unfavorable effect on renal function or graft survival at 12mo post-transplant. Long CS/MP time detrimentally affects early DCD/DBD kidney transplant outcome when grafts were mainly preserved by MP; prolonged CS time before MP has a particularly negative impact in DCD kidney transplantation.

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Introduction

While the number of kidney transplant candidates on the wait list continues to increase, donation rates have not increased in recent years [1]. Consequently, waiting time for deceased donor kidney transplantation increased significantly, and more patients are removed from the list every year without getting transplanted. To increase the number of potentially transplantable organs, more kidneys are recovered from donors after death determined by cardiocirculatory criteria (DCD) [1,2]. Kidney grafts recovered from DCDs, however, have higher risks of developing delayed graft function (DGF) and graft loss [1].

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Kidneys recovered from DCD donors can be preserved by either hypothermic machine perfusion (MP) or static cold storage (CS) before transplantation [3–5]. Retrospective studies have shown that MP, as compared with conventional simple CS, decreases DGF rates [6–8] and organ discard rates [6]. Subsequently, a European multicenter randomized controlled trial involving 336 kidney pairs (including 42 DCD kidney pairs) demonstrated the lower incidence of DGF (MP: 21% vs. CS: 27%) and better graft survival (94% vs. 90% at 1 year and 91% vs. 87% at 3 years, respectively) when grafts were preserved by MP [9,10]. The outcomes of the extended data set from this trial focusing on DCD kidneys (European DCD trial with median cold ischemia time (CIT) of 15 h, 82 pairs) were reported separately, again showing the lower DGF rate (54% vs. 70%) but no difference in 1-year graft survival (94% vs. 95%) [11]. Although these trials demonstrated the favorable outcomes of MP, a UK multicenter randomized controlled trial of DCD kidney pairs with mean CIT of 14 h was terminated after inclusion of 45 pairs, when the interim analysis showed no differences in terms of DGF rates (58% vs. 56%) and 1-year graft survival (93% vs. 98%) between MP and CS preserved kidney transplants [12].

The impact of prolonged CIT on DCD kidney grafts remains unclear when grafts were preserved by MP. The main difference between the aforementioned European and UK randomized trials was their approach to MP [13]. While the European trial started MP immediately after retrieval [11], it was frequently delayed in the UK trial [12]. In fact, before being placed on MP, the grafts were preserved in CS for 31% (mean) of the total CIT (mean CS time of 3.9 h and mean CIT of 14 h) in the UK trial. Because our center has used a similar method as the UK trial to preserve kidney grafts (i.e., CS at retrieval followed by MP immediately upon arrival at the transplant center) with relatively long CITs, we sought to investigate the impact of prolonged CS, MP, and overall CIT in DCD kidney transplantation in comparison with donation after brain death (DBD) kidney transplantation.

Materials and methods

We retrospectively analyzed all consecutive kidney transplant recipients who received kidney-alone grafts from DCDs at a single center (University of Miami) between November 2004 and December 2011. To compare the impact of CS and MP time on DCD and DBD kidneys, matched control patients were selected from the entire cohort of kidney-alone transplant patients who received kidneys from DBDs during the same time period. With transplant outcome blinded, 3 DBD transplants to each DCD transplant were matched for transplant year (±1.5 years), recipient age (±7 years), donor age (±7 years), and expanded criteria donor (ECD) status. When multiple potential matched controls were available, a patient who matched CS time (±2 h), MP time (±6 h), donor and recipient gender, race, and dual kidney transplant status was selected. The primary outcome measure was the incidence of DGF (defined as the requirement for dialysis during the first week post-transplant); secondary outcome measures were renal function and graft survival at 12 months post-transplant.

All transplanted kidneys were initially placed in CS using University of Wisconsin (Belzer UW) solution at retrieval (i.e., during transportation from the time of donor cross-clamp). All grafts were placed on MP following arrival at our center and remained on MP until the time of transplant (Fig. 1). All kidneys received pulsatile MP preservation with the RM3 Renal Preservation Machine (Waters Medical Systems, Rochester, MN, USA), using a DCM-100 Cassette and Belzer-MPS Machine Perfusion Solution (Trans-Med Corporation, Elk River, MN, USA) as the perfusate (set at 4°C). As detailed in our previous publications [14,15], the initial perfusion pressure was set to 40 mmHg systolic; mannitol was added periodically with the pressure being increased up to 50 mm Hg if needed to improve flow.

All recipients received induction immunosuppression with high-dose corticosteroids and either Thymoglobulin, an anti-IL2 receptor antibody, or both combined as dual induction [16]. Maintenance immunosuppression consisted of tacrolimus and mycophenolate; corticosteroids were often withdrawn early (7–10 days). The

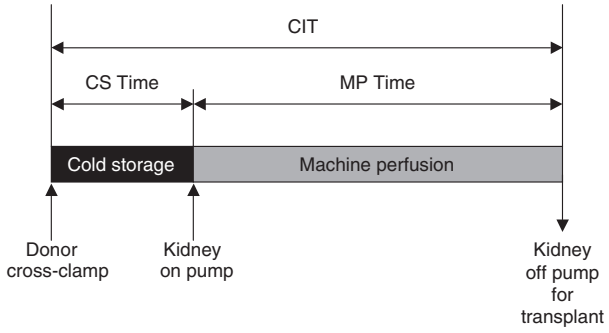

Figure 1 Flow diagram of kidney preservation. A kidney graft was initially preserved in simple cold storage (CS) at retrieval, subsequently preserved on machine perfusion (MP) following arrival at our center and remained on MP until the time of transplant.

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schedule of nonimmunosuppressive adjunctive therapy was the same as in our previous protocols [17.]

Statistical methods

Data are shown as mean ± standard deviation (unless otherwise stated) for continuous values and number and percentage for categorical values. Student's *t*-tests and analysis of variance were used in comparing the mean for a continuous variable across patient subgroups, with Pearson's chi-square (or Fischer's exact) test being used in testing the association between categorical variables. Stepwise logistic regression was used to determine a multivariable set of significant predictors for (i) DCD status (yes/no) and (ii) DGF incidence (yes/no), considering as potential predictors DCD status (in the latter model), CS time, MP time, both recipient and donor age, gender, and race, ECD status, donor terminal serum creatinine, donor history of hypertension, donor cause of death, and receiving a dual kidney transplant, with no variables being retained at the initial stage. Significance criterion for inclusion (and retention) in the DCD status model (as well as in testing for any interaction effects) was 0.05, and given the relatively small number of patients who experienced DGF, significance criterion for inclusion (and retention) in the DGF incidence model was 0.10. It was also planned that propensity scores for DCD status determined by the first logistic model would be included as a single control variable in the stepwise logistic regression analysis of DGF incidence (i.e., retained as a single variable at the initial stage). Cutpoints for CS time (e.g., <6 vs. ≥6 h), MP time (e.g., <36 vs. ≥36 h), recipient age, and donor age were considered in the DGF analysis, but only if the test of association of the continuous variable with DGF incidence was significant (note: receiver operating characteristic (ROC) curves were also used to identify appropriate cutoff points for ischemia time). Stepwise linear regression was used to determine a multivariable set of significant predictors for CS time and MP time (no variables retained at the initial stage). Stepwise linear regression was also used to determine a multivariable set of significant predictors for estimated glomerular filtration rate (eGFR) at 12 months post-transplant using the abbreviated modification of diet in renal disease (MDRD) formula [18]. Cox's model was used to specifically test the simultaneous impact of DCD status, longer CIT, and DGF occurrence on (death uncensored) graft survival, with Kaplan–Meier curves and log-rank tests also being used to estimate and compare graft survival rates.

Results

Demographics

Distributions of selected baseline characteristics for DCD and DBD recipients are presented in Table 1. Based on the selection criteria used in matching 3 DBD recipients to each DCD recipient, as expected, there were no significant differences between the two groups with respect to recipient and donor age, gender, and race, ECD status, donor terminal creatinine, donor history of hypertension, donor cause of death, and receiving a dual kidney transplant. However, the mean CS time trended shorter among DCD recipients (mean ± SD: 5.4 ± 2.7 h vs. 6.2 ± 3.0 h among DBD recipients, $P = 0.10$), and the mean MP time was significantly longer among DCD recipients (mean ± SD: 32.3 ± 9.0 h vs. 29.2 ± 9.4 h among DBD recipients, $P = 0.02$).

Stepwise logistic regression of the probability of being a DCD recipient found one significant predictor, MP time ($P = 0.02$), with an odds ratio estimate of 1.038 and a 95% CI (confidence interval) of 1.004–1.072. The score test to include CS time into the logistic model yielded $P = 0.16$. Frequency distributions of CS time, MP time, and CIT by DCD status appear in Table 2. The percentage of DCD and DBD recipients with CS time ≥6 h was 39.0% and 50.9%, respectively, and the percentage with CS time ≥12 h was 6.8% in both groups. The percentage of DCD and DBD recipients with MP time ≥24 h was 89.8% and 73.4%, respectively, and the percentage with MP time ≥36 h was 30.5% and 28.2%, respectively. The percentage of patients with CIT ≥42 h was 32.2% and 26.6% in the DCD and DBD groups, respectively.

Stepwise linear regression of longer CS time yielded one significant predictor: shorter MP time ($P = 0.05$). Stepwise linear regression of longer MP time yielded two significant multivariable predictors: older donor age ($P = 0.01$) and DCD recipient ($P = 0.02$). Thus, patients with longer CS times were more likely to also have shorter MP times, whereas recipients of older donor and DCD kidneys were more likely to have longer MP times.

DGF

Overall, the percentage of DCD and DBD recipients who developed DGF was 30.5% (18/59) and 7.3% (13/177), respectively ($P < 0.0001$). Of note, none of the 236 patients in this cohort had primary nonfunction.

Table 1. Demographics by DCD status.

	DCD (N = 59)	DBD (N = 177)	P-value
Recipient			
Age	50.4 ± 12.9	50.7 ± 12.7	0.88
Gender (Male)	47 (79.7%)	122 (68.9%)	0.11
Race (Black)	27 (45.8%)	80 (45.2%)	0.94
Donor			
Age	35.8 ± 15.0	36.0 ± 14.3	0.92
Gender (Male)	35 (59.3%)	117 (66.1%)	0.35
Race (Black)	10 (16.9%)	32 (18.1%)	0.84
Expanded Criteria Donor	8 (13.6%)	24 (13.6%)	1.00
Donor Age ≥50 years	12 (20.3%)	36 (20.3%)	1.00
Donor Serum Cr ≥1.5 mg/dl	14 (23.7%)	56 (31.6%)	0.25
Donor History of hypertension	11 (18.6%)	40 (22.6%)	0.52
Donor Cause of Death: CVA	20 (33.9%)	71 (40.1%)	0.40
Dual Kidney Transplant	5 (8.5%)	16 (9.0%)	0.90
Allograft Preservation			
CS Time (h)	5.4 ± 2.7	6.2 ± 3.0	0.10
MP Time (h)	32.3 ± 9.0	29.2 ± 9.4	0.02
CIT (h)	37.7 ± 9.0	35.3 ± 9.6	0.09

Data are shown as mean ± standard deviation for continuous values and number and percentage (in parenthesis) for categorical values.

CS, cold storage; CIT, cold ischemia time; CVA, cerebrovascular accident; DBD, donation after brain death; DCD, donation after circulatory death; MP, machine perfusion.

Table 2. Frequency distributions of CS time, MP time, and CIT by DCD status.

	DCD, % (N = 59)	DBD, % (N = 177)
CS Time (h)		
<6	61.0 (36/59)	49.1 (87/177)
6-11	32.2 (19/59)	44.1 (78/177)
≥12	6.8 (4/59)	6.8 (12/177)
MP Time (h)		
<24	10.2 (6/59)	26.6 (47/177)
24–29	28.8 (17/59)	23.2 (41/177)
30–35	30.5 (18/59)	22.0 (39/177)
≥36	30.5 (18/59)	28.2 (50/177)
CIT (h)		
<30	13.6 (8/59)	27.1 (48/177)
30–35	23.7 (14/59)	23.2 (41/177)
36–41	30.5 (18/59)	23.2 (41/177)
≥42	32.2 (19/59)	26.6 (47/177)

DBD, donation after brain death; DCD, donation after circulatory death.

Stepwise logistic regression of DGF incidence yielded 4 significant multivariable predictors of higher DGF (see Table 3) (listed here by order of selection): DCD recipient ($P < 0.0001$), longer CS time ($P = 0.0002$), male recipient ($P = 0.02$), and longer MP time ($P = 0.08$). Of note, if the single significant predictor of DCD status (MP time) was retained first in the logistic regression model (equivalent to adjusting for the propensity score), then the same 3 other variables would still be selected. Tests to include interaction effects among any two pairs of these 4 variables were not significant.

If cutpoints of <6 vs. ≥6 h for CS time and <36 vs. ≥36 h for MP time were used in place of their continuous expressions, then the following 4 variable logistic model predicting higher DGF risk was obtained: DCD recipient ($P < 0.0001$), CS time ≥6 h ($P = 0.01$), male recipient ($P = 0.04$), and MP time ≥36 h ($P = 0.003$). Estimated odds ratios and corresponding 95% CI's for these 4 variables were 6.80 (2.83–16.30) for DCD recipient, 3.14 (1.28–7.73) for CS time ≥6 h, 3.86 (1.06–14.04) for male recipient, and 3.75 (1.55–9.03) for MP time ≥36 h. The likelihood ratio test for equality of the 4 logistic model coefficients was not significant ($P = 0.55$); thus, a simple count of the number of unfavorable characteristics would provide an effective way to stratify patients according to DGF risk. The observed DGF incidence for patients having 0, 1, 2, 3, and 4 unfavorable characteristics was 0.0% (0/19), 1.4% (1/71), 15.3% (15/98), 26.8% (11/41), and 100.0% (4/4), respectively.

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Table 3. Stepwise logistic regression results for DGF incidence.

Baseline variable	Univariable *P*-value	Selection into the model (√) and multivariable *P*-value	Odds ratio estimate (95% CI)
DCD Recipient	<0.0001	(√) <0.0001	6.44 (2.67–15.56)
Recipient Age	0.23		
Male Recipient	0.01	(√) 0.02	4.67 (1.24–17.64)
Black Recipient	0.71		
Donor Age	0.63		
Male Donor	0.41		
Black Donor	0.44		
Expanded Criteria Donor	0.65		
Donor Age ≥50 years	0.42		
Donor Serum Cr ≥1.5 mg/dl	0.45		
Donor History of HTN	0.89		
Donor Cause of Death: CVA	0.68		
Dual Kidney Transplant	0.87		
CS Time (h)	0.009	(√) 0.0002	1.25 (1.10–1.42)
MP Time (h)	0.06	(√) 0.08	1.04 (0.995–1.09)
CIT Time (h)	0.008		

To more clearly show the impact of longer CS time and longer MP time on DGF risk, Table 4 displays the observed percentage of patients developing DGF stratified by donor type (DBD vs. DCD), CS time (< 6 h vs. ≥ 6 h), and MP time (< 36 h vs. ≥ 36 h). One clearly sees the unfavorable effect of DCD recipient across each CS time and MP time stratum; one also clearly sees an unfavorable effect of MP time ≥ 36 h across each donor type and CS time stratum. While the unfavorable effect of CS time ≥ 6 h is clearly seen among DCD recipients, it is not seen among DBD recipients. In fact, in the four variable logistic model that included categorized representations of CS time and MP time, the interaction effect of DCD recipient with CS time ≥6 h was significant ($P = 0.04$). Also of note, if recipients with MP time ≥36 h were excluded, then there was no longer any unfavorable impact of longer MP time (results not shown).

Renal function and graft survival at 12 months post-transplant

Stepwise linear regression of eGFR at 12 months post-transplant found 3 significant multivariable predictors of lower eGFR (listed by order of selection): older donor age ($P < 0.0001$), non-Black recipient ($P = 0.03$), and DGF occurrence ($P = 0.04$). DCD status, CS time, and MP time were not associated with eGFR at 12 months post-transplant in either univariable ($P = 0.24$, 0.45, and 0.18, respectively) or multivariable ($P = 0.51$, 0.36, and 0.62, respectively) analyses.

Nineteen patients had graft loss during the first 12 months post-transplant (actuarial graft survival ± standard error for the whole cohort at 12 months post-transplant was 92% ± 2%). Graft survival at 12 months was significantly less favorable among patients who experienced DGF ($P = 0.01$), with an estimated hazard ratio (95% CI) of 3.34 (1.27–8.78). Univariable tests of DCD status and longer CIT (as a continuous variable) with graft survival were not significant ($P = 0.69$ and 0.09, respectively), and multivariable tests to include these factors into the Cox model controlling for DGF Status were also not significant ($P = 0.25$ and 0.20, respectively). These results are also shown by the Kaplan–Meier graft survival curves in Fig. 2a–c. Figure 2(a) displays actuarial graft survival estimates ± standard error at 12 months post-transplant of 94% ± 2% for the 205 patients not having DGF (13 events) vs. 81% ± 7% for the 31 patients having DGF (6 events) ($P = 0.01$). Figure 2(b) shows no unfavorable effect of DCD status on graft survival once DGF status is controlled ($P = 0.25$), and Figure 2c shows no unfavorable effect of CIT ≥42 h on graft survival once DGF status is controlled ($P = 0.68$).

Discussion

This single-center retrospective study demonstrated the detrimental effect of prolonged CIT in DCD kidneys similarly to DBD kidneys even when the grafts were mainly preserved by MP. We believe that this retrospective study is particularly important as the overall

Table 4. Observed percentage of patients developing DGF stratified by donor type, CS time, and MP time.

Donor type	CS time, h	MP time, h	%DGF
DBD	<6	<36	1.8 (1/57)
DBD	<6	≥36	16.7 (5/30)
DBD	≥6	<36	5.7 (4/70)
DBD	≥6	≥36	15.0 (3/20)
DCD	<6	<36	13.0 (3/23)
DCD	<6	≥36	23.1 (3/13)
DCD	≥6	<36	44.4 (8/18)
DCD	≥6	≥36	80.0 (4/5)

DBD, donation after brain death; DCD, donation after circulatory death; DGF, delayed graft function.

median MP time and CIT (static CS time+MP time) were 30 and 36 h, respectively, both noticeably longer than those reported in most of the other studies [7,9,11,12]. This study also revealed the distinctive impact of (pre-MP) CS time and MP time on early transplant outcomes of DCD and DBD kidney transplantation. Significant multivariable predictors of higher DGF incidence included DCD recipient, longer static CS time, male recipient, and longer MP time. Patients with a static CS time of ≥6 h were at significantly higher risk of developing DGF, and this result was particularly apparent among DCD recipients. The lesser impact of CS time ≥6 h among DBD recipients may indicate that a longer threshold time is required (possibly as long as ≥18 h) to produce a similarly unfavorable effect of longer CS time among DBD recipients [14,19]. Conversely, the significantly higher risk of developing DGF seen among patients with MP time ≥36 h was consistent across DBD and DCD recipients. However, we saw no unfavorable effect of a longer MP time if recipients with MP time ≥36 h were excluded; thus, in the presence of reasonably short static CS times, our data suggest that MP times up to 36 h (but not any longer) may provide effective preservation of both DBD and DCD kidneys prior to transplant. Furthermore, other than the known unfavorable influence of DGF, we observed no directly unfavorable effect of a longer MP time or longer CIT on renal function or graft survival at 12 months post-transplant.

From a practical standpoint, the results of this article suggest that when a DCD kidney graft is imported from a distant donor hospital in static CS, prolonged CS time will negatively affect early transplant outcome (i.e., a higher DGF rate), even if the graft is subsequently preserved on MP until transplant. It is possible that the

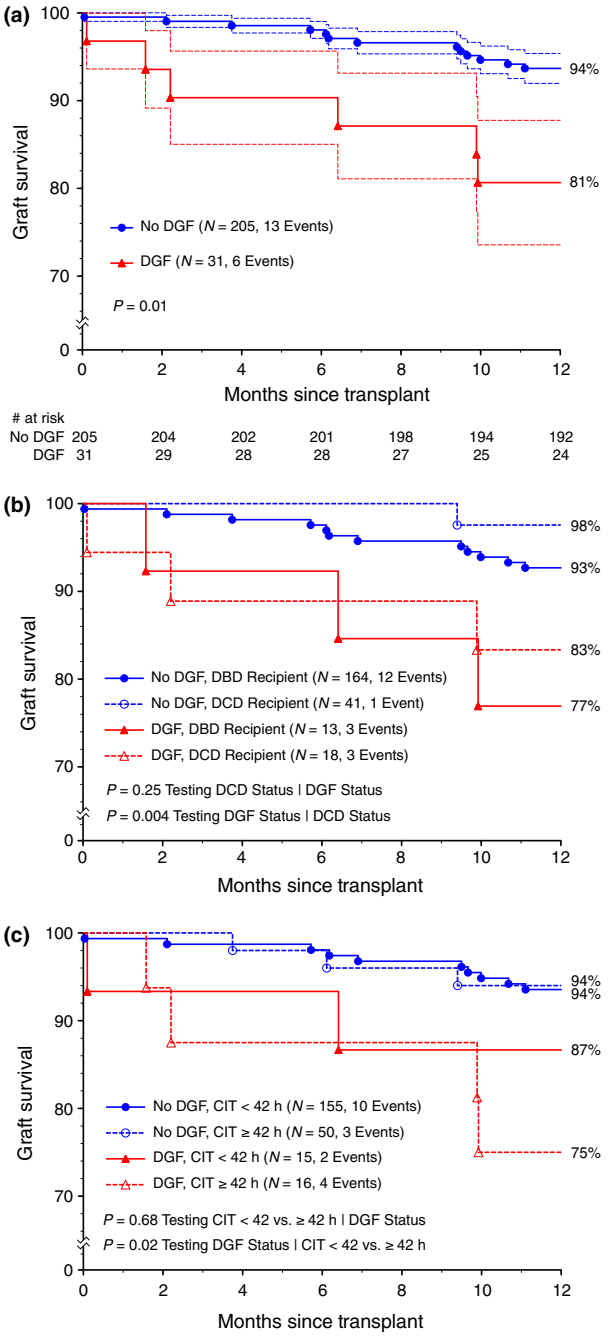

Figure 2 (a) Kaplan–Meier estimate ± standard error (dashed lines) of graft survival by presence/absence of DGF. (b) Graft survival by DBD/DCD status and presence/absence of DGF. (c) Graft survival by CIT <42 vs. ≥42 h and presence/absence of DGF. DGF, delayed graft function; DBD, donation after brain death; DCD, donation after circulatory death; CIT, cold ischemia time.

lack of benefit of MP (vs. CS) on the DGF rate in the UK DCD randomized trial vs. the benefit of MP seen in the European DCD randomized trial is explained by the fact that the European trial started MP immediately after retrieval, whereas the UK trial used CS before MP

[11–13]. It is also important to note that both trials used shorter CITs (15 and 14 h, respectively) than the current study.

While there have been no large-scale randomized trials conducted in the United States, Cannon *et al.*, using United Network for Organ Sharing data, demonstrated the decreased incidence of DGF by MP in three separate analyses: propensity matched, paired kidney, and multivariable (odds ratios: 0.64, 0.61, and 0.63, respectively as compared with CS alone) [20]. Graft survival, however, was not different in any of these analyses (hazard ratios: 0.98, 1.02, and 0.99, respectively). In current practice in the United States, it is common that MP is used only for a small part of the entire preservation period, potentially explaining the lack of any survival benefit in the analyses. Similar to our center's protocol, a kidney graft may be preserved by MP only after the graft is transferred to a local organ recovery agency; a kidney may also be preserved by MP only for a short period of time (e.g., 4 h) just to assess the quality of the graft by perfusion parameters [21, 22]. Because registry data analyses do not consider the effect of CS time before or after MP and treat all MP kidneys equally regardless of the proportion of MP time [20,23,24], the current study with detailed CS and MP time data will supplement the findings of such registry studies, revealing the negative impact of prolonged (pre-MP) CS time. This is particularly important in the United States, because recent changes in the kidney allocation policy have significantly increased the percentage of regional and national kidney sharing, and shared kidney grafts are often transported in CS [25].

In a porcine kidney perfusion and transplantation model, the concept of "hypothermic recondition" has been tested, showing similar beneficial effects of short-term MP after a longer period of CS as applying MP from organ recovery until transplantation [26]. The magnitude of reconditioning effect, however, seems to vary with perfusion settings (e.g., oxygenation, pulsatility), and this concept cannot be immediately applied to current clinical practice [27–29]. Indeed, in a similar porcine DCD kidney perfusion model with 4-h MP after short warm ischemia (10 min) and long CS (14 h) time, other investigators found no advantage to the addition of end-ischemic reconditioning by MP [30], suggesting that the negative impact of prolonged CS is not always reversible by subsequent MP.

The results of current study may seem to contradict the results of our earlier publications showing favorable outcomes with longer MP time [14,15]. However, the demographics and approaches of this study contain certain differences from our previous studies. First, one major goal of this study was to explore the effect of prolonged ischemia time among 59 consecutive DCD kidney transplants, whereas the previous studies included only small numbers of DCD transplants, 12 (3.5%) and 2 (1.5%) [14,15]. Second, mean MP time (and thus, mean CIT) was longer for patients in the current study, and we therefore considered larger cutoff values for MP time (i.e., 36 h) and CIT (i.e., 42 h) than previously considered. Shorter cutoff values were not discriminatory here, and in fact, when a MP cutoff time of 24 h was used as in the previous studies, we no longer found a significant difference between the longer and shorter MP time groups (data not shown). Third, in this study, we did not analyze the main favorable outcome measure of the previous studies (i.e., lower incidence of acute rejection with longer MP time) [14,15].

The limitations of our report are inherent in the nature of performing a single-center retrospective study with a relatively small number of patients. Although some of the recipient and donor characteristics were matched between DCD and DBD transplants, there may be other confounding factors that influence DGF incidence (primary outcome) as well as renal function and graft survival at 12 months post-transplant (secondary outcomes). For instance, there was a tendency for some of the higher-risk kidneys (i.e., kidneys from DCD and older donors) to receive MP for a longer period of time; thus, the observed unfavorable effect of longer MP time on DGF incidence may be due to an unaccounted for type of selection bias (i.e., other variables not included in the study). MP has been used at our center in virtually all deceased donor kidney transplants for more than 20 years [31], and the noticeably longer MP times have been mainly due to ongoing operating room scheduling issues at our busy county hospital, not part of any planned protocol. It is possible that the consistently low DGF rates observed at our center over the years have translated into some of the higher-risk kidneys being intentionally placed on MP for longer periods of time (selection bias) with the goal of reaching more acceptable perfusion parameters. Thus, another study limitation was that perfusion parameters (e.g., flow, resistance) and an assessment of their degree of improvement prior to transplant were not included.

Warm ischemia time of DCD kidneys was also not included in this analysis due to inconsistent definitions for warm ischemia time being used and hemodynamic parameters after extubation not being the same [32]. Nonetheless, its effect may not be substantial given our

center's relatively strict acceptance criterion for warm ischemia with the majority of DCDs being Maastricht category III [33].

Each deceased donor kidney retrieved by our organ procurement organization undergoes a biopsy before deciding whether or not to place it on MP, and kidneys of poor quality are usually discarded before being placed on MP. However, information as to whether any kidneys were discarded after prolonged MP was not available.

Another study limitation includes the fact that patient-specific information on immunosuppression (type of induction received, maintenance drug dosing and levels, etc.) and acute rejection information (date of occurrence, pathological grade, etc.) was not available. Nonetheless, the 59 recipients of DCD donors were carefully identified at this single center along with proper matching of 3 DBD recipients per DCD case (as described above) and a carefully planned statistical analysis.

In conclusion, our study, the first of its kind, indicates that prolonged CS time (particularly ≥6 h) before MP has a negative impact on DGF occurrence in DCD kidney transplantation. Long MP time (≥36 h) (and thus CIT ≥42 h) detrimentally affects DGF occurrence in both DCD and DBD kidney transplant recipients even when the grafts were mainly preserved by MP. To further elucidate the cause(s) of contradictory results existing among studies of MP, a large-scale prospective study to specifically address the effect of CS before MP is warranted.

Authorship

JS, GC, and GB: designed the study. SP, JS, and LC: collected data and performed the study. JS and JJG: analyzed the data. SP, JS, and JJG: drafted the manuscript. All authors critically reviewed and revised the manuscript and approved the final version.

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Conflict of interest

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