ORIGINAL ARTICLE

Renal resistance thresholds during hypothermic machine perfusion and transplantation outcomes – a retrospective cohort study

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SUMMARY

Renal resistance (RR), of allografts undergoing hypothermic machine perfusion (HMP), is considered a measure of organ quality. We conducted a retrospective cohort study of adult deceased donor kidney transplant (KT) recipients whose grafts underwent HMP. Our aim was to evaluate whether RR is predictive of death-censored graft failure (DCGF). Of 274 KT eligible for analysis, 59% were from expanded criteria donor. RR was modeled as a categorical variable, using a previously identified terminal threshold of 0.4, and 0.2 mmHg/ml/min (median in our cohort). Hazard ratios (HR) of DCGF were 3.23 [95% confidence interval (CI): 1.12–9.34, $P = 0.03$] and 2.67 [95% CI: 1.14–6.31, $P = 0.02$] in univariable models, and 2.67 [95% CI: 0.91–7.86, $P = 0.07$] and 2.42 [95% CI: 1.02–5.72, $P = 0.04$] in multivariable models, when RR threshold was 0.4 and 0.2, respectively. Increasing risk of DCGF was observed when RR over the course of HMP was modeled using mixed linear regression models: HR of 1.31 [95% CI: 1.07–1.59, $P < 0.01$] and 1.25 [95% CI: 1.00–1.55, $P = 0.05$], in univariable and multivariable models, respectively. This suggests that RR during HMP is a predictor of long-term KT outcomes. Prospective studies are needed to assess the survival benefit of patients receiving KT with higher RR in comparison with staying wait-listed.

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Key words
graft failure, hypothermic machine perfusion, kidney transplantation, renal resistance

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Introduction

Hypothermic machine perfusion (HMP) is a preservation technique that has been shown to decrease ischemia reperfusion injury and delayed graft function (DGF) and improve graft outcomes in kidney transplant (KT) recipients [1–5]. Parameters such as pressure, resistance, time, and flow are continuously measured during HMP, and those have been linked to short-term graft outcomes. Although, lower flow and higher renal resistance (RR) were associated with higher DGF [6–20], some publications suggest that RR during HMP demonstrated a poor predictive performance for the same outcome [8,11]. Table 1 summarizes the evidence, to date, on the relationship between pump parameters and KT outcomes.

Despite contradictory reports on the relationship between RR and short-term graft outcomes, RR has

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Table 1. Literature review of studies evaluating kidney transplant outcomes by parameters of hypothermic machine perfusion.

Reference	Machine used	Parameter	Threshold	Unit employed for RR	Sample size*	Donor type	Follow up	Outcome
Sandal *et al.* [5]	LifePort IL	Resistance	0.4 and 0.2	mmHg/ml/min	274	59% ECD	5 years	RR during HMP is associated with DCGF
Parikh *et al.* [7]	LifePort IL	Flow and resistance	None	mmHg/ml/min	671	31% ECD, 26% DCD	6 months	1-h flow associated with DGF and last RR with higher 6-month eGFR
Burgos Revilla *et al.* [8]	LifePort IL	Resistance	0.3 and 0.4	mmHg/ml/min	90	100% ECD	1 year	RR not predictive of graft survival
Yushkov *et al.* [9]	LifePort IL	Resistance	<0.2, 0.2–0.3, >0.3	?mmHg/ml/min	454	41% ECD, 10% DCD	1 year	RR >0.3 was predictive of 1-year graft survival
Patel *et al.* (pg 2207) [19]	LifePort IL	Flow and resistance	None	mmHg/ml/min	190	N/A	1 year	Initial resistance associated with 1-year graft survival
Patel *et al.* (pg 2202) [19]	LifePort IL	Pressure	23 mmHg	mmHg/ml/min	73	27% ECD, 4%DCD	1 year	Higher pressure associated with an increased risk for DGF but similar 1-year graft survival
de Vries *et al.* [10]	Gambro PF-3B	Resistance	≤1, 1–1.99, ≥2	mmHg/ml/min per 100 g kidney weight	440	All DCD	15 years	Initial RR associated with PNF and DGF but not graft survival
Jochmans *et al.* [11]	LifePort IL	Resistance	None	mmHg/ml/min	336	27% ECD, 12% DCD	1 year	Terminal RR predictive of graft failure
Matsuno *et al.* [12]	LPS-2, Nikiso, Japan	Flow	0.45–0.90	ml/min/g machine perfusion flow	88	84% DCD	Immediate	Low flow associated with PNF and ATN
Nyberg *et al.* [14]	MOX100, MN	Resistive index	0.2–0.55	ml/min/100 g renal mass	425	N/A	4 year	RR associated with inferior graft function
Mozes *et al.* [13]	Waters RM-3	Resistance	≤0.4 vs. 0.4–0.6	mmHg/ml/min	280	N/A	Immediate	RR not predictive of immediate function
Kwiatkowski *et al.* [15]	MOX 100, MN	Resistance and flow	None	mmHg/ml/min/g	260	N/A	Immediate	RR associated with DGF
Polyak *et al.* [17]	MOX 100, MN	Resistance	None	N/A	111	All ECD†	Immediate	Higher RR predictive of early graft dysfunction
Tesi *et al.* [18]	MOX 100, MN	Resistance and flow	≤0.4, ≥70 for flow	mmHg/ml/min	82	N/A	2 year	Pump parameters not predictive of DGF
Henry *et al.* [16]	MOX 100, MN	Resistance	None	N/A	254	N/A	Immediate	RR >0.289 predictive of DGF
Sampson *et al.* [6]	Belzer LI-400 & MOX 100	Flow	<80, 80–100, >100	ml/min	100	N/A	1 year	Flow not associated with creatinine values

ATN, acute tubular necrosis; DCD, donation after cardiac death; DCGF, death censored graft failure; DCGS, death censored graft survival; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; ECD, expanded criteria donor; N/A, not available; PNF, primary non function; RR, renal resistance.

*Some kidneys were discarded.

†ECD defined as all grafts needing a biopsy.

been used as an indicator of graft quality, at times to guide decisions on organ utilization [21,22]. It has been reported that more than 15% of kidneys undergoing HMP are discarded annually in the USA, partly based on elevated RR [22,23]. This is despite a paucity of evidence on the predictive value of these parameters in relation to graft outcomes beyond 1 year. Hence, many have advocated against discarding kidneys strictly based on parameters during HMP [11,13,22–24]. In the light of these knowledge gaps, the aim of our study was to evaluate RR as an independent predictor of long-term graft survival in KT recipients whose allografts underwent HMP.

Methods

Study design and population

We conducted a retrospective cohort study in adult, deceased donor KT recipients at the McGill University Health Centre (MUHC) between November 1, 2007 and October 31, 2013. Multi-organ transplant recipients and grafts with missing perfusion parameters were excluded. The Research Ethics Board at the MUHC approved this study.

Data sources

Recipient and donor demographics, histocompatibility data, laboratory investigations, treatments employed, histology data, and clinical outcomes were obtained from our in-centre transplant database. Pump parameters measured at the time of HMP were downloaded from the LifePort apparatus.

Immunosuppression

Until 2011, the induction immunosuppression protocol consisted of anti-thymocyte globulin (Thymoglobulin®; Genzyme Canada, Mississauga, ON, Canada) and intraoperative methylprednisolone. Maintenance regimen included tacrolimus (Prograf®; Astellas, Mississauga, ON, Canada), mycophenolate mofetil (CellCept®; Hoffmann-LaRoche, ON, Canada), and prednisone. From November 2011 onwards, the induction immunosuppression protocol consisted of alemtuzumab (Campath®; Genzyme Canada) and intraoperative methylprednisolone. Maintenance regimen was long-acting tacrolimus (Advagraf®; Astellas Pharmaceuticals, Markham, ON, Canada) and mycophenolate sodium (Myfortic®; Novartis, Dorval, QC, Canada).

Organ preservation

Kidneys were recovered from adult deceased donors and flushed with SPS-1 (Belzer solution; Organ Recovery Systems (ORS), Itasca, IL, USA). Kidneys were shipped on ice. On arrival to our centre, grafts were placed on the LifePort Kidney Transporter device (ORS), and perfused with KPS-1 solution (ORS) supplemented with mannitol (2.5 g/l). Initially, the device was set for a systolic pressure of 30 mmHg. At the discretion of the surgeon on call, occasionally, this was reduced to 25 mmHg when flow rates were extremely high. HMP was continued until transplantation. At our center, HMP is considered the standard of care and is used whenever possible for deceased donor KT. By agreement of all surgeons and nephrologists involved in the transplant program, organs that were deemed clinically acceptable for transplantation were not discarded based on pump parameters alone.

Exposure and outcome measurements

The exposure of interest was RR calculated using the formula $\frac{\text{pressure (mmHg)}}{\text{flow (ml/min)}}$. Terminal RR, representing the mean RR measured over the last 15 min of HMP, was modeled as a categorical variable using a threshold of 0.4 mmHg/ml/min, based on prior publications [11]. We also used a threshold of 0.2 mmHg/ml/min, representing the median terminal RR in our study sample. Finally, to flexibly capture the relationship between our study endpoints and RR over the entire course of HMP, we used mixed linear regression models assuming a random slope and intercept [25,26]. The main outcome of interest was death-censored graft failure (DCGF). All-cause graft failure, patient death with function, DGF, and primary nonfunction (PNF) were secondary endpoints.

Definitions

Expanded criteria donor (ECD) refers to all brain-dead donors aged 60 years or older, and donors aged 50–59 years with two or more of the following comorbidities: history of hypertension, death resulting from cerebrovascular accident, and terminal serum creatinine ≥1.5 mg/dl or 133 μmol/l. Standard criteria donors (SCD) included all non-ECD donors. Donation after cardiac death (DCD) donors was those designated by the United Network for Organ Sharing as nonheart-beating donors. DGF was defined as the need for dialysis within the first week post-transplant. PNF was

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defined as the failure of the transplanted kidney to function within the first 3 months post-transplant. Estimated GFR (eGFR) was calculated using the chronic kidney disease epidemiology collaboration equation [27].

Model covariates

Recipient characteristics that were considered for inclusion in the model included recipient age, sex, race, BMI, peak panel reactive antibody, cause of end-stage renal disease, and dialysis modality. Donor characteristics included age, sex, race, BMI, type of donor (ECD, DCD), and terminal eGFR. Other transplant variables included cold ischemia time (CIT), human leukocyte antigen mismatch, and, induction and maintenance immunosuppression regimens. Dialysis modality was missing in 6% of the final cohort, and a missing indicator was used in the multivariable analysis. Other variables were missing in ≤5% of the analytic cohort.

Statistical analysis

The distributions of recipient, donor, and transplant characteristics at the time of transplantation were evaluated by RR categories using the *t*-test or Kruskal–Wallis test for continuous variables, and chi-square or Fisher's exact test for categorical variables. The relationships between terminal RR thresholds, modeled as categorical variables, (using thresholds of 0.4 and 0.2 mmHg/ml/min) and DCGF as well as all-cause graft failure and death with function, were estimated using the Kaplan–Meier method. Univariable and multivariable Cox proportional hazards models were employed to assess the independent relationship between terminal RR and time-to-event outcomes. RR estimates from the mixed linear regression models were similarly incorporated into univariable and multivariable Cox proportional hazards models [25,26]. To ensure the relationship was robust, we conducted sensitivity analyses whereby the models were also fit in a subcohort excluding patients with PNF. We were planning to introduce an interaction term and assess whether the effect of RR on study endpoints was modified within prespecified subgroups; however, the number of events was too small to conduct such an analysis. Statistical analyses were performed using STATA/IC 12.0 (StataCorp, College Station, TX, USA, www.stata.com) and R version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria, www.R-projects.org). Missing covariate data were handled by multiple imputations using STATA's "ice" command and R's "mice" package. A two-tailed P value of <0.05 was considered statistically significant.

Results

During the study period, 422 deceased donor KT were performed. Of these, 274 were eligible for analysis. The study flow diagram is presented in Fig. 1. In our cohort, 59% of the KT were from ECD and 8% from DCD donors. Mean donor eGFR was 98.30 ± 29.32 ml/min/1.73 m^2. Allografts underwent HMP over a median of 8.5 h [95% confidence interval (95% CI): 6.1–11.5]. In most allografts, RR measurements decreased to a trough corresponding to the terminal RR. In some allografts, however, temporal changes in RR from initiation of HMP to transplantation demonstrated other patterns (e.g. U-shaped curve or rising to a peak corresponding to terminal RR measurement). Figure 2 presents changes in RR measurements over the course of HMP in eight allografts from our cohort.

Baseline characteristics by terminal RR category

Baseline donor, recipient, and transplant characteristics of the analytic cohort are presented in Table 2. Of the 274 kidneys, 259 (94.5%) had a RR of <0.4 mmHg/ml/min and 15 (5.5%) had a RR of ≥0.4 mmHg/ml/min. Donor eGFR was lower in allografts with a higher RR measuring 99.47 ± 28.72 in those with RR <0.4 mmHg/ml/min vs. 78.43 ± 33.14 in those with RR ≥0.4 mmHg/ml/min ($P < 0.01$). Otherwise, donor and recipient characteristics were equally distributed. When applying the median

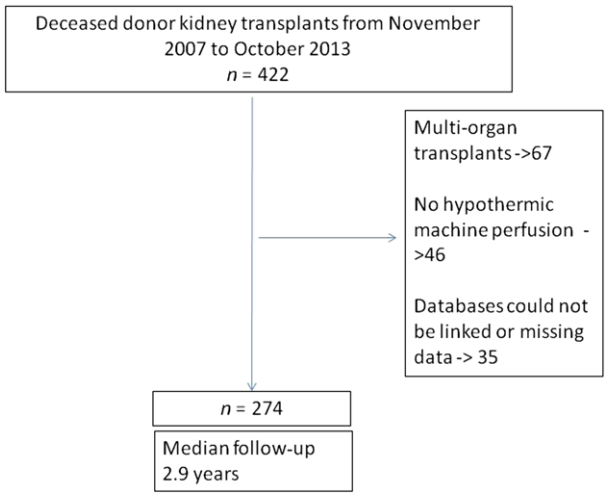

Figure 1 Study flow diagram.

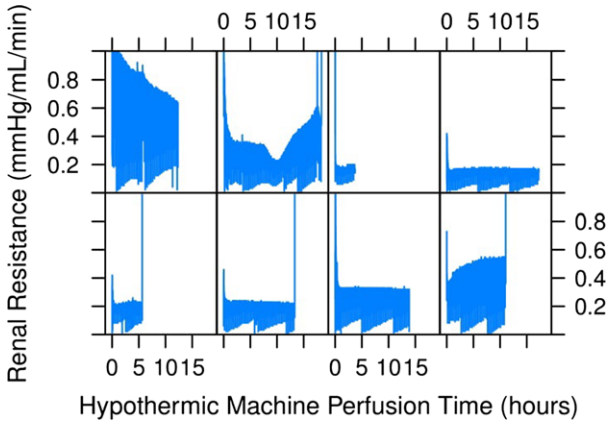

Figure 2 Patterns of change in renal resistance over the course of hypothermic machine perfusion.

terminal RR in our population as a threshold (0.2 mmHg/ml/min), kidneys with higher RRs tended to be from female donors (50% vs. 38%, $P = 0.01$) and had a lower eGFR (102.72 ± 27.51 vs. 94.49 ± 30.36, $P = 0.01$).

Death-censored graft failure

Median follow-up in our cohort was 2.9 years [interquartile range (IQR): 1.3–4.4]. By the end of the study, 80% of the recipients were alive with a functioning graft, 10% experienced graft loss, and 10% died with a functioning graft. Kaplan–Meier curves of DCGF by terminal RR thresholds are depicted in Fig. 3. Results of univariable and multivariable Cox proportional hazards models evaluating the association between terminal RR and DCGF are presented in Table 3. An increased risk of DCGF was observed in univariable models with hazard ratio (HR) of 3.23 [95% CI: 1.12–9.34, $P = 0.03$] and 2.67 [95% CI: 1.14–6.31, $P = 0.02$] when applying terminal RR thresholds ≥0.4 and ≥0.2 mmHg/ml/min, respectively. In multivariable models (adjusted for ECD, CIT, DGF, and recipient age), HRs of 2.67 [95% CI: 0.91–7.86, $P = 0.07$] and 2.42 [95% CI: 1.02–5.72, $P = 0.04$] were observed when applying terminal RR thresholds of ≥0.4 and ≥0.2 mmHg/ml/min, respectively. An increasing risk of DCGF was also observed when repeated measurements of RR over the course of HMP were modeled using mixed linear regression models: HR 1.31 [95% CI: 1.07–1.59, $P < 0.01$] and 1.25 [95% CI: 1.00–1.55, $P = 0.05$] in univariable and multivariable analysis, respectively.

Secondary endpoints

Kaplan–Meier curves for all-cause graft failure and death with function by terminal RR thresholds are depicted in Fig. 3. When RR representing measurements over the course of HMP were modeled using mixed linear regression models, HRs for all-cause graft failure and death with function of 1.20 [95% CI: 0.98–1.45, $P = 0.07$] and 1.04 [95% CI: 0.75–1.45, $P = 0.79$] were observed in univariable analysis, and HR of 1.08 [95% CI: 0.88–1.34, $P = 0.49$] and 0.91 [95% CI: 0.64–1.28, $P = 0.59$] were observed in multivariable analysis (adjusted for ECD, DGF, and recipient age), respectively.

Delayed graft function was observed in 66 (24.1%) and PNF in 8 (2.9%) of the KT recipients. When applying a threshold of 0.4 mmHg/ml/min, 23.5% with RR <0.4 and 33.3% with RR ≥0.4 experienced DGF. When applying a threshold of 0.2 mmHg/ml/min, a 23.2% with RR <0.2 and 24.8% with RR ≥0.2 experienced DGF. An adjusted odds ratio for DGF, when RR measurements over the course of HMP were modeled using mixed linear regression models, was 1.01 [95% CI: 0.77–1.32, $P = 0.95$].

Sensitivity analysis

When repeating the analysis in a subcohort that excluded patients with PNF, univariable analysis demonstrated HR of 2.28 [95% CI: 0.53–9.84, $P = 0.27$] and 3.64 [95% CI: 1.21–10.89, $P = 0.02$], and multivariable analysis (adjusted for ECD, CIT, DGF, and recipients age) demonstrated HR of 1.34 [95% CI: 0.28–6.31, $P = 0.71$] and 3.42 [95% CI: 1.16–10.48, $P = 0.03$] for DCGF when applying terminal RR thresholds ≥0.4 and ≥0.2 mmHg/ml/min, respectively. When RR representing measurements over the course of HMP were modeled using mixed linear regression models, HRs for DCGF of were 1.29 [95% CI: 1.01–1.66, $P = 0.05$] and 1.24 [95% CI: 0.93–1.66, $P = 0.15$] in univariable and multivariable analysis, respectively.

Discussion

This study sought to assess whether RR measured during HMP is an independent predictor of long-term graft outcomes in KT recipients. We found that terminal RR using a threshold of 0.2 mmHg/ml/min was associated with DCGF. Although underpowered to detect a statistically significant difference, when applying a threshold of 0.4 mmHg/ml/min, a greater effect size for risk of DCGF was observed. Finally, an increased risk of DCGF was also observed in analyses considering RR measured repeatedly over the entire course of HMP. Taken together, these findings suggest that RR is an independent predictor of long-term graft survival.

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Table 2. Baseline characteristics of total cohort and subgroups by terminal renal resistance threshold of hypothermic machine perfused kidney allografts.

Variable	Total cohort $n = 274$	RR <0.4 $n = 259$	RR ≥0.4 $n = 15$	*P* value	RR <0.2 $n = 125$	RR ≥0.2 $n = 149$	*P* value
Age (years)	56.08 ± 12.69	55.86 ± 12.80	59.93 ± 10.23	0.89	54.63 ± 12.96	57.30 ± 12.37	0.96
Sex *n* (%)							
Female	102 (37.2)	94 (36.3)	8 (53.3)	0.18	51 (40.8)	51 (34.2)	0.26
Male	172 (62.8)	165 (63.7)	7 (46.7)		74 (59.2)	98 (65.8)	
BMI (kg/m^2)	27.40 ± 5.20	27.43 ± 5.12	26.77 ± 6.86	0.33	28.05 ± 5.27	26.84 ± 5.10	0.03
Race *n* (%)							
Caucasian	182 (66.4)	173 (66.8)	9 (60.0)	0.59	88 (70.4)	94 (63.1)	0.2
Non-Caucasian	92 (33.6)	86 (33.2)	6 (40.0)		37 (29.6)	55 (36.9)	
Dialysis modality *n* (%)							
Pre-emptive	21 (7.7)	20 (7.7)	1 (6.7)	0.48	10 (8)	11 (7.4)	0.29
Hemodialysis	225 (82.1)	213 (82.2)	12 (80.0)		107 (85.6)	118 (79.2)	
Peritoneal dialysis	22 (8.0)	21 (8.1)	1 (6.7)		6 (4.8)	16 (10.7)	
Missing	6 (2.2)	5 (1.9)	1 (6.7)		2 (1.6)	4 (2.7)	
Cause of ESRD *n* (%)							
Diabetes	79 (28.8)	75 (29)	4 (26.7)	0.63	40 (32)	39 (26.2)	0.53
Polycystic kidney disease	34 (12.4)	32 (12.4)	2 (13.3)		13 (10.4)	21 (14.1)	
Glomerulonephritis	71 (25.9)	67 (25.9)	4 (26.7)		28 (22.4)	43 (28.9)	
Pyelonephritis/ interstitial nephritis	49 (17.9)	48 (18.5)	1 (6.7)		23 (18.4)	26 (17.4)	
Other	41 (15.0)	37 (14.3)	4 (26.7)		21 (16.8)	20 (13.4)	
HLA mismatch *n* (%)							
0	1 (0.4)	1 (0.4)	0	0.41	0	1 (0.67)	0.91
1	11 (4.0)	10 (3.9)	1 (6.7)		6 (4.8)	5 (3.4)	
2	24 (8.8)	22 (8.5)	2 (13.3)		9 (7.2)	15 (10.1)	
3	79 (28.8)	72 (27.8)	7 (46.7)		37 (29.6)	42 (28.2)	
4	89 (32.5)	86 (33.2)	3 (20.0)		39 (31.2)	50 (33.6)	
5	51 (18.6)	50 (19.3)	1 (6.7)		24 (19.2)	27 (18.1)	
6	18 (6.6)	17 (6.6)	1 (5.6)		10 (8)	8 (5.4)	
Missing	1 (0.4	1 (0.4)	0		0	1(0.7)	
Donor age (years)	51.27 ± 16.00	50.91 ± 16.98	57.6 ± 15.41	0.94	48.21 ± 15.67	53.84 ± 15.87	1
Donor sex *n* (%)							
Female	123 (44.9)	112 (43.2)	11 (73.3)	0.08	48 (38.4)	75 (50.3)	0.01
Male	147 (53.6)	143 (55.2)	4 (26.7)		77 (61.6)	70 (47)	
Missing	4 (1.5)	4 (1.5)	0		0	4 (2.7)	

Table 2. Continued.

Variable	Total cohort $n = 274$	RR <0.4 $n = 259$	RR ≥0.4 $n = 15$	*P* value	RR <0.2 $n = 125$	RR ≥0.2 $n = 149$	*P* value
Donor BMI (kg/m^2)	28.00 ± 12.49	28.14 ± 12.81	25.47 ± 3.19	0.21	27.99 ± 6.11	28.00 ± 16.00	0.5
Donor type *n* (%)							
ECD	162 (59.1)	151 (58.30)	11 (73.33)	0.19	66 (52.8)	96 (64.4)	0.05
DCD	22 (8.0)	22	0	0.28	12 (9.6)	10 (6.7)	0.38
Donor eGFR* (ml/min per 1.73 m^2)	98.30 ± 29.32	99.47 ± 28.72	78.43 ± 33.14	<0.01	102.72 ± 27.51	94.49 ± 30.36	0.01
CIT (h)	18.31 ± 6.14	18.23 ± 6.00	19.65 ± 8.40	0.8	17.85 ± 6.07	18.69 ± 6.19	0.89
Induction immunosuppression *n* (%)							
Anti-thymocyte globulin	167 (61)	162 (62.5)	5 (33.3)	0.023	79 (63.2)	88 (59.1)	0.4
Alemtuzumab	66 (24.1)	62 (23.9)	4 (26.7)		31 (24.8)	35 (23.5)	
IL2R inhibitor	25 (9.1)	21 (8.1)	4 (26.7)		11 (8.8)	14 (9.4)	
Missing	16 (5.8)	14 (5.4)	2 (13.3)		4 (3.2)	12 (8.1)	
Maintenance immunosuppression *n* (%)							
Tacrolimus	264 (96.3)	251 (96.9)	13 (86.7)	0.1	123 (98.4)	141 (94.6)	0.1
Mycophenolate	268 (97.8)	254 (98.1)	14 (93.3)	0.29	125 (100)	143 (96)	0.02
Prednisone	205 (74.8)	197 (76.1)	8 (53.3)	0.05	99 (79.2)	106 (71.1)	0.13

DCD, donation after cardiac death; CIT, cold ischemia time; eGFR, estimated glomerular filtration rate; ESRD, end stage renal disease; ECD, expanded criteria donor; HLA, human leukocyte antigen; RR, renal resistance.

*Calculated using the chronic kidney disease epidemiology collaboration equation.

Our study provides the longest follow-up to date when evaluating the transplant outcomes in relation to pump parameters measured using the LifePort Kidney Transporter preservation system. In a study using the Gambro PF-3B preservation system and using three different RR thresholds in recipients who received grafts from DCD donors, no difference was observed in graft survival over a 15-year follow-up [10]. In a study using the MOX 100 preservation system, with a 4-year follow-up, higher RR was found to correlate with inferior graft outcomes [14]. Table 1 outlines the variability across studies with regard to the choice of kidney transport preservation system, the units of pump parameters considered, and the thresholds applied when verifying a relationship between pump parameters and graft outcomes. Six different devices were used across different studies. Yushkov *et al.* [9] suggested that thresholds for RR differ between different machines. For example, machines using a pressure controlled roller pump to deliver the perfusate create sinusoidal flow curves, and others that use a flow controlled pumping system give rise to alternate pressure waveforms [11]. When the effect of the device used for HMP was evaluated in relation to graft outcomes by Wszola *et al.* [28], a flow driven device was found to result in inferior graft outcomes when compared to a pressure driven device. Hence, caution should be exercised when comparing the relationship between HMP parameters and outcomes across studies.

When using the LifePort Kidney Transporter preservation system, two studies reported no relationship between RR and graft survival, and three studies showed an association; however, follow-up was limited to 1 year or less in all these five studies [8,9,11,19,20]. Jochmans *et al.* [11] showed that RR was an independent risk factor for 1-year graft failure in both unadjusted and adjusted Cox proportional hazards models; however, the authors only considered donor age as a covariate. When applying the RR threshold of ≥0.4 mmHg/ml/min identified by Jochmans *et al.*, the relationship between RR and DCGF in our sample did not reach statistical significance. However, our analysis was likely underpowered to detect a difference, considering the small number of participants with RR exceeding 0.4 mmHg/ml/min. When using the median RR in our study sample (0.2 mmHg/ml/min) as a threshold, on the other hand, we did find a statistically significantly increased risk for DCGF as a function of RR with an expected lower effect size than that estimated for organs with RR greater than 0.4 mmHg/ml/min.

Although these analyses confirm that RR is an independent risk factor for DCGF, our findings do not suggest that organs with terminal RR exceeding either of these thresholds should influence decisions on organ utilization or discard. This is also supported by the fact that we observe a gradual increase in the risk for DCGF when considering changes across the range of RR measurements over the course of HMP. Evidence suggests that multiple intrinsic qualities of an allograft determine graft survival, such as donor's gender, age, height, and weight [7,11,29]. For instance, in our cohort, allografts with RR ≥0.2 mmHg/ml/min demonstrated a lower eGFR and were more likely to originate from female donors; however, our sample was too small to pursue subgroup analyses by donor sex. In addition to donor characteristics, recipient and transplant characteristics are also important determinants of graft outcomes. For example, donor–recipient sex mismatch, in particular, KT from female donors to male recipients, has been linked to inferior graft outcomes [30]. Realizing the multifactorial nature of predictors of kidney transplant outcomes, the United Network of Organ Sharing is currently using a new allocation policy that risk-stratifies deceased donors using 10 different donor factors, that are associated with all-cause graft survival [29,31]. Thus, a higher RR is unlikely to be the sole determinant of kidney transplant outcomes but rather one of several characteristics that inform on the quality of the graft.

In contrast to prior studies, in our cohort, RR was not associated with DGF (Table 1). The reasons for this could be manifold. First, our donor population included a large proportion of grafts from ECD. In a large registry analysis, we have recently shown that in some high-risk grafts from ECD, HMP use did not lower the odds of DGF [5]. The mechanism of DGF is not limited to ischemia-induced damage to the kidney tubules, but a constellation of thrombotic and inflammatory effects mediated by cytotoxins, and both the innate and adaptive immune responses [32]. This may also serve to explain why RR measured during HMP in our population with a relatively large proportion of ECD kidneys is not associated with DGF. Second, while the standard definition of DGF warrants administration of at least one dialysis session during the first week post-transplant, the number of dialysis sessions administered may vary. Consequently, the presence of DGF may not necessarily gauge the severity of graft dysfunction [4]. Third, and as mentioned previously, in addition to the considerable variability in terms of the preservation system used, no uniform practices exist across physicians and centers in terms of perioperative care including that

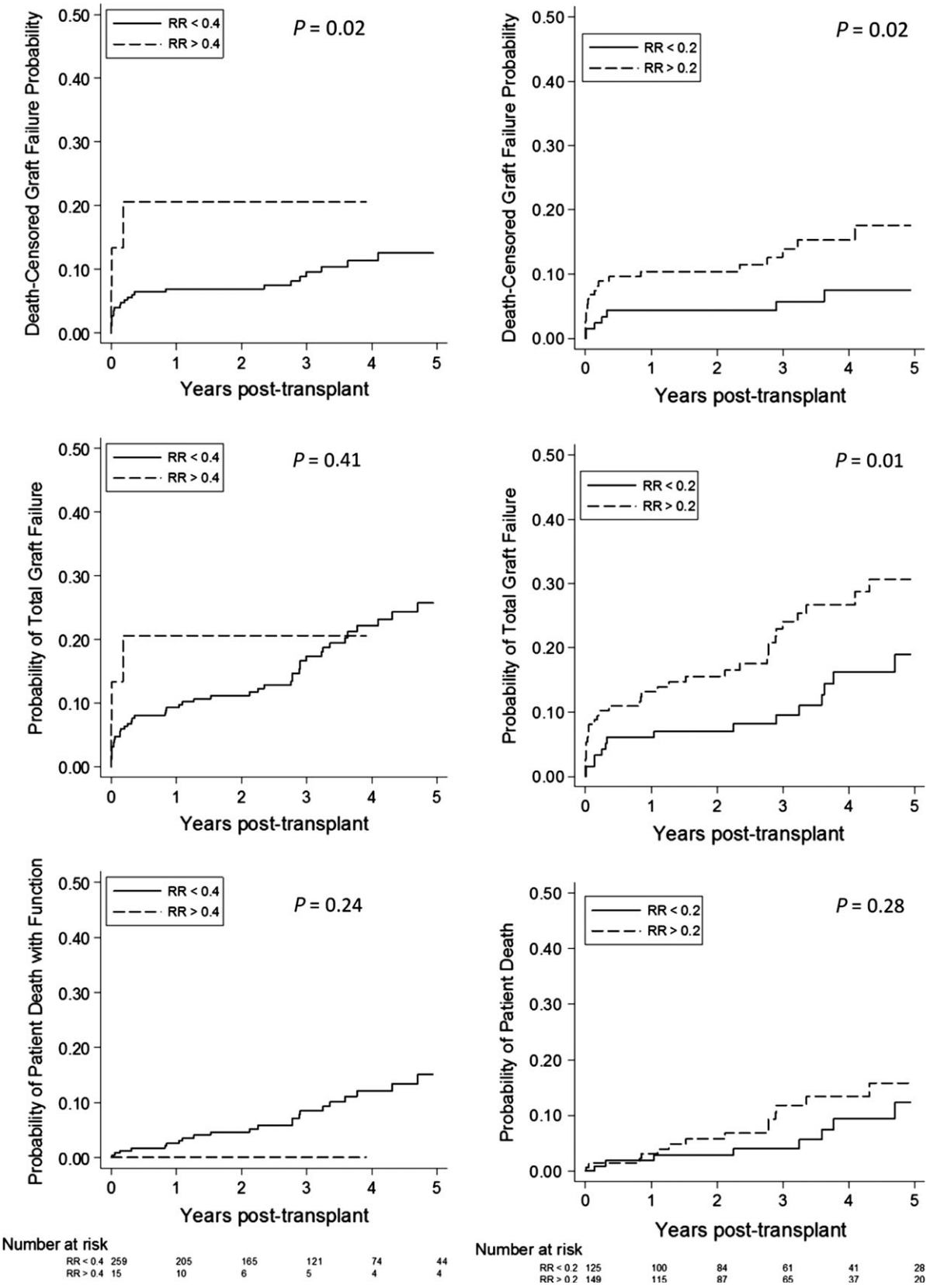

Figure 3 Death-censored graft failure, all-cause graft failure and death with function by terminal renal resistance threshold of 0.4 and 0.2 mmHg/ml/min.

Table 3. Hazard ratio for graft outcomes by renal resistance measured during hypothermic machine perfused kidney allografts.

Variable	Univariable Cox proportional hazards model		Multivariable Cox proportional hazards model*	
Death censored graft failure				
Terminal RR ≥0.4	$_{1.12}3.23_{9.34}$	0.03	$_{0.91}2.67_{7.86}$	0.07
Terminal RR ≥0.2	$_{1.14}2.67_{6.31}$	0.02	$_{1.02}2.42_{5.72}$	0.04
RR over course of HMP	$_{1.07}1.31_{1.59}$	<0.01	$_{1.00}1.25_{1.55}$	0.05
All-cause graft failure				
Terminal RR ≥0.4	$_{0.55}1.53_{4.24}$	0.82	$_{0.34}1.00_{2.85}$	0.98
Terminal RR ≥0.2	$_{1.14}2.02_{3.59}$	0.02	$_{0.95}1.70_{3.05}$	0.07
RR over course of HMP	$_{0.98}1.20_{1.45}$	0.07	$_{0.88}1.08_{1.34}$	0.49
Death with function				
RR over course of HMP	$_{0.75}1.04_{1.45}$	0.79	$_{0.64}0.91_{1.29}$	0.59

CIT, cold ischemia time; DGF, delayed graft function; ECD, expanded criteria donor; HMP, hypothermic machine perfusion; RR, renal resistance.

*Multivariable Cox proportional hazards models of graft outcomes by terminal renal resistance were adjusted for ECD, CIT, DGF, and recipient age and multivariable models of death censored graft failure by renal resistance over the course of HMP modeled using mixed linear regression models was adjusted for ECD, DGF, and recipient age.

may affect the risk of experiencing reversible and short-term graft outcomes like DGF [33,34].

As is apparent from Table 1, our study provides information on the risk of adverse transplant outcomes associated with increasing RR measures in allografts undergoing HMP using the LifePort Kidney Transporter preservation system over the longest post-transplant follow-up to date. Our study population of KT recipients includes a large proportion of ECD allowing the evaluation of the predictive role of RR in a relatively high risk donor population. Moreover, rather than relying on terminal RR alone, we apply mixed linear regression models to better capture variability in RR both between allografts and within allografts over time. Despite these strengths, some limitations need to be considered. First, this is a single-center study, the findings of which may not be applicable to all centers. As with any study evaluating the effect of HMP parameters on kidney transplant outcomes, our study is also prone to inherent bias due to selection. Mitigating this risk, however, is the fact that it is the standard practice at our center to use HMP for all deceased donor kidneys. Furthermore, if the clinical characteristics of the graft are deemed acceptable, grafts are transplanted regardless of RR. The observational nature of this study makes it vulnerable to residual confounding. For example, previous studies found histological findings such as glomerulosclerosis, tubular interstitial scarring, and arterial disease in kidney allografts demonstrating lower flow rates and higher RR [9,35]. However, histological findings on procurement biopsies are not available for all our study participants, and, consequently, could not be accounted for in multivariable analyses. Finally, the small sample size and number of events limits our ability to adjust for all relevant confounders in the multivariable analysis and may compromise the power to detect associations between RR and study secondary endpoints.

In conclusion, our findings suggest that terminal RR and repeated measurements of RR in kidney allografts undergoing HMP are associated with DCGF. These findings, however, should not be interpreted to suggest that a particular RR measurements should be used for decisions on organ utilization or discard but rather that this is one of the several graft characteristics that need to be accounted for in organ allocation. Our observations warrant evaluation in larger, prospective, multicenter cohort studies (e.g. trial ISRCTN15821205 underway). These studies, in addition to informing on the added risk of graft failure, must also evaluate on how ascending RR may affect not only allograft survival but also the survival of kidney transplant recipients in comparison with end-stage renal disease patients remaining on dialysis.

Authorship

SS: contributed to conception and design of the study, acquisition and interpretation of data, drafting and revising the article, providing intellectual content, final approval of the version to be published. SP and MC: contributed to conception and design of the study,

interpretation of data, revising the article, providing intellectual content, final approval of the version to be published. DB, PC, JT: performed conception of the study, interpretation of data, revising the article, providing intellectual content, final approval of the version to be published. RS-P: performed conception and design of the study, analysis and interpretation of data, revising the article, providing intellectual content, final approval of the version to be published.

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Conflict of interest

The authors have declared no conflicts of interest.

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