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The Impact of Hypothermic Pulsatile Machine Perfusion Versus Static Cold Storage: A Donor-Matched Paired Analysis in a Scenario of High Incidence of Delayed Kidney Graft Function

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Background: The present study analyzed the impact of hypothermic pulsatile machine perfusion (MP) following a long period of static cold (SC) storage in the peculiar Brazilian scenario of high incidence of delayed graft function (DGF), despite good donor characteristics.

Material/Methods: A retrospective analysis, with a 1-year follow-up, of 206 recipients of donor-matched paired kidneys was performed. Of the 206 donor kidneys, 103 were maintained exclusively in static cold storage (SC group) and 103 were kept on machine perfusion after a period of SC preservation (MP group). All donors were brain dead.

Results: Only 4.9% of the kidneys were from expanded-criteria donors. Static cold ischemia time (CIT) in the SC group was 20.8±4.1 hours *vs.* 15.8±6.2 hours in the MP group ($P<0.001$). Dynamic CIT in the MP group was 12.3±5.7 hours. MP significantly reduced DGF incidence (29.1% *vs.* 55.3%, $P<0.001$), and this effect was confirmed in multivariable analysis (OR, 1.115; 95% CI, 1.033–1.204, $P=0.001$). No differences were observed between the groups with regard to DGF duration, length of hospital stay, incidence of primary nonfunction and acute rejection, graft loss, death, or renal function.

Conclusions: In this Brazilian setting, MP following a long period of SC preservation was associated with reduced DGF incidence in comparison with SC storage without MP.

MeSH Keywords: Delayed Graft Function • Organ Preservation • Pulsatile Flow

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Background

Brazil has the second-highest kidney transplantation (KT) program in the world, performing about 6,000 kidney transplants per year [1,2]. Brazilian studies have reported delayed kidney allograft function (DGF) incidences varying between 50% and 82%. These rates are 2- to 3-fold higher than those described by European and USA cohorts, without obvious explanation based on recipient and donor demographics [3–8]. Poor donor maintenance before and during the organ procurement process and longer cold ischemia time (CIT) should explain these results [9]. In this scenario, measures to reduce DGF are warranted.

Previous studies showed that machine perfusion (MP) reduces the incidence of DGF [10,11]. However, MP is not widely used in Brazil and there is scarce evidence on the benefits of MP in Brazilian patients.

A Brazilian multicenter, prospective, randomized, controlled study showed a significant reduction in DGF incidence (61% *vs.* 45%, *P*=0.031), without any impact on DGF duration, primary nonfunction (PNF), renal function, or graft loss [12]. In that clinical trial, kidneys were connected to MP immediately after retrieval surgery, which is not the usual current Brazilian practice. In most regions of our country, after retrieval surgery, kidneys are kept in SC storage while the allocation and distribution process occurs. Once a KT candidate accepts the kidney, the transplant center decides how the organ will be preserved until transplant surgery and implantation of the kidney. Importantly, in this multicenter study, 54% of the kidneys were obtained from expanded-criteria donors (ECD), and the median Kidney Donor Profile Index (KDPI) was 75%, which does not reflect the national numbers (about 30% ECD) [5,8,13]. This high percentage of ECD probably impacted the incidence of DGF, resulting in a large effect size and favoring the significance of the 26% reduction in DGF incidence.

Another Brazilian study reported MP results in a scenario closer to that of most kidney transplantation scenarios in Brazil: kidneys were kept on pulsatile perfusion after a long time in SC storage. About 20% were ECD. As a result, MP was associated with lower DGF incidence (79.2 *vs.* 61.1%, *P*=0.002), lower DGF time (11 *vs.* 5 days, *P*<0.001) and lower length of hospital stay (18 *vs.* 13 days, *P*<0.001) [14]. Of note, Euro-Collins solution was used during all static CIT, which might have contributed to these results [15].

Brazil is a large country, with significant regional disparities [13]. In our region (Ceará, in the northeast area of the country), standard criteria deceased kidney donor (SCD) transplants are predominant, and Custodiol HTK is the main perfusion solution; nevertheless, DGF incidence remains high [16]. There are 2 main transplant centers. In 1 of these 2, MP became available in 2012. The present study aimed to analyze the impact of pulsatile perfusion following a long period of SC storage due to this peculiar scenario.

Material and Methods

Study design and population

This retrospective cohort analysis included 206 donor-matched recipients of 103 pairs of deceased-donor kidneys, in which 1 kidney was maintained exclusively on SC storage preservation (SC group), and the contralateral organ was placed on MP following an initial period of SC storage (MP group). Transplants were performed at 2 transplant centers located in northeastern Brazil, between July 2013 and December 2017. Donors whose recipients lost the graft or died within 7 days after KT were excluded. All donors were brain dead (DBD).

The study was performed following the ethical standards of National Health Council Resolution 466/12 and the Declaration of Helsinki, and was approved by the local Institutional Review Board (IRB) (number 3.660.383). Data were retrospectively collected by a systematic review of medical charts and the electronic database after obtaining informed consent from patients.

Objectives

The main objective was to analyze the impact of MP on the incidence and duration of DGF. Secondary objectives included analysis of the incidence of PNF, length of hospital stay, renal function at 1 year post-transplantation, and incidence of acute rejection, graft loss, and death.

Logistics and definitions

Kidneys transplanted in the 2 main centers of the Ceará region of Brazil were included in the study. At Site 1, kidneys were maintained in SC storage. At Site 2, kidneys meeting 1 of the following criteria were preserved by MP: donor age ≥50 years; final serum creatinine (sCr) >1.5 mg/dL, estimated CIT ≥20 h, severe hemodynamically unstable donors, small children, and immunologically high-risk recipients. These kidneys were kept on MP (LifePort Kidney Transporter, Organ Recovery Systems, Chicago, IL USA) for at least 6 hours using Kidney Preservation Solution-1 (KPS-1). Intra-renal resistance and flow were closely monitored and no kidney was discarded based only on hemodynamic parameters.

DGF was defined as the need for at least 1 dialysis session in the week after KT [17]. DGF duration was assessed by 2 measures: the time to the last dialysis session (days) and the number of

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Table 1. Donor demographics and clinical characteristics.

	N=103	
Age (years), median (IQR)	30	(21–40)
Death cause, n(%)		
Traumatic	74	(71.8)
Cerebrovascular	19	(18.4)
Anoxic encephalopathy	6	(5.8)
Cerebral tumor	2	(1.9)
Other	2	(1.9)
Hypertension, n(%)	5	(4.9)
Diabetes, n(%)	0	(0.0)
Final sCR (mg/dL), median (IQR)	1.1	(1.1–1.6)
Expanded criteria donor, n(%)	5	(4.9)
KDPI (%), median (IQR)	27	(17–45)
Perfusion solution used during SC storage, n(%)		
Custodiol – histidine-tryptophan-ketoglutarate (HTK)	91	(88.3)
Static preservation solution (SPS-1)/UW	8	(7.8)
Institut Georges Lopez-1 (IGL-1)	4	(3.9)

sCr – serum creatinine; KDPI – Kidney Donor Profile Index; SC – static cold; IQR – interquartile range.

dialysis sessions performed during this period. ECD were defined using the United Network for Organ Sharing (UNOS) definition: a) donors >60 years, or b) donors 50–59 years with at least 2 of the following: sCr >1.5 mg/dL, history of hypertension, or cardiovascular death [18]. Kidney Donor Profile Index was assessed using the Organ Procurement and Transplantation Network (OPTN) online calculator [19]:

https://optn.transplant.hrsa.gov/resources/allocation-calculators/kdpi-calculator/.

Statistical analysis

Nominal variables are presented as absolute frequency and percentage and compared using Chi-square or Fisher tests. Normally distributed continuous variables are expressed as mean and standard deviation and compared using the *t* test. Non-normally distributed continuous variables are expressed as median and interquartile range and compared by Mann-Whitney test. Continuous dependent variables were compared using Wilcoxon test. Graft function was assessed by estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, adjusting for graft losses, deaths, and losses to follow-up (Last Observation Carried Forward analysis), as follows: for patients who lost the graft, eGFR was given as 0 mL/min; for those who died or were lost to follow-up, the last available eGFR was used. A multivariable logistic regression model was fitted to compute covariate-adjusted odds ratios (OR) for DGF. Factors with univariable association of $P<0.15$ were considered to be of sufficient statistical significance for inclusion in multivariable analysis. For all other analyses, statistical significance required a P value of <0.05. Statistical analysis was performed using SPSS v.24 (SPSS, Inc., Chicago, IL, USA).

Results

Demographics

Donors were predominantly young adults (median age, 30 years) who died from trauma. Only 4.9% were ECD and the median KDPI was 27%. During SC storage, the main perfusion solution was Custodiol HTK (Table 1).

Ninety-eight (95.1%) kidneys from the MP group were transplanted at Site 2. Five (4.9%) of these MP kidneys were implanted in patients from Site 1 due to clinical/immunological problems with Site 2 candidates. Eighty (77.7%) kidneys from the SC group were transplanted at Site 1.

Due to the clinical protocol for MP use, patients in the MP group were younger (39.9±18.2 *vs.* 45.9±15.0 years, $P=0.011$) and a higher percentage presented preformed donor-specific antibodies (15.5% *vs.* 2.9%, $P=0.003$). In addition, this group had lower body-mass index than the SC group (22.6±4.7 *vs.* 24.5±4.7 kg/m^2, $P=0.004$). The mean static CIT was 20.8±4.1 h in the SC group versus 15.8±6.2 h in the MP group ($P<0.001$)

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Table 2. Recipient demographic and clinical characteristics.

	SC group N=103	MP group N=103	P value
Gender – Male, n(%)	59 (57.3)	58 (56.3)	1.000
Age (years), mean±SD	45.9±15.0	39.9±18.2	0.011
BMI (Kg/m²), mean±SD	24.5±4.7	22.6±4.7	0.004
Ethnicity, n (%)			0.857
Caucasian	9 (8.7)	11 (10.7)	
Mixed/Hispanic	89 (86.4)	86 (83.5)	
Afro-Brazilian	5 (4.9)	6 (5.8)	
ESRD etiology, n (%)			0.059
Unknown	29 (28.3)	41 (39.8)	
Hypertension	28 (27.2)	27 (26.2)	
Diabetes	18 (17.5)	9 (8.7)	
Glomerulonephritis	8 (7.8)	6 (5.8)	
Urological	16 (15.5)	8 (7.8)	
PKD	3 (2.9)	10 (9.7)	
Other	1 (1.0)	2 (1.9)	
Diabetes, n (%)	19 (18.4)	10 (9.7)	0.108
Time on dialysis (months), median (IQR)	35 (20–58)	36 (18–60)	0.937
Prior KT, n (%)	9 (8.7)	12 (11.7)	0.646
PRA class I (%), median (IQR)	0 (0–1.9)	0 (0–17)	0.360
PRA class II (%), median (IQR)	0 (0–0)	0 (0–0)	0.804
Preformed DSA, n(%)	3 (2.9)	16 (15.5)	0.003
HLA Mismatches, median (IQR)	3 (3–4)	3 (3–4)	0.299
Total CIT (hour), mean±SD	20.8±4.1	28.1±6.3	<0.001
Static CIT (hour), mean±SD	20.8±4.1	15.8±6.2	<0.001
Dynamic CIT (hour), mean±SD	n.a	12.3±5.7	n.a
VAT (min), mean±SD	36.0±12.3	36.6±9.5	0.690
rATG induction, n(%)	101 (98.1)	102 (99.0)	1.000

SC – static cold; MP – machine perfusion; BMI – body mass index; ESRD – end-stage renal disease; KT – kidney transplant; PKD – polycystic kidney disease; PRA – panel reactive antibodies; DSA – donor specific antibodies; HLA – human leukocyte antigen; CIT – cold ischemia time; VAT – vascular anastomosis time; rATG – rabbit antithymocyte globulin; na – not applicable; IQR – interquartile range; SD – standard deviation.

and dynamic CIT was 12.3±5.7 h in the MP group. Induction therapy with rabbit antithymocyte globulin was used in 98.5% of the patients, without any significant differences between the groups. More detailed information on recipient demographics and clinical characteristics is available in Table 2.

Machine perfusion hemodynamic parameters

As expected, a significant reduction in intra-renal resistance [0.44 (0.30–0.60) mmHg/mL/min reduced to 0.22 (0.18–0.28) mmHg/mL/min, *P*<0.001] and increase in flow [(52 (36–79) mL/min increased to 107 (88–129) mL/min, *P*<0.001] occurred from the beginning to the end of the machine perfusion period.

Outcomes

There was a significant reduction in DGF incidence in the MP group (55.3 *vs.* 29.1%, *P*<0.001). When we excluded patients who underwent a single dialysis session on the immediate postoperative day, motivated by hyperkalemia or hypervolemia, the incidence of DGF was 46.6% in the SC group and 25.2% in the MP group (*P* =0.002). There were no differences in duration of DGF,

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Table 3. Transplant outcomes at 1 year.

	SC group N=103	MP group N=103	P value
DGF, n(%)	57 (55.3)	30 (29.1)	<0.001
DGF excluding 1o dialysis session, n(%)	48 (46.6)	26 (25.2)	0.002
PNF, n(%)	2 (1.9)	1 (1.0)	1.000
Time on DGF (days), median (IQR)	7 (2–13.5)	6 (2–16.3)	0.964
Dialyses sessions, median (IQR)	3 (1–6)	2 (1–7)	0.630
Length of hospital stay (days), median (IQR)	14 (9–24)	13 (9–18)	0.204
BPAR, n (%)	2 (1.9)	5 (4.9)	0.445
Graft loss, n (%)	3 (2.9)	2 (1.9)	1.000
Late vascular thrombosis	1	0	
PNF	2	1	
Perigraft abscess	0	1	
Death, n (%)	4 (3.9)	2 (1.9)	0.683
Cardiovascular event	1	0	
Infection	3	2	
eGFR (mL/min/1.73 m²), mean±SD	67.7 ± 23.3	62.6±24	0.124

SC – static cold; MP – machine perfusion; DGF – delayed graft function; PNF – primary non-function: BPAR – biopsy-proven acute rejection; eGFR – estimated glomerular filtration rate; IQR – interquartile range; SD – standard deviation.

Table 4. Risk factors for DGF*.

	OR	95% CI	P value
Recipient BMI (Kg/m²)	1.115	1.033–1.204	0.001
Time on dialysis (months)	1.011	1.004–1.019	0.004
Donor age (years old)	1.035	1.006–1.065	0.019
Donor final creatinine (mg/dL)	2.910	1.666–5.085	<0.001
Machine perfusion	0.316	0.160–0.626	0.001

BMI – body mass index. * Multivariable analysis adjusted for the following variables: recipient age, gender, race, pretransplant diabetes, class I and II panel reactive antibodies, preformed donor specific antibodies, HLA mismatches; history of prior kidney transplant, donor history of hypertension, death cause, cold ischemia time, vascular anastomosis time.

as measured by the time until the last dialysis session [7 (2–13.5) days *vs.* 6 (2–16.3) days, *P*=0.964] or by the number of required dialysis sessions [3 (1–6) *vs.* 2 (1–7), *P*=0.630]. Only 2 patients in the SC group and 1 in the MP group had PNF (*P*=1.000). There were also no differences in the length of hospital stay, acute rejection incidence, graft loss, death, or renal function (Table 3).

Risk factors for DGF

Multivariable analysis demonstrated that MP was associated with DGF reduction (OR, 0.316; 95% CI, 0.160–0.626; *P*=0.001). Risk factors for DGF occurrence were recipient BMI, time on dialysis, donor age, and donor final creatinine (Table 4).

Discussion

This study demonstrated that pulsatile machine perfusion reduced DGF incidence in KT in which the donor kidneys had been previously maintained in SC storage. Interestingly, we observed a 47% reduction in DGF incidence, a markedly greater impact than those observed in previous studies [10,12,14].

The benefit of kidney reconditioning with hypothermic pulsatile perfusion after a period of SC storage was previously demonstrated in experimental models and clinical settings [20–23]. The strategy usually adopted in Brazil is to only place donor kidneys with high predicted risk of DGF on MP, following an

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initial period of SC storage. Besides the hemodynamic issues involved in this strategy, using different perfusion solutions may be another concern, since the impact of electrolytic and osmotic environment changes on tubular renal cells is unclear.

Our results are aligned with robust and consistent evidence showing that MP reduces DGF incidence, regardless of KT modality (kidneys from SCD or ECD; donation after brain death or circulatory/cardiac death) [11,24–27]. However, we observed no impact on other transplant outcomes, despite previous results showing an inhomogeneous impact on DGF duration, PNF incidence, acute rejection incidence, graft survival, patient survival, and long-term renal function [24,25,28]. Of note, available studies list heterogeneous donor and recipient characteristics, static and dynamic ischemia times, and immunosuppressive regimens as affecting long-term outcome, in addition to short-term follow-up results.

Despite its impact being limited to DGF incidence (and not DGF duration), MP may be cost effective in our scenario of high DGF incidence. Using data from the Brazilian multicenter trial [12], Tedesco-Silva et al. evaluated the cost-effectiveness of MP in the context of public health assistance. The authors concluded that MP is a cost-effective alternative to SC preservation, with an incremental cost-effectiveness ratio of USD $22 117, adjusted for quality-adjusted life years [29]. Cost-effectiveness studies in distinct Brazilian scenarios are required. In addition, considering the scarcity of resources, a major challenge is to determine those patients who will most benefit from this strategy.

Other potential uses of MP not explored in this study are: logistic benefits; allowing transplantation with longer CIT without increasing DGF [30]; and better evaluation and improvement of hemodynamic parameters of intra-renal vasculature, reducing discard rates [21,31].

The present study has some limitations inherent in any retrospective study design in a limited number of patients. On the other hand, this study complements the existing set of clinical studies, providing evidence of the benefits of MP in the scenario of high DGF incidence despite KT with ideal donors. In addition, this study was performed in a Brazilian real-life scenario, in which MP occurred after a long period of SC ischemia. In our study, kidneys in the SC group were perfused with Custodiol HTK, a solution associated with similar outcomes when compared with KPS-1 [15], which served to minimize the influence of perfusion solution on outcomes. Another strength of the study was that it was a paired-kidney analysis, reducing donor-related biases.

Conclusions

In conclusion, MP use after a long period of SC preservation was associated with reduced DGF incidence. This result supports the benefit of this strategy in countries where machine perfusion equipment is not available at the time of the retrieval surgery. Studies with larger sample sizes are needed to define which patient subgroups are most likely to benefit from this strategy.

Statement

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