Favorable Outcomes With Machine Perfusion and Longer Pump Times in Kidney Transplantation: A Single-Center, Observational Study

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Background. Hypothermic machine perfusion (MP) preservation is used for all deceased donor kidney transplants at our center. Kidneys are placed in cold storage at retrieval, then transferred to MP on arrival. Because a lack of consensus regarding optimal use of MP still exists, we evaluated the overall impact of using MP at our center and the prognostic value of MP (Pump) time.

Methods. We retrospectively analyzed 339 adult, primary deceased donor kidney transplant recipients who were pooled across three prospective, randomized immunosuppression trials (since 2000) at our center. In addition to providing overall results for delayed graft function (DGF) (requirement for dialysis in the first week), slow graft function (SGF), first biopsy-proven acute rejection (BPAR), and graft failure, stepwise logistic and Cox regression analyses were used to determine the prognostic value of pump time, particularly after controlling for other significant prognosticators.

Results. Mean cold storage and pump times were 6.6 and 26.7 hr, consistent across immunosuppression protocols. Overall DGF and SGF rates were 4.4% (15/339) and 12.1% (41/339). DGF was equally low for pump time less than 24 vs. more than or equal to 24 hr, 5.2% (6/116) vs. 4.0% (9/223) ($P=0.63$), with similar results after adjusting for known DGF predictors. A significantly lower first BPAR rate was observed for longer pump time (as a continuous variable) among more immunologically active recipients (those having more risk factors: DGF, age <50 yr, and non-white) (univariable $P=0.005$; multivariable $P=0.009$), with an estimated hazard ratio of 0.43 ($P=0.006$) favoring pump time more than or equal to 24 hr among those with more than or equal to two risk factors.

Conclusions. In this single-center, observational study, MP with prolonged pump times was associated with low DGF/SGF and first BPAR rates, supporting continued use of MP.

Keywords: Kidney transplantation, Machine perfusion, Static cold storage, Duration of machine perfusion (pump) time, Delayed graft function, Biopsy-proven acute rejection, Graft failure.

(*Transplantation* 2010;90: 882–890)

A major issue in optimizing kidney transplant outcome has been how to best preserve deceased donor (DD) kidneys after retrieval for transplantation. With static cold storage (CS) preservation, longer cold ischemia time (CIT) results in significantly higher rates of delayed graft function (DGF) (*1–10*) and subsequent graft failure (*10–16*). Associations of DGF with significantly higher biopsy-proven acute

Partial salary support was provided by Institut Georges Lopez (IGL), parent company of the manufacturer (Waters Instruments, Inc.) of the RM3 Renal Preservation Machine (with a grant of $50,000) for the completion of this study to the Kidney and Kidney/Pancreas Transplant Program at the University of Miami.

Representatives of IGL had no direct involvement in this study, and none of the co-authors have any financial interest in IGL.

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Ciancio, Gaynor, and Burke participated in the research design, performance of the research, data analysis, and writing of the article; Sageshima, Chen, Roth, Kupin, and Guerra participated in the research design and performance of the research; Tueros, Zarak, Hanson, and Ganz, participated in performance of the research; and Ruiz, O'Neill, Livingstone, participated in performance of the research and writing of the article.

Received 20 May 2010. Revision requested 11 June 2010.

Accepted 19 July 2010.

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ISSN 0041-1337/10/9008-882

DOI: 10.1097/TP.0b013e3181f2c962

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rejection (BPAR) and graft failure rates also implicate longer CS time as an unwanted characteristic. In fact, the conclusion of one recent article (*16*) stated that DD kidneys "should ideally be transplanted within 18 hr."

Results of the early randomized trials (*17*, *18*) comparing hypothermic machine perfusion (MP) versus CS preservation were equivocal, with overall high DGF rates reported. More recent data suggest both reduced DGF and improved graft survival with MP (*9*, *19*, *20*), including the recently reported randomized clinical trial of 336 paired DD kidneys (*21*). More favorable DGF rates with MP have also been reported among standard criteria donor and expanded criteria donor (ECD) recipients combined (*3*, *21–25*), ECD recipients alone (*21*, *26–30*), and donation after cardiac death (DCD) recipients alone (*21*, *31*, *32*). At our center, all DD kidneys are initially placed in CS at retrieval, and on arrival at our medical center placed on MP. In our experience, MP use for all DD kidneys has resulted in a low DGF rate even in the context of prolonged CIT.

Because a lack of consensus regarding optimal use of MP still exists, we retrospectively analyzed 339 DD kidney transplant recipients who were pooled across three of our previously reported prospective, randomized immunosuppression trials (*33–38*). The primary goals of this single-center, observational study were 2-fold: (1) to describe our overall experience with MP preservation on the rates of developing DGF, slow graft function (SGF—defined below), first BPAR, graft failure (censoring deaths and never functioning grafts), and death with a functioning graft and (2) to determine the prognostic value of duration of MP (i.e., pump) time on these rates, particularly after controlling for other significant prognosticators.

RESULTS

Demographics

Distributions of selected baseline characteristics and mean CS time, pump time, and CIT stratified by randomized trial are presented in Table 1. Mean recipient and donor ages±SD were 50.2±13.5 and 35.0±15.1, respectively; African Americans and Hispanics comprised 28.6% (97/339) and 33.0% (112/339), respectively (Table 1). Percentages receiving ECD and DCD kidneys were 10.9% (37/339) and 3.5% (12/339), respectively; 80.8% (274/339) received locally retrieved kidneys. Overall mean CS time, pump time, and CIT±SD were 6.6±4.5 hr, 26.7±8.4 hr, and 33.2±8.4 hr, respectively; these mean values were reasonably consistent across the three randomized trials (Table 1). Median CS time was 4 hr (range, 2–12) for locally retrieved versus 14 hr (range, 5–25) for imported kidneys, with mean CS time being significantly shorter for locally retrieved kidneys, 4.7±1.8 hr vs. 14.2±4.2 hr for imported kidneys ($P<0.000001$). Only 14% (47/339) of patients had a CS time more than or equal to 13 hr (all imported kidneys); only 3% (11/339) had a CS time more than or equal to 18 hr. Thus, although our average CIT is much longer compared to most other centers, this reflects our rather long "duration on machine" (pump) times; in fact, most patients had relatively short CS times.

DGF and SGF

Observed percentages of patients developing DGF and SGF were 4.4% (15/339) and 12.1% (41/339), respectively. Five patients had never functioning grafts (i.e., developed graft failure during the first 2 weeks posttransplant) due to primary nonfunction (n=3), renal vein thrombosis (n=1), and ruptured kidney (nonimmunologic) (n=1) (4/5 had DGF; 5/5 had SGF).

Receiving a DCD kidney was the only factor associated with a significantly higher DGF rate ($P=0.0004$), with estimated odds ratio (95% confidence interval) of 8.8 (2.1–36.5). Two factors, non-white race/ethnicity (all but Caucasian) and pretransplant diabetes mellitus, were independently associated with significantly higher rates of SGF (multivariable $P=0.04$ and 0.04, respectively), with estimated odds ratios (95% confidence interval) of 2.4 (1.04–5.7) and 2.0 (1.01–4.1), respectively. Using these results, low-, medium-, and high-risk strata were defined as follows: donation after brain death (DBD) recipients who were white and non-diabetic (n=92), DBD recipients who were non-white or diabetic (n=235), and DCD recipients (n=12). Percentages developing DGF in these three strata were 1.1% (1/92), 4.7% (11/235), and 25.0% (3/12), respectively ($P=0.0007$). Similarly, percentages developing SGF were 5.4% (5/92), 14.0% (33/235), and 25.0% (3/12), respectively ($P=0.04$).

With the exception of the small percentage who received a DCD kidney, no significantly unfavorable effect of any other donor-specific variable on DGF or SGF was found, that is, older donor age ($P\geq0.10$), receiving an ECD kidney ($P\geq0.15$), longer CS time ($P\geq0.30$), longer pump time ($P\geq0.30$), and longer CIT ($P\geq0.30$). For instance, the observed percentage developing DGF by CS time less than 13, 13 to 17, and more than or equal to 18 hr was 4.1% (12/292), 5.6% (2/36), and 9.1% (1/11), respectively ($P=0.69$, although consistent with greater DGF for CS time ≥18 hr). Percentages developing DGF by pump time less than 24, 24 to 29, and more than or equal to 30 hr were 5.2% (6/116), 7.3% (7/96), and 1.6% (2/127), respectively ($P=0.11$, see Table 2); percentages developing SGF by pump time less than 24, 24 to 29, and more than or equal to 30 hr were 9.5% (11/116), 15.6% (15/96), and 11.8% (15/127), respectively ($P=0.39$). Finally, Table 2 compares the percentages developing DGF and SGF by pump time less than 24 vs. more than or equal to 24 hr, stratified by (1) risk category (low, medium, and high) and (2) donor age (<35 and ≥35 yr)—no significant differences by pump time within any of the various strata were observed. For instance, the percentage developing DGF by pump time less than 24 vs. more than or equal to 24 hr yielded 3.2% (2/63) vs. 2.9% (3/102) among recipients of donors younger than 35 yr ($P=0.93$), and 7.5% (4/53) vs. 5.0% (6/121) among recipients of donors aged 35 yr or older ($P=0.50$). The results in Table 2 clearly indicate the lack of a detrimental effect of longer pump time on DGF and SGF rates. In addition, DGF and SGF rates were low regardless of donor age, pump time, and immunosuppressive protocol (*33–38*).

First BPAR

The observed percentage developing a first BPAR during the first 6 months, 12 months, and at any time posttransplant was 5.6% (19/339), 9.1% (31/339), and 18.3% (62/339),

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TABLE 1. Distributions of selected baseline characteristics and CS time, pump time, and CIT by randomized trial (N=339, unless specified otherwise)

Baseline characteristic	Mean±SD if continuous, % if categorical
Recipient age at transplant (yr)	50.2±13.5 (median: 52 yr; range:14–78)
Recipient age ≥50 yr	56.6% (192/339)
Race/ethnicity	
Caucasian	33.3% (113/339)
Hispanic	33.0% (112/339)
African American	28.6% (97/339)
Other	5.0% (17/339)
Sex	
Male	68.4% (232/339)
Female	31.6% (107/339)
Pretransplant diabetes	
No	75.8% (257/339)
Yes	24.2% (82/339)
No. HLA mismatches	4.1±1.1 (median: 4; range: 0–6)
Donor age (yr)	35.0±15.1 (median: 35 yr; range: 1–73)
Donor age ≥50 yr	18.9% (64/339)
Received ECD kidney	
No	89.1% (302/339)
Yes	10.9% (37/339)
Received DCD kidney	
No	96.5% (327/339)
Yes	3.5% (12/339)
Kidney retrieval	
Local	80.8% (274/339)
Imported	19.2% (65/339)
CS time (hr)	6.6±4.5 (median: 5; range: 2–25)
CS time ≥13 hr	13.9% (47/339)
CS time ≥18 hr	3.2% (11/339)
Pump time (hr)	26.7±8.4 (median: 26; range: 1–54)
CIT (hr)	33.2±8.4 (median: 34; range: 7–59)

	Accrual dates	No. DD recipients	Mean±SD		
			CS time[b]	Pump time[b]	CIT[b]
Randomized trial[a]					
First	May 2000–December 2001	121	5.7±4.3	26.5±7.9	32.2±7.3
Second	November 2002–September 2004	90	7.0±4.7	27.0±7.7	33.9±7.9
Third	December 2004–February 2006	128	7.1±4.4	26.6±9.3	33.7±9.5

[a] See the Materials and Methods section for a more detailed description of each trial.

[b] While comparisons of the mean pump time and mean CIT across the 3 trials revealed no differences (P=0.92 and .25, respectively), the mean CS time was significantly shorter in the first trial in comparison with the other 2 trials (P=0.03 comparing the 3 groups; P = 0.01 comparing the mean in the first trial vs. the average of the means in the other 2 trials).

CS, cold storage; CIT, cold ischemia time; HLA, human leukocyte antigen; ECD, expanded criteria donor; DCD, donation after cardiac death; DD, deceased donor.

respectively. Grades of the 62 episodes were IA (n=28), IB (n=16), and more than or equal to IIA (n=18).

Three unfavorable characteristics were independently associated with higher rates of first BPAR in multivariable analysis (see Table 3): developed DGF (P=0.003); age at transplant younger than 50 yr (P=0.006); and non-white race/ethnicity (P=0.002). Magnitudes of the Cox model coefficients were not significantly different (P=0.51), implying that a reasonable prognostic score could be defined according to the number of risk factors present (0, 1, 2, or 3). Figure 1(A) shows a highly significant association of this prognostic score with freedom-from-first BPAR (P=0.000001).

Multivariable tests to include other variables into the Cox model were nonsignificant (P>0.10), with the exception of a favorable trend (negative Cox model coefficient) for longer pump time (P=0.09 as a continuous variable). Figure 1(B) shows a more favorable (although nonsignificant) freedom-from-first BPAR among patients having pump time

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TABLE 2. Percentages of patients developing DGF and SGF by pump time: unstratified, stratified by risk category, and stratified by donor age

	Pump time	% Developing DGF	% Developing SGF
	Unstratified comparison[a]		
	<24 hr	5.2% (6/116)	9.5% (11/116)
	24–29 hr	7.3% (7/96)	15.6% (15/96)
	≥30 hr	1.6% (2/127)	11.8% (15/127)
Risk category	Comparison stratified by risk category[b]		
Low	<24 hr	0.0% (0/28)	3.6% (1/28)
	≥24 hr	1.6% (1/64)	6.3% (4/64)
Medium	<24 hr	4.8% (4/84)	9.5% (8/84)
	≥24 hr	4.6% (7/151)	16.6% (25/151)
High	<24 hr	50.0% (2/4)	50.0% (2/4)
	≥24 hr	12.5% (1/8)	12.5% (1/8)
Donor age	Comparison stratified by donor age[c]		
<35 yr	<24 hr	3.2% (2/63)	7.9% (5/63)
	≥24 hr	2.9% (3/102)	8.8% (9/102)
≥35 yr	<24 hr	7.5% (4/53)	11.3% (6/53)
	≥24 hr	5.0% (6/121)	17.4% (21/121)

[a] $P=0.11$ comparing the 3 groups for DGF; $P=0.39$ comparing the 3 groups for SGF. In comparing pump time <24 vs. ≥24 hr, $P=0.63$ for DGF, and $P=0.29$ for SGF.

[b] Low risk: DBD recipients who were white and non-diabetic (N=92); medium risk: DBD recipients who were either non-white or diabetic (N=235); high risk: DCD recipients (N=12). None of the comparisons of DGF and SGF by pump time (<24 vs. ≥24 hr) were significant within any of the risk categories ($P\geq0.24$ for DGF; $P\geq0.14$ for SGF).

[c] None of the comparisons of DGF and SGF by pump time (<24 vs. ≥24 hr) were significant within either of the donor age categories ($P\geq0.50$ for DGF; $P\geq0.31$ for SGF).

DGF, delayed graft function; SGF, slow graft function; DCD, donation after cardiac death; DBD, donation after brain death.

more than or equal to 24 hr ($P=0.18$). However, a refined Cox model using the prognostic scoring variable "number of risk factors" (developed DGF, recipient age <50 yr, and non-white) present found a significant interaction effect of pump time (as a continuous variable) with this scoring variable ($P=0.009$, see Table 3). The negative Cox model coefficient for this interaction effect implies that a significantly favorable impact of longer pump time (hr) exits, but it only becomes apparent as there are more risk factors present. For instance, Figure 1(C) shows equally favorable freedom-from-first BPAR outcomes for pump time less than 24 vs. more than or equal to 24 hr among patients having less than or equal to 1 risk factor present ($P=0.59$), with actuarial freedom-from-first BPAR plateaus of 91% vs. 87%, respectively. However, among the more immunologically active patients, that is, those with more than or equal to two risk factors present, Figure 1(D) shows a significantly more favorable freedom-from-first BPAR for pump time more than or equal to 24 hr ($P=0.006$; estimated hazard ratio: 0.43), with actuarial freedom-from-first BPAR estimates at 84 months posttransplant being 44% vs. 67% for pump time less than 24 vs. more than or equal to 24 hr, respectively.

Graft Failure and Death With a Functioning Graft

Thirty-three patients developed graft failure beyond 2 weeks posttransplant (i.e., excluding the five never functioning grafts); death with a functioning graft occurred in 27 patients. Causes of graft failure were chronic allograft injury (n=22), recurrent disease (n=4), polyoma viral infection (n=2), acute rejection (n=2), and other (n=3). Causes of death with a functioning graft were cardiovascular (n=9), malignancy (n=8), infection (n=6), and other (n=4).

Four unfavorable characteristics were independently associated with higher rates of graft failure (beyond 2 weeks posttransplant) in multivariable analysis: developed DGF ($P=0.0001$), age at transplant less than 50 yr ($P=0.02$), non-white race/ethnicity ($P=0.01$), and donor age 50 yr or older ($P=0.001$). For death with a functioning graft, age at transplant 50 yr or older was the single characteristic independently associated with a higher rate ($P=0.00004$). Multivariable tests to include other variables were nonsignificant ($P>0.10$). Although a similar trend of longer pump time and lower graft failure rates was observed, for example, graft failure (beyond 2 weeks posttransplant) occurred in 13.8% (16/116) vs. 7.6% (17/223) of patients having pump time less than 24 vs. more than or equal to 24 hr ($P=0.10$, log rank test), no main or interaction effect of pump time was statistically significant in the Cox model.

Overall Graft Survival

A total of 65 of 339 patients had graft loss (including five never functioning grafts). Median follow-up (range) among 274 patients who were still alive with a functioning graft was 47 (24–93) months posttransplant; numbers of patients still alive with a functioning graft at 1, 3, 5, and 7 years were 317, 195, 99, and 30, respectively. For the whole cohort (Fig. 2), actuarial graft survival ±SE at 1, 3, 5, and 7 years was 94%±1%, 86%±2%, 77%±3%, and 72%±3%, respectively. Death-censored graft survival ±SE at 1, 3, 5,

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TABLE 3. Cox model results for the hazard rate of developing a first BPAR (62 events)

	Baseline variable[a,b]		
	Univariable *P*	Multivariable *P*	Model Coeff±SE
Initially selected Cox model			
Developed DGF	0.002	0.003	1.305±0.471
Recipient age <50 yr	0.0009	0.006	0.722±0.269
Non-white	0.0002	0.002	1.142±0.383
Pump time(hr)[c]	0.14	0.09	
Refined Model			
No. risk factors[b]	<.000001	<.000001	1.471±0.260
No. risk factors × pump time (hr)[c]	.005	.009	−0.022±0.008

[a] The first 3 variables listed in the table were defined as dichotomous, 0–1 variables (i.e., 0 if DGF did not occur, 1 if DGF occurred; 0 if recipient age ≥50, 1 if recipient age <50; and 0 if recipient race/ethnicity was white, 1 if recipient race/ethnicity was non-white.

[b] The Cox model coefficients for the 3 selected variables (developed DGF, recipient age <50 yr, and non-white race/ethnicity) were not significantly different from one another ($P=0.51$). Thus, a refined model using just one variable, a count of the number of risk factors (0, 1, 2, or 3) was used.

[c] The Cox model coefficient for pump time (hr) as a main effect was negative, both as a single factor as well as after controlling for the 3 selected factors, suggesting more favorable outcomes (even if nonsignificant) for patients with a longer pump time. In the refined model, a significant interaction effect of pump time (hr) with the number of risk factors was found, implying that a significantly favorable impact of longer pump time (hr) exits, but it becomes more pronounced as there are more risk factors present.

BPAR, biopsy-proven acute rejection; Coeff, Coefficient; DGF, delayed graft function.

and 7 years was 97%±1%, 92%±2%, 86%±2%, and 83%±3%, respectively.

DISCUSSION

The results of this single-center, observational study in which MP preservation was used for all DD kidneys can be summarized as follows: (1) our overall DGF and SGF rates of 4.4% and 12.1%, respectively, among 339 DD recipients were compellingly low, clearly lower than a consensus DGF estimate of 22% to 24% reported by registry-based studies (using mainly CS preservation) (*1*, *5*, *8*); (2) our favorable DGF/SGF rates occurred in the presence of relatively short static CS times and were unaffected by length of pump time, short or long (i.e., ≥24 hr); (3) a longer (≥24 hr) pump time was actually associated with a significantly lower first BPAR rate, but mainly among patients (representing approximately 35% of the total cohort) at higher immunologic risk of developing a first BPAR episode (i.e., those with two or more of the following risk factors: developing DGF, being younger than 50 yr, and being non-white); and (4) the low rates of both DGF/SGF and first BPAR (9.1% at 12 months and 18.3% overall) translated into favorable graft survival outcomes. In fact, our long-term (5–7 yr) graft (death-uncensored) survival results, across numerous immunosuppressive protocols, approach living donor graft survival rates (*39*–*42*), even with our heterogeneous (by race/ethnicity) cohort. For instance, the 5-year (unadjusted) graft survival reported by U.S. Organ Procurement Transplant Network/Scientific Registry of Transplant Recipients (*42*) for all patients transplanted in the United States during 2000–2005 was 80.2% for all LD and 69.8% for all non-ECD DD recipients, in comparison with our whole cohort's result of 77%.

These results suggest that in the presence of relatively short static CS times, use of MP provides a truer, more successful form of hypothermic preservation and superior early outcomes, even with pump times as long as 48 hr. Of note, longer pump (and thus, cold ischemia) times were due to ongoing operating room (24 hr/d) scheduling issues at our busy county hospital (with limited resources resulting in restricted operating room access)—not part of a planned protocol (i.e., there was no intentional lengthening of pump time). We believe that our threshold to use early posttransplant dialysis is comparable with other major transplant centers as more than 200 kidney transplants are performed per year at our center. In addition, because our low SGF rate (independent of the use of early dialysis) supports our low DGF rate, the reason for low DGF/SGF must be independent of our criteria for dialysis. Finally, because we follow the same protocol in allocating DD kidneys to all eligible patients, randomized or nonrandomized (the latter usually decided by patient choice), we believe that there was no selection bias in favor of better quality DD kidneys being allocated to randomized patients.

All DD kidneys retrieved by our Organ Procurement Organization undergo a pathological biopsy reading before deciding whether the kidney (or pair of kidneys) warrants being placed on MP. Kidneys of such poor quality are discarded before being placed on MP. In a recent evaluation of 661 consecutive DD kidneys retrieved by our Organ Procurement Organization between November 2004 and October 2007, a total of 65 kidneys were discarded (9.8%). Of the 65 discarded kidneys, 25 were never placed on MP due to a poor biopsy finding. Of the 40 discarded kidneys that were placed on MP, a poor biopsy finding was the main reason for eventual discard in most cases, with poor perfusion flow being a major factor in only five cases.

The recently reported randomized trial of Moers et al. (*21*) demonstrated a significantly lower risk of DGF, improved renal function during the first 2 weeks, and improved graft survival at 1 year posttransplant for MP versus CS preservation ($P=0.01$, 0.01, and 0.04, respectively). However, their reported DGF rate with MP of 20.8% (70/336) was significantly higher than our DGF rate of 4.4% (15/339) ($P<0.000001$). In the subgroup of DBD recipients, Moers et al. reported a DGF rate of 16.3% (48/294) in the MP arm. Because their median DD age was clearly older than that in our study (51 vs. 35 yr), our DGF rate in the subgroup of DBD recipients having donor age 45 yr or older (median=52 yr) was 4.9% (5/103)—still yielding a significant difference ($P=0.003$). Thus, differences in donor characteristics seem to account for only a small part of the significantly more favorable DGF rates existing in our study. Therefore, type of preservation machine may play a role in determining the initial posttransplant performance, as the Waters and Lifeport machines differ in their pump engineering, cooling system, and perfusate oxygenation. Second, similar to all centers using the

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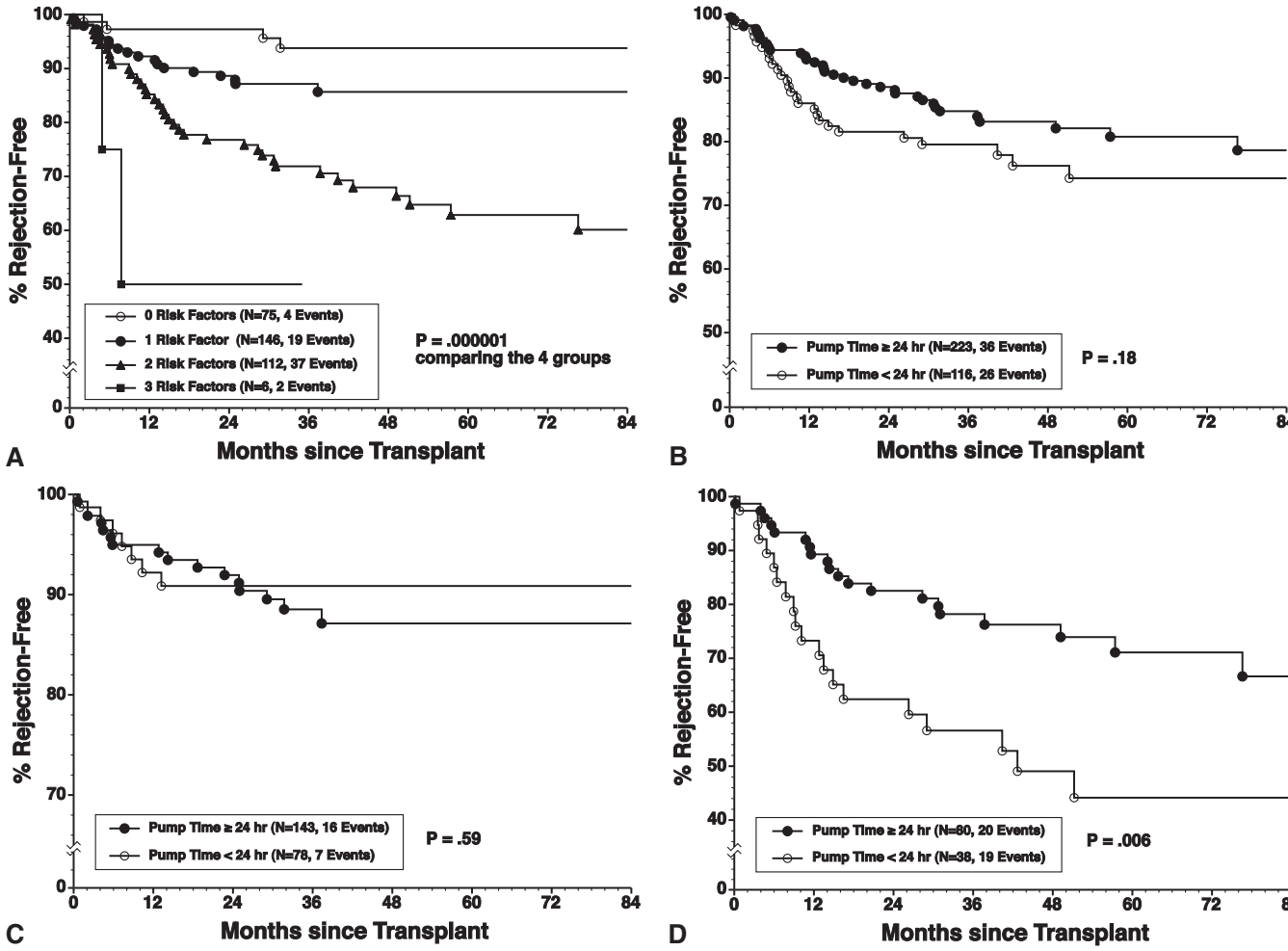

FIGURE 1. (A) Freedom-from-first BPAR by the number of risk factors (developed DGF, recipient age <50yr, and non-white race/ethnicity) present (0, 1, 2, or 3). (B) Freedom-from-first BPAR by pump time <24 vs. ≥24 hr. (C) Freedom-from-first BPAR by pump time <24 vs. ≥24 hr among the less immunologically active recipients, that is, those with ≤1 risk factor present. (D) Freedom-from-first BPAR by pump time <24 vs. ≥24 hr among the more immunologically active recipients, that is, those with ≥2 risk factors present. DGF, delayed graft function; BPAR, biopsy-proven acute rejection.

Waters machine, the kidney is proactively monitored while on the machine, whereas in the study by Moers et al. (*21*), there were "no changes in perfusion settings throughout the perfusion period."

Because our favorable DGF result is single-center based, one could question its generalizability. One clear conclusion is that in a program which machine pumps all kidneys, patients enrolled in clinical trials had rates of DGF/SGF lower than typical national averages. Other centers may be left to wonder whether this outcome reflects universal use of MP, more restrictive donor selection, or a greater requirement for some other center-specific process. Although our data were prospectively obtained, they still represent an empirical study, and thus, lack power to avoid potential selection bias mostly overcome in a randomized study. In an attempt to address these limitations, we reviewed the results of seven other single centers using the Waters machine and Belzer's perfusate in 100% of DD kidneys (*19*, *22*, *25*, *26*, *43–45*). DGF rates reported for 140, 82, 40, and 36 locally retrieved DD kidneys (thus, similarly short CS times as in our study) were 2% (*39*), 6% (*44*), 5% (*25*), and 2.3% (*45*), respectively—comparable with our results. DGF for 33 imported kidneys (mean CS time of 17 hr) was 15.2% in one study (*45*), demonstrating that longer CS times result in greater DGF. Polyak et al. (*19*) reported a DGF rate of 9% among 237 standard criteria donor recipients, and their reported longer mean CS time of 14.7 hr possibly explains the doubling of DGF rate. DGF rates among 165 and 114 ECD recipients were 14% (*19*) and 11% (*26*), respectively (indicating a higher DGF rate for ECD recipients). Finally, Sellers et al. (n=568) (*22*) reported a DGF rate of 8.8% among recipients having CS times less than 5 hr; however, 55% of recipients were African American (again, possibly explaining the doubling of DGF rate in comparison with ours). In summary, the published literature adds credence to our premise that long-lasting (24–48 hr) preservation with physiologically based MP is possible.

A longer pump time may actually protect against BPAR incidence, as a significantly more favorable freedom-from-first BPAR for longer pump time (i.e., ≥24 hr) was observed among the more immunologically active recipients. This surprising clinical finding must now be verified in an independent study. Although it is possible that more pumping

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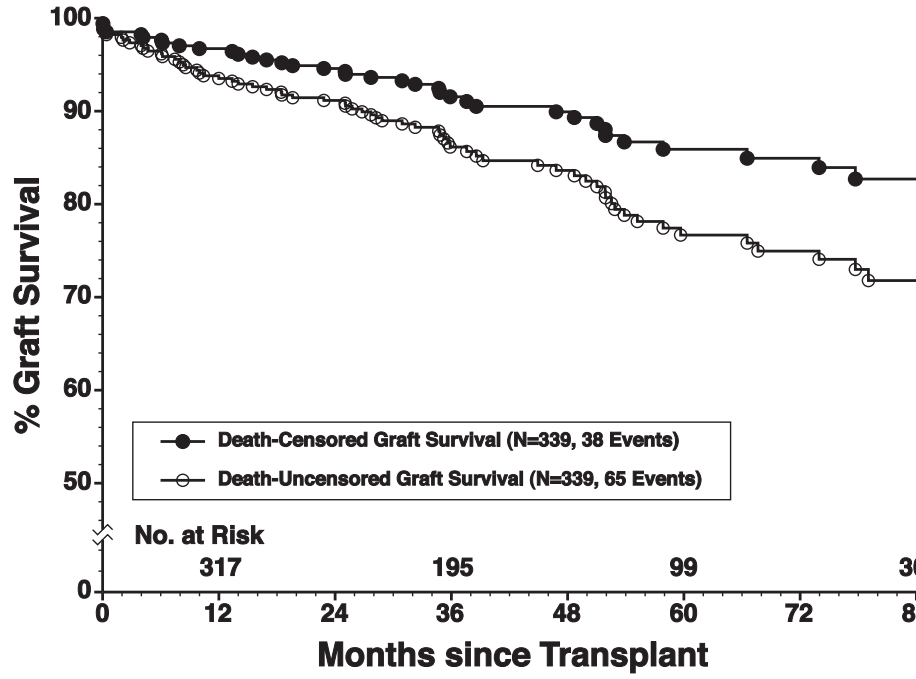

FIGURE 2. Death-censored and death-uncensored graft survival for the whole cohort.

directly helps to flush out immunogenicity from the DD kidney, MP may simply maintain better hypothermic conditions, reducing oxidative and metabolic stress (*46*) as well as somatic cell senescence (*47*) and vascular injury (*48*). We are currently collecting pre- and post-MP kidney biopsies to evaluate differential gene expression and tissue markers of apoptosis, immunogenicity, hibernation (*49*), and senescence.

In conclusion, although a nonrandomized, observational study cannot fully rule out selection bias (even with our best attempts to minimize confounding), our results with better rates of DGF/SGF and BPAR support continued use of MP preservation (minimizing static CS time), not only for ECD/DCD donors but also for all DD kidneys. Optimization of the machine's performance (i.e., type, proactive monitoring) should receive careful attention (possibly including a head-to-head comparison of machine type). The extra expense incurred at the front end of the transplant process will hopefully translate into early (reduced length-of-stay (*50*)) and late (improved long-term graft survival) benefits.

MATERIALS AND METHODS

We analyzed three of our previously published, prospective, randomized immunosuppression trials in kidney transplantation. Between May 2000 and December 2001, a randomized trial of 150 adult, primary kidney transplant recipients (121/150 receiving DD kidneys) was performed comparing tacrolimus/sirolimus versus tacrolimus/mycophenolate mofetil (MMF) versus cyclosporine microemulsion/sirolimus (50 patients per arm) (*33–35*). All patients received daclizumab induction and corticosteroids. Between November 2002 and September 2004, a randomized trial of 90 adult, primary kidney transplant recipients of DD kidneys was performed comparing induction with thymoglobulin versus alemtuzumab versus daclizumab (30 patients per arm) (*36*, *37*). Tacrolimus, MMF, and corticosteroids were given in the thymoglobulin and daclizumab arms, whereas one-half of the regular MMF dose, tacrolimus, and no corticosteroids was scheduled in the alemtuzumab arm. Between December 2004 and February 2006, a randomized trial of 150 adult, primary kidney transplant recipients (128/150 receiving DD kidneys) was performed comparing tacrolimus/MMF versus tacrolimus/enteric-coated mycophenolate sodium as maintenance, with an induction regimen consisting of both thymoglobulin and daclizumab, and early (1 week) discontinuation of corticosteroids (75 patients per arm) (*38*). Combining the DD recipients from these three studies yielded a total of 339 patients for the present study. Previously reported comparisons of clinical results across study arms will not be provided here. Minimum follow-up of 2 years posttransplant existed for each patient.

All transplanted DD kidneys were initially placed in CS at retrieval (i.e., from the time of donor crossclamp). On arrival at our medical center, the DD kidney (or pair of kidneys) was placed on MP and remained there till the time of transplant.

All transplanted DD kidneys received MP preservation with the RM3 Renal Preservation Machine (Waters Instruments Inc., Rochester, MN), using a DCM-100 Cassette and Belzer-MPS Machine Perfusion Solution (Trans-Med Corporation, Elk River, MN) as the perfusate (set at 4°C). Additives included: mannitol (3.75 g/L), dexamethasone (16 mg/L), insulin (100 units/L), and ampicillin (250 mg/L). For adult kidneys, the initial perfusion pressure was set to 40 mm Hg systolic, with the pressure being increased (if needed) to improve flow (by 5 mm Hg increments up to 50 mm Hg). Up to three additional doses of mannitol were added (generally, at 12 hr apart) when the flow was suboptimal (<100 mL/min/100 g renal mass as a guide, but also at the discretion of the kidney transplant surgeon). DGF was defined as the requirement for dialysis during the first week posttransplant. SGF was defined as DGF or a serum creatinine decreasing less than 0.5 mg/dL during the first 24 hr posttransplant.

Statistical Methods

Stepwise logistic regression was used to determine multivariable predictors of DGF and SGF, and Cox stepwise regression was used to determine multivariable predictors of the hazard rates of developing a first BPAR, graft failure (censoring deaths and never functioning grafts), and death with a functioning graft. Univariable and multivariable *P* values were determined using the Score chi-square test criterion, and a multivariable selection criterion of *P* less than or equal to 0.05 was used. To avoid potentially spurious findings, only those factors having a multivariable *P* value less than or equal to 0.15 throughout the stepwise procedure were considered for inclusion into a final multivariable model. Kaplan-Meier curves (along with log rank tests) were also used for graphical display of the results.

Baseline variables that were considered for their prognostic value included recipient and donor age (continuous and categorical representations), recipient gender, race and ethnicity, body mass index, presence of diabetes

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pretransplant, percentage of panel reactive antibody, number of human leukocyte antigen mismatches, ECD status, DCD status, type of DD kidney retrieval (local vs. imported), CIT, CS time, and Pump time. The occurrence of DGF and SGF was also considered for their prognostic value regarding subsequent clinical outcomes.

ACKNOWLEDGMENTS

The authors acknowledge the dedication of our talented and experienced perfusionist team. They also acknowledge Waters Instruments Inc. (and its parent company, Institut Georges Lopez) for their ongoing collaboration in helping us maintain optimal performance of our pulsatile perfusion equipment.

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