ORIGINAL ARTICLE

Preliminary experience of sequential use of normothermic and hypothermic oxygenated perfusion for donation after circulatory death kidney with warm ischemia time over the conventional criteria - a retrospective and observational study

Matteo Ravaioli[1], Vanessa De Pace[1], Giorgia Comai[2], Irene Capelli[2], Olga Baraldi[2], Antonietta D'Errico[3], Valentina Rosa Bertuzzo[1], Massimo Del Gaudio[1], Chiara Zanfi[1], Giovanni Liviano D'Arcangelo[2], Vania Cuna[2], Antonio Siniscalchi[4], Gabriela Sangiorgi[5] & Gaetano La Manna[2]

1 Unit of General and Transplant Surgery, Department of Medical and Surgical Sciences, University of Bologna Sant'Orsola Malpighi Hospital, Bologna, Italy
2 Unit of Nephrology, Dialysis and Transplantation, Department of Experimental Diagnostic and Specialty Medicine, University of Bologna Sant'Orsola Malpighi Hospital, Bologna, Italy
3 Unit of Oncology and Transplant Pathology, Department of Experimental Diagnostic and Specialty Medicine, University of Bologna Sant'Orsola Malpighi Hospital, Bologna, Italy
4 Unit of Anesthesiology, Department of Medical and Surgical Sciences, University of Bologna Sant'Orsola Malpighi Hospital, Bologna, Italy
5 Emilia Romagna Transplant Reference Center, Department of Medical and Surgical Sciences, University of Bologna Sant'Orsola Malpighi Hospital, Bologna, Italy

Correspondence
Matteo Ravaioli, PhD Department of Medical and Surgical Sciences, University of Bologna, Sant'Orsola-Malpighi Hospital, Via Massarenti 9, 40138 – Bologna, Italy.
Tel.: 39.051.2144810
fax: 39.051.2143105
e-mails: mrava1@hotmail.com; matteo.ravaioli@aosp.bo.it

SUMMARY

Donation after circulatory death (DCD) is a potential source of reducing organ demand. In Italy, DCD requires a 20-min no-touch period that prolongs warm ischemia and increases delayed graft function (DGF) risk and graft loss. We report here our preliminary experience of sequential use of normothermic regional perfusion (NRP), as standard procedure, and hypothermic oxygenated perfusion (HOPE), as an experimental technique of organ preservation, in 10 kidney transplants (KT) from five DCD Maastricht III with extensive functional warm ischemia time (*f*WIT) up to 325 min. During NRP, renal function tests were evaluated to accept organs which were retrieved according to standard fashion with biopsy. While waiting for pathology and cross-match results, organs were preserved with HOPE through pressure- and temperature-controlled arterial pulsatile flow. All grafts with Karpinski score ≤4 were used for conventional single KT with mean cold ischemia time of 584 ± 167 min and mean *f*WIT of 151 ± 132 min. At the end of HOPE, lactate levels increased significantly in all cases with DGF ($P = 0.0095$), which were 3/10 (30%). No primary nonfunctions were recorded, and all patients had sCr < 1.5 mg/dl at 6-month post-KT. NRP and HOPE for DCD may overcome *f*WIT limits safely, and lactate during HOPE predicts DGF.

Transplant International 2018; 31: 1233–1244

Key words
delayed graft function, donation, donation after cardiac death, graft survival, ischemia reperfusion injury, kidney transplantation, organ preservation and procurement

Received: 28 January 2018; Revision requested: 9 March 2018; Accepted: 19 June 2018; Published online: 20 July 2018

doi:10.1111/tri.13311

Introduction

Despite the increase in kidney transplantation (KT) with living donor and extended criteria grafts, the organs available for transplantation are not enough for the candidates on the waiting list [1]. Donation after circulatory death (DCD) is a potential source of reducing the constant demand for organs. However, DCD grafts are subject to warm ischemic damage because of the time required for death determination. In Italy, death declaration for circulatory arrest (CA) can only be made after 20 min of absent electrocardiographic activity, known as the no-touch period, compared to the 5 min approved by other countries [2].

Donation after circulatory death transplants have higher rates of delayed graft function (DGF) [3,4] and graft loss [4] compared to transplantations from brain-dead donors. However, the balance among mortality risk during dialysis on the waiting list and mortality after KT with marginal grafts is in favor of the second option [5].

A crucial aspect to reduce the postoperative risks of DCD transplants is to mitigate the organ damage occurring during warm ischemia time (WIT), including functional warm ischemia time (*f*WIT).

Normothermic regional perfusion (NRP) was reported as a beneficial strategy to restore cellular metabolism and to evaluate organ function through the oxygenated blood flow of kidneys, liver and pancreas after death until organ retrieval [6–8].

A further strategy, with the same aim, was the application of normothermic [9–12] or hypothermic [13–17] machine perfusion *ex situ* to decrease the preservation injury (PI) for marginal kidneys.

Here, we present the findings of our preliminary experience on DCD kidney transplant with extended *f*WIT following a sequential use of NRP as standard procedure to select a potential DCD as eligible for kidney transplantation and hypothermic oxygenated perfusion (HOPE) as an experimental technique of organ preservation to improve ischemic and preservation injuries.

Patients and methods

Between January 2016 and February 2017, five DCD Maastricht IIIA category patients, defined as having controlled and potential donor status awaiting CA in intensive care unit [18], were allocated at the Bologna Transplant Center and evaluated as suitable for KT during NRP, retrieved and preserved by HOPE before transplantation. In this retrospective and observational study using data gathered prospectively, we report the clinical outcomes of the patients receiving DCD kidneys after the application of NRP and HOPE in compliance with the Declarations of Helsinki and Istanbul. The local medical ethics committee of the University of Bologna Sant'Orsola-Malpighi Hospital approved the study, and informed consent was signed by recipients before transplantation.

A decisional algorithm to evaluate the eligibility of the potential DCD and the subsequent treatments is reported in Fig. 1.

Normothermic regional perfusion

Following withdrawal of life-supporting treatment (WLST) and CA, electrocardiographic activity recording was started. Circulatory death was declared after 20 min of asystole. Upon donation approval by the family, NRP was performed by means of extracorporeal membrane oxygenator (ECMO). Following an incision in the right groin, dissection and cannulation of the femoral artery and vein by 18–26-Fr cannulae, a Fogarty balloon catheter was placed and inflated in the supraceliac aorta to separate the abdominal circulation before WLST.

Fogarty balloon placement was confirmed as being correct by transient balloon inflation and X-ray with radiologist support. After priming the pump with 500 ml of Ringer's lactate solution, the arterial and venous cannulae were connected after the no-touch period to the ECMO system (Maquet Rotaflow Centrifugal Pumps with Quadrox-D oxygenators, Maquet, Rastatt, Germany). ECMO pump flow was maintained >1.7 l/min, and pH 7.35–7.45 by continuous adjustments with 1 mol/l bicarbonate. Intravenous heparin was administered immediately after CA (50–100 units/kg) and during NRP (4 units/kg/h without bleeding).

According to Italian Transplant Center protocol, antemortem and postmortem interventions were performed as approved by the National Bioethics Committee.

Hemodynamic parameters of DCD were monitored: pump flow and mixed airflow were modified to obtain central venous oxygen saturation of 60–75%; the oxygen level was set to maintain the arterial oxygen pressure at more than 150 mmHg and arterial oxygen saturation not <95%.

Blood pressure was maintained at an MAP target level of 75–90 mmHg.

During perfusion *in situ*, the ultrafiltrate volume was observed and blood samples were collected every 30 min to measure ALT, AST, bilirubin, creatinine and urea levels.

Transplant International 2018; 31: 1233–1244

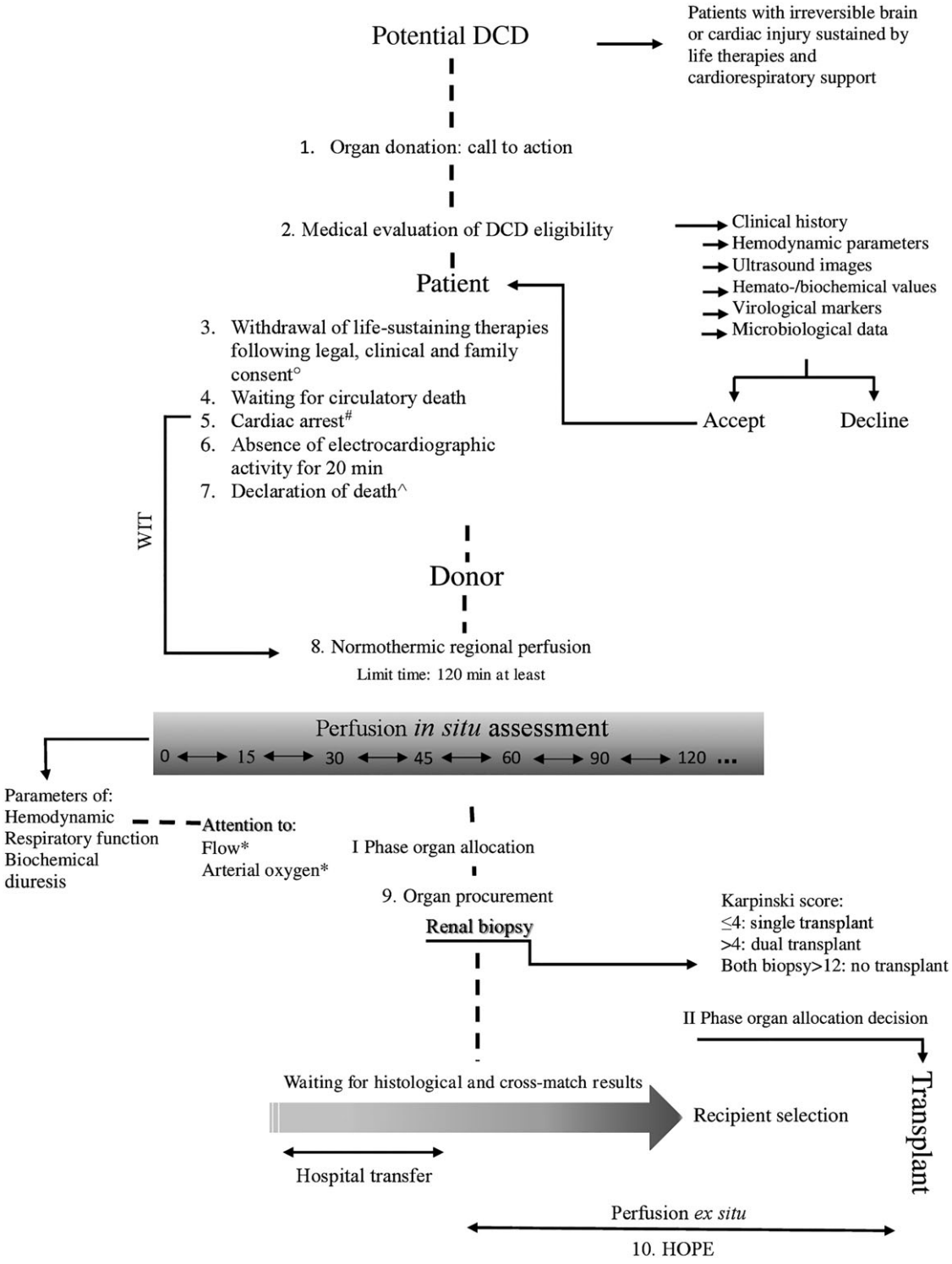

Figure 1 Evaluation algorithm of eligibility of the potential DCD and the timeline plan of organ preservation. According to Italian Transplant Center protocol antemortem and postmortem interventions were performed. °Before withdrawal of life-supporting treatment, an incision is performed in the right groin, followed by dissection and cannulation of the femoral artery and vein, and placement and inflation of the Fogarty balloon catheter in the supraceliac aorta to separate the abdominal circulation. #Immediately after the cardiac arrest, intravenous heparin administration is allowed except in bleeding cases. ^Organ preservation procedures, including external cardiac massage, may be performed only following cardiac death determination. *Limit to donation: If recorded values are absent or too low, this will stop NRP and the donation will be declared as concluded with failure. Warm ischemia time (WIT) is the time without the circulation of oxygenated blood from circulatory arrest (CA) to normothermic regional perfusion (NRP). Abbreviations: donor or donation after circulatory death (DCD); hypothermic oxygenated perfusion (HOPE).

Quality assessment and organ procurement

Following the first step of organ selection during NRP, organs were retrieved in standard fashion [19]. Livers were retrieved and transplanted except donor 1 liver which was discarded; instead, the lungs were not retrieved because they were excluded after a preliminary assessment because of donor age (donor 3) or organ disease (donors 1, 2, 4 and 5) before the start of the NRP. Following organ retrieval, a renal biopsy was performed for histological evaluation. Renal tissue was collected by means of a punch, obtaining a small cylindrical core of capsule and renal cortex. Following formalin fixation and appropriate staining (hematoxylin and eosin, periodic acid–Schiff, Masson's trichrome and periodic acid–methenamine silver), pathological analysis was performed. Kidney pathological state was classified according to Karpinski's score [20], and organ allocation was based on the Remuzzi's criteria [21] (Karpinski score 0–3: single transplant; 4–6: double transplant; 6–7: organ discard). However, according to our organ allocation system, kidneys with score 4 were used for single transplant if the donor was <75 years of age and had normal sCr level.

Organs were preserved by static cold storage (SCS) using Celsior as organ solution during hospital transfer and back-table procedure.

Hypothermic oxygenated perfusion

Sequentially, *ex situ* organ perfusion was performed in the operating room by Kidney Assist (Organ Assist, Groningen, The Netherlands). A pump unit produced a pulsatile flow to the renal artery with Celsior solution, oxygenation [oxygen dioxide partial pressure (pO_2) = 600–750 mmHg], temperature (4 °C) and arterial pressure (25 mmHg) auto-regulated and monitored on the display of the device together with the resistance values. A membrane oxygenator, included in the sterile disposable set together with the sterile perfusion reservoir and tubing, provided oxygen, as well as removal of CO_2. Carbon dioxide partial pressure (pCO_2), pO_2, pH and lactate were measured during HOPE by means of gas analysis of the effluent fluid perfusion every 15 min.

To assay bacterial or fungal contamination related to HOPE, perfusion fluid was collected before (T0) and after (T1) organ perfusion.

Recipient selection process

Adult patients, aged over 18 years, were selected with informed consent during the DCD NRP procedure and during HOPE when cross-match was completed. Recipient data are detailed in Table 1.

Transplantation, outcome and follow-up

Kidney transplants were performed by the same surgeon according to the standard procedure, implanting the grafts into the iliac fossa. Ureter–bladder anastomoses were constructed over a single stent and artery and vein anastomoses on the external iliac vessels with similar vascular anastomoses times (mean = 30 ± 10 min) among enrolled recipients without any effect on the post-transplant outcome.

Postoperative management was conducted following the standard protocol [22]. Immunosuppression therapy was based on the conventional scheme: 2 Thymoglobuline dose, steroid dose decreasing, tacrolimus and mycophenolate mofetil.

Delayed graft function, slow graft function (SGF) and immediate graft function (IGF) were assessed as outcome parameters and according to the literature definition [23].

Recipient follow-up was performed regularly and reported up to 6 months.

Definition of DCD variables

Definitions and terminology regarding DCD adopted in the study are reported in detail [18]:

1 CA corresponds to the absence of the cardiac activity that leads to the cessation of blood circulation.

2 Donor or DCD is organ donation after CA.

3 WIT is the time without the circulation of oxygenated blood from CA to the start of organ preservation (NRP).

4 *f*WIT is defined as the interval from when systolic blood pressure is <50 mmHg or oxygen saturation is <70% for at least 2 min after WLST until the start of organ preservation (NRP).

5 Agonal phase is the time from WLST to asystole (patient death).

6 Total WIT is the interval from WLST to the start of organ preservation (NRP).

Statistical analyses

Data are reported as mean ± SD or median and ranges. Differences between continuous variables of outcome were determined using the unpaired *T* test. *P* values <0.05 were considered statistically significant. SPSS, version 20.0 (IBM, Armonk, New York, USA), and

Transplant International 2018; 31: 1233–1244

Table 1. Recipient characteristics.

Recipient	Age at KT	Sex	BMI	BG	Etiology	Dialysis	Age_Dialysis	Creatinine	Previous KT
1	58	Male	25.5	B−	IgA nephropathy (Berger's disease)	HD	25.2	8.2	No
2	62	Male	25.5	B−	Polycystic kidney disease	PD	28.4	12.53	No
3	50	Female	25.3	A+	Nephropathy HIV-related	HD	91.1	4.32	No
4	69	Male	18.4	A+	Chronic renal insufficiency (uncertain etiology)	HD	21.7	4.42	Yes
5	59	Male	27.2	0+	Chronic glomerulonephritis	HD	56.4	13.02	No
6	70	Male	25.2	0+	Chronic glomerulosclerosis	HD	98.6	6.2	No
7	52	Male	26.9	0+	Chronic glomerulonephritis	PD	39.3	7.71	Yes
8	54	Male	30.8	0+	Polycystic kidney disease	HD	60.5	9.73	No
9	53	Male	28	A+	Diabetic nephropathy	HD	27.6	7.21	No
10	70	Male	27	A+	Chronic glomerulonephritis	HD	12.6	5.54	No

Age at dialysis is reported as months.

KT, kidney transplantation; BMI, body mass index; BG, blood group; HD, hemodialysis; PD, peritoneal dialysis; Cr, creatinine (mg/dl).

GraphPad Prism 5.0 software (La Jolla, San Diego, California, USA) were used for statistical analysis.

Results

Donor evaluation during perfusion *in situ*

Donation after circulatory death information before CA is reported in Table 2. During NRP, ultrafiltrate volume did not change and sCr values were normal overall, except donor 1 with a final sCr value of 2.11 mg/dl. Histological analyses reported a Karpinski score between 0 and 4, and all grafts were declared suitable for single transplantation.

No circulatory failure was observed during NRP performed with median perfusion flow rate of 2 l/min (1.7–4 l/min) at 37 °C. NRP was continued for at least 2 h to evaluate the function of abdominal organs overall, and it was prolonged for up to 5 h at most according to the time required for organ retrieval coordination (mean of NRP time = 207.2 ± 70.4 min).

Hemodynamic parameters were in the ranges of the DCD protocol.

The lactate levels were low for donors 3, 4 and 5 and constantly high for donors 1 and 2 following the different times of *f*WIT and WIT (Table 2). *f*WIT was 300 min and more for donors 1 and 2, respectively, while it was 51 min for donors 3, 4 and 5. Mean cold ischemia time (CIT) was 10 ± 3 h (Table 2).

Graft evaluation during perfusion *ex situ*

Flow and resistance values showed good perfusion parameters for all grafts according to the previous reports [8,9] (Table 3 and Fig. 2 panel a). Total mean flow was 61.4 ± 28.2 ml/min, and total mean resistance was 0.49 ± 0.23 Ru. Oxygenation was kept to the standard recommendations during the treatments as shown by the high values of pO2 at T1 (Table 3). pCO_2 was <6 mmHg constantly, before and after *ex situ* kidney perfusion, because an oxygenator that removes CO_2 was used. Lactate value increased at T1 versus T0 only for grafts 1, 4, 8 and 9 by 50%, 89%, 60% and 54%, respectively (Table 3), with statistical significance.

Perfusion time varied with a mean of 240 ± 146 min (Table 3) according to the length of time on dialysis required for all patients prior to renal transplantation.

No bacterial or fungal growth was detected by perfusate cultures defined as pathogen contamination.

Table 2. Donor characteristics in the organ selection process

Donor features											Normothermic regional perfusion					
Donor	Kidney	Age	Sex	BMI	BG	Cause of death	K score	Cr before CA	fWIT	WIT	Time	Lat T0	Lat T1	Cr T0	Cr T1	CIT (min)
1	1	22	Male	23.15	0+	Traumatic brain injury	4	1.12	300′	50′	310	78.37	172.07	1.12	2.11	460
	2						4									760
2	3	47	Male	16.25	0+	Myocardial infarction	3	0.99	325′	35′	255	90.09	>180	0.7	0.82	420
	4						3									720
3	5	33	Female	24.22	0+	Traumatic brain injury	0	0.3	40′	25′	210	71.17	34.23	0.6	0.5	500
	6						0									900
4	7	63	Male	21.6	0+	Traumatic brain injury	0	1.05	40′	22′	121	73.33	35.04	0.82	0.94	430
	8						0									660
5	9	59	Female	24.22	A+	Traumatic brain injury	4	0.47	51′	38′	140	61.98	53.06	0.6	0.75	630
	10						4									360

BMI, body mass index; BG, blood group; K score, Karpinski score; Lat, lactate (mg/dl); Cr before CA, serum creatinine values before circulatory arrest (mg/dl); *f*WIT, functional warm ischemia time; NRP, normothermic regional perfusion; WIT, warm ischemia time; CIT, cold ischemia time; T0, before NRP; T1, after NRP. *f*WIT, WIT and CIT are expressed as minutes.

Table 3. Perfusion and biochemical data before (T0) and after (T1) HOPE

Kidney	Flow	Pressure	Resistance	Temperature, °C	Time	pH T0	pCO_2 T0	pO_2 T0	Lat T0	pH T1	pCO_2 T1	pO_2 T1	Lat T1
1	25	25	0.88	3	165	6.99	<5	664	<2	6.84	7	746	11
2	36.5	25	0.7	3	220	6.96	<5	700	3	6.96	<5	690	5
3	108	24	0.22	4	120	6.98	<5	639	4	6.98	<5	396	2
4	38	25	0.63	3	277	6.97	<5	660	2	6.87	5	447	18
5	90	25	0.26	4	160	7.28	<6	747	<2	7.18	<6	672	7.2
6	31	26	0.81	3	420	7.32	<6	732	<2	7.32	<6	630	2.7
7	80	25	0.3	4	150	7.34	<6	721	<2	7.24	<6	720	7.2
8	42	25	0.57	3	590	7.34	<6	629	<2	7.21	<6	613	12.6
9	79	25	0.31	4	205	7.29	<6	730	<2	7.19	<6	740	11.7
10	85	25	0.29	4	90	7.22	<6	711	<2	7.02	<6	685	6.3

Flow (ml/min), pressure (mmHg) and resistance (Ru) data are expressed as median, and the time is expressed as minutes.

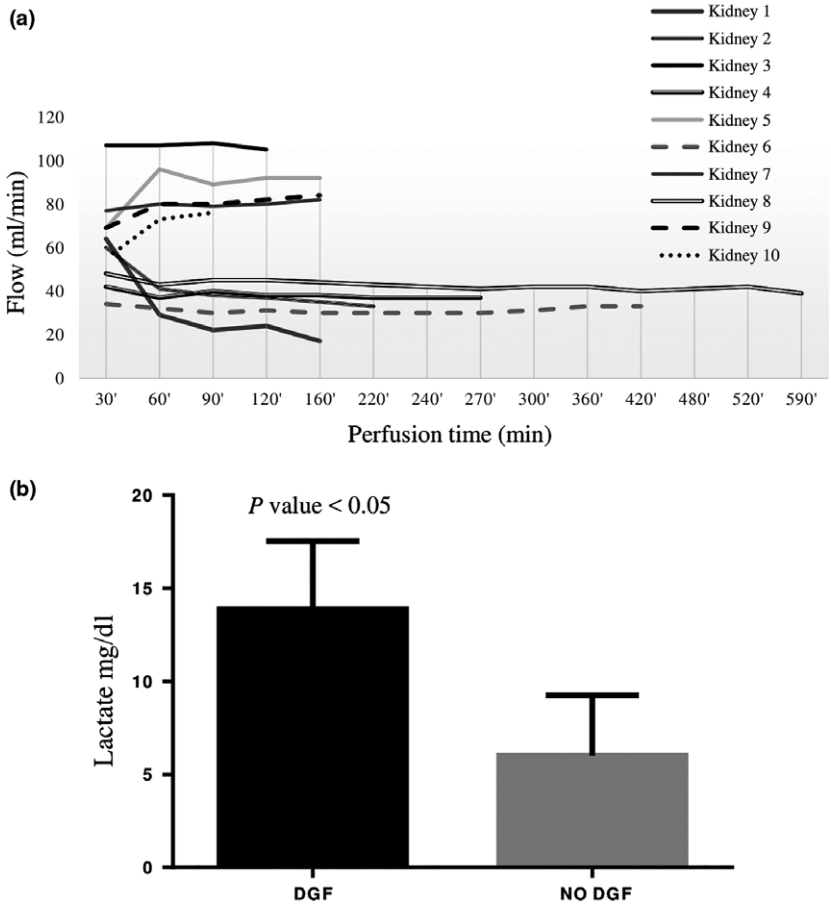

Figure 2 Panel a – Trend of flow (ml/min) during hypothermic oxygenated perfusion (HOPE). Panel b - Lactate value at the end of HOPE. Its statistical correlation with significance to the delayed graft function (unpaired *T* test, $P = 0.0095$). Delayed graft function (DGF).

Transplant outcome and its correlations with organ perfusion variables

Data on clinical outcome of DCD kidney transplants are reported in Table 4. No cases of primary nonfunction (PNF) were reported for any of the included study cases.

Delayed graft function developed in three of 10 cases (30%), and renal replacement therapy for these recipients was performed for 2–9 days.

SGF was recorded for three patients (30%) and IGF for four patients (40%). At discharge, recipients had low sCr levels (mean = 2.18 ± 0.74 mg/dl) (Fig. 3), eGFR mean of 43.8 ± 13.6 and normal diuresis (mean = 2137 ± 495 ml/day). Median follow-up was 449.5 (201–627) days.

Delayed graft function had no statistical correlation with demographic or clinical features of recipients and donors, such as age, BMI, sCr, *f*WIT, WIT and the lactate values during NRP (Table 5). Indeed, donors 1 and 2 with prolonged *f*WIT and increased lactate levels in NRP showed DGF in one graft, but not in the other one from the same donor. In addition, flow and resistance during *ex situ* perfusion and CIT did not show any relation to the DGF (Table 5).

The only correlation to the DGF was the increased lactate level at T1 compared to T0 during HOPE as reported in Fig. 2 panel b (P value = 0.0095).

Other postoperative complications were as follows: urinary tract infections in two of 10 cases and anemia in one of 10 cases treated with medical therapy and classified as grade II of the Clavien–Dindo system.

Mean length hospital stay was 16 ± 4 days, and sCr levels of all recipients at 6-month post-transplant were below 1.5 mg/dl. Data in detail are reported in Table 4.

Discussion

In Italy, the no-touch period of 20 min limits the use of DCD, because of the organ damage occurring as a result of the extension of *f*WIT.

Strategies to reduce the ischemic injury and to evaluate the functional state of organs are therefore advocated. Several studies limited *f*WIT to 60–120 min in the KT and 5 min of no-touch period to avoid irreversible graft damage [24–26]. A recent consensus about

Table 4. Clinical outcome of donation after circulatory death kidney transplants

Recipient	PNF	DGF	Time of dialysis	Cr at discharge	Length of hospital stay	Cr at 1-month KT	Cr at 3-month KT	Cr at 6-month KT
1	No	Yes	2	1.96	16	1.3	1.2	1.15
2	No	No	0	2.08	18	1.6	1.8	1.36
3	No	No	0	1.6	14	1.38	1.13	1.21
4	No	Yes	9	2.8	22	2.24	1.6	1.13
5	No	No	0	1.51	21	1.46	1.56	1.33
6	No	No	0	1.26	21	1.36	1.31	0.87
7	No	No	0	2.1	11	1.91	1.74	1.43
8	No	Yes	2	3.73	14	1.73	1.61	1.35
9	No	No	0	2.85	13	1.9	1.54	1.07
10	No	No	0	1.52	12	2.21	1.8	1.11

PNF, primary nonfunction; DGF, delayed graft function; Cr 1-, 3- and 6-month post-KT, creatinine (mg/dl) 1, 3 and 6 months after kidney transplant. Time of dialysis and length of hospital stay are expressed as days.

uncontrolled DCD process has reported a safe limit of total WIT between 120 and 150 min so as not to affect transplant outcomes negatively [18].

On the other hand, new technologies are being developed to decrease the PI of grafts, such as normothermic perfusion and hypothermic machine perfusion [9–17].

Our center reported a preliminary experience of controlled DCD renal transplants with favorable outcomes above the conventional limits reported in the literature [27].

Among reported study cases, four of 10 DCD organs were subjected to very long *f*WIT with higher values of 300 and 325 min for kidneys 1 and 2, 3 and 4, respectively (Table 2). Despite these unfavorable conditions, we did not experience PNF, and DGF was recorded only for kidneys 1 and 4. However, all grafts were functioning at the time of hospital discharge and at 6 months of follow-up post-KT.

Similarly, Reid *et al.* [28] suggested an extension of *f*WIT limit up to 4 h with good transplant outcome. Our data extended the limit up to 5 h of *f*WIT with the sequential use of NRP and HOPE. To the best of our knowledge, we did not find any successful kidney transplantation with DCD and *f*WIT of 5 h and WIT of 50 min.

Several clinical studies reported the impact of WIT on the outcome of renal transplant; this procedure damages the proximal tubular cells [29] and induces acute tubular necrosis which leads to delayed or primary nonfunction [30]. According to the Eurotransplant cohort study, in the 5 years of post-transplant follow-up, the longer the WIT is prolonged and the more the graft failure increases. Nevertheless, these results were obtained without reporting the use of organ preservation techniques for DCD [31].

Our preliminary experience based on the strategy of sequential NRP and HOPE seems to overcome the problems related to the WIT, and other interesting information comes from the variables measured during these procedures.

Grafts with DGF failed to recover aerobic metabolism during HOPE, and they showed adenosine triphosphate (ATP) catabolism with consequent lactate release.

Lactate levels and their changes during NRP were well related to the WIT: donors with longer *f*WIT showed an increased lactate level during perfusion (Table 2), and in the first case sCr also increased to 2.11 mg/dl. On the other hand, the lactate value evaluated during NRP was not related to PNF and DGF, so it may not be considered a contraindication to the use of the graft in our preliminary experience [32].

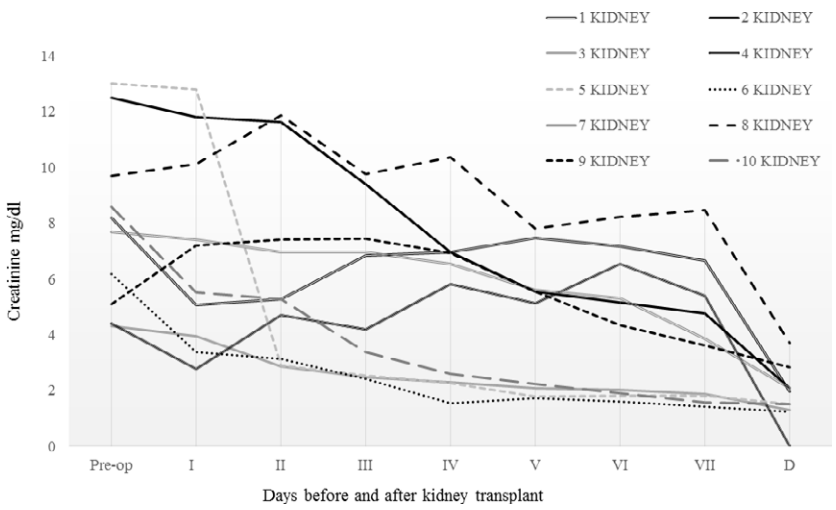

Figure 3 Renal outcome assessment by the values of serum creatinine in the post-transplantation period. Changes in the serum creatinine (mg/dl) post-transplant.

Table 5. Analysis of delayed graft function predictors

	Delayed graft function (DGF)	NO_DGF	*P* value
Recipient			
Age	60.33 ± 6.34	59.42 ± 7.70	0.8768
BMI	24.9 ± 5.08	26.48 ± 1.03	0.5006
Creatinine	7.41 ± 2.27	8.21 ± 3.18	0.7347
Donor			
Age	44 ± 16.87	45.14 ± 14.81	0.9260
BMI	20.33 ± 2.95	22.55 ± 2.72	0.3337
Creatinine	1.29 ± 0.58	0.91 ± 0.51	0.3834
*f*WIT	221.66 ± 128.86	121 ± 121.38	0.3222
WIT	35.66 ± 11.44	33.28 ± 9.19	0.3282
Lactate_ECMO	129.02 ± 66.56	80.24 ± 61.10	0.3435
Organ—HOPE			
Flow	35 ± 7.25	72.78 ± 26.29	0.0598
Time	344 ± 179.85	195.71 ± 100.15	0.1756
Lactate	13.86 ± 2.99	6 ± 3	0.0095*
CIT	613.33 ± 111.15	571.42 ± 185.04	0.7524

Creatinine (mg/dl); WIT, *f* WIT, time HOPE, CIT (minutes); flow (ml/min); lactate_ECMO and lactate of HOPE (mg/dl). Results are reported as mean ± standard deviation, and statistical significance ($P < 0.05$)* is calculated with *T* test.

At the same time, sCr level and anuria during NRP, considering that all our donors had anuria, may not be considered absolute criteria for discarding the kidneys, as instead suggested by other authors [33].

Lactates during HOPE, as the only parameter showing correlation to the DGF, probably indicate a specific ischemic injury of the graft. At the same time, the lactate increase during HOPE may not be considered a contraindication for transplantation, because these grafts had no PNF and they were functioning properly at patient discharge and at 6-month post-KT.

From our results, it may be assumed that the lactate values have different meanings between NRP and HOPE. Lactate production during normothermic regional perfusion is because of the abdominal organs, while during hypothermic oxygenated perfusion it is because of specific organ justifying the use of HOPE after NRP, although some authors perform only NRP in renal DCD transplant [8]. If the lactate significance is confirmed in clinical trials, HOPE might be a powerful procedure for evaluating organ function as well as for improving organ preservation with increase in ATP production [34].

Lactic acid is a simple trackable parameter, and it may be a metabolic injury marker for establishing an endpoint of graft quality during HOPE. Transplant outcome may be predicted by monitoring lactate levels during organ perfusion *ex situ*.

Results and suggestions derived from our preliminary experience in DCD are limited by the small number of cases involved. Furthermore, we had no PNF or other severe adverse events, which are required to establish exclusion criteria.

Therefore, we may only have theoretical organ acceptance criteria to be applied in DCD and our good results reported with *f*WIT time suggest it may be possible to further extend other criteria.

Basically, we had young DCD, the NRP was functioning properly, the creatinine level during ECMO remained stable in most cases, and the kidney biopsy did not show any severe tubular necrosis or other unfavorable histological features.

The lactic acid during ECMO was also influenced by liver perfusion, and we therefore believe lactate assessment during HOPE to be more appropriate for kidney evaluation, but even if this parameter was related to DGF we had no experience of PNF, so it may be not considered an absolute criterion for discarding the graft.

Our experience may thus help to overcome the standard limit of *f*WIT, but we do not know where to stop. We hope to prove the insights into the predictive value of lactic acid with the results of the HOPE clinical trial on human marginal kidney transplant (ClinicalTrials.gov ID: NCT03031067).

An additional study limitation was the unavailability of a case–control matching analysis. In the past, before these clinical cases, DCD were not considered for transplant in our center because of high risk of warm ischemia damage. Therefore, we have no previous data on the outcome of KT from DCD without organ perfusion.

Interesting results are contemplated by other international groups on kidney transplants from different types of DCD and with different organ recovery techniques or even without them.

Unlike other authors [13–15], the UK experience of Watson and colleagues on the use of cold machine perfusion versus static cold storage in the preservation of DCD renal grafts showed no difference in the incidence of DGF between these treatments [35]. However, these results referred to a mean WIT of 15 (4–35) minutes that is lower than the WIT mean of our study cases which was 29 (13–50) minutes. The Spanish experience of Minambres *et al.* [8], with just application of NRP in DCD without *ex situ* machine perfusion, reported a DGF of 27% and a graft survival of 91% at 1-year post-KT, but *f*WIT was <30 min. From a large transplant center at Michigan University, controlled DCD abdominal organ transplantation was performed with extracorporeal support at normothermia. In addition, cold machine perfusion was applied to renal grafts before transplantation. DGF incidence for 29 kidney transplants was 31%, and 1 graft had PNF, reporting fWIT values <90 min [36]. Another USA experience from Farney *et al.* [37] reported a substantial reduction in DGF rate from 55% to 21% for renal grafts by controlled DCD with fWIT of 24 ± 15 when extracorporeal interval support for organ recovery was used. However, our study with the application of HOPE as well as NRP had a DGF rate of 30% (3/10), but WIT rose to 50 min and fWIT exceeded 300 min in four cases.

Focusing our attention on organ perfusion strategies *ex situ* showed that HOPE is one of many that have been explored [38]. Furthermore, in renal preservation SCS was compared to hypothermic machine perfusion [39] and normothermic machine perfusion (NMP) [40]. Recently, other organ preservation strategies have been developed, such as sub-normothermic dynamic preservation (20–25 °C) and controlled oxygenated rewarming. Each scientific group contributes to improve (abdominal) organ preservation, but no data from published clinical studies confirm one preservation strategy as better than others. However, dynamic perfusion with oxygenation has been shown to be a potential preservation method for retrieving vulnerable grafts [38,40]. In the clinical field, only normothermic machine perfusion was applied to marginal donor kidney transplants [10] and it is superior in the evaluation of organ metabolism, but some considerations are noteworthy. First, NMP triggers the reperfusion injury and its extent depends on the length of the initial donor WIT [38]; in contrast, HOPE prevents oxidative stress through the activation of mitochondrial repair mechanisms [41]. Second, an accidental event of pump failure can be very harmful in a normothermic system, while HOPE preserves the graft in the SCS even if a machine failure occurs [40]. Third, ATP production of HOPE is very easy to obtain at basal levels of metabolism in contrast to NMP that increases ATP required at high metabolic activity overall for DCD.

Therefore, *ex vivo* organ perfusion with HOPE might be a promising preservation strategy in the field of transplantation [38].

In conclusion, our preliminary experience suggests the clinical feasibility of the sequential use of NRP and HOPE for DCD with prolonged *f*WIT and a role for lactate as the metabolic marker to predict DGF during HOPE.

Authorship

MR: designed the study, performed kidney transplant, interpreted the data, drafted the article and approved the final version to be published as the author accountable for all aspects of the work. VDeP: performed organ perfusion, collected and analyzed the data, drafted the article and approved the final version to be published. GC: involved in recipient selection in the pretransplant phase, clinical activity in the post-transplant outcome and revision of the article. IC: involved in clinical activity in the post-transplant outcome and revised the article. OB: involved in clinical activity in the post-transplant outcome and revised the article. AD: performed histological evaluation in the pretransplant phase and revised the article. VB: involved in DCD management, transplant activity and revision of the article. MDelG: involved in transplant activity and revised the article. CZ: involved in transplant activity and revised the article. GLD: involved in clinical activity in the post-transplant outcome and revised the article. VC: involved in clinical activity in the post-transplant outcome and revised the article. AS: involved in DCD management, clinical activity in the post-transplant outcome and revision of the article. GS: involved in DCD management and revised the article. GLM: involved in recipient selection in the pretransplant phase, clinical activity in the post-transplant outcome and revision of the article.

Funding

The authors have declared no funding.

Conflicts of interest

The authors have declared no conflicts of interest.

Acknowledgements

For their important scientific contribution, the authors thank: Matteo Cescon, Professor, and Maria Cristina Morelli, MD. Furthermore, we are grateful to Elisa Lodi and Marcella Gambino for their machine perfusion support (Kidney Assist of Organ Assist, Groningen, The Netherlands—distributed by Avionord).

REFERENCES

1. Sistema Informativo Trapianti (SIT), https://trapianti.sanita.it.
2. Geraci PM, Sepe V. Non-heart-beating organ donation in Italy. *Minerva Anestesiol* 2011; **77**: 613.
3. Bellingham JM, Santhanakrishnan C, Neidlinger N, *et al.* Donation after cardiac death: a 29-year experience. *Surgery* 2011; **150**: 692.
4. Lim WH, McDonald SP, Russ GR, *et al.* Association between delayed graft function and graft loss in donation after cardiac death kidney transplants – a paired kidney registry analysis. *Transplantation* 2016; **101**: 1139.
5. Głyda M, Włodarczyk Z, Czapiewski W. Results of renal transplantation from expanded criteria deceased donors – a single-center experience. *Ann Transplant* 2012; **17**: 35.
6. Fondevilla C. Is extracorporeal support becoming the new standard for the preservation of DCD grafts? *Am J Transplant* 2010; **10**: 1341.
7. Oniscu GC, Randle LV, Muiesan P, *et al. In situ* normothermic regional perfusion for controlled donation after circulatory death—the United Kingdom experience. *Am J Transplant* 2014; **14**: 2846.
8. Miñambres E, Suberviola B, Dominguez-Gil B, *et al.* Improving the outcomes of organs obtained from controlled donation after circulatory death donors using abdominal normothermic regional perfusion. *Am J Transplant* 2017; **17**: 2165.
9. Hosgood SA, Saeb-Parsy K, Hamed MO, Nicholson ML. Successful transplantation of human kidneys deemed untransplantable but resuscitated by *ex vivo* normothermic machine perfusion. *Am J Transplant* 2016; **16**: 3282.
10. Nicholson ML, Hosgood SA. Renal transplantation after *ex vivo* normothermic perfusion: the first clinical study. *Am J Transplant* 2013; **13**: 1246.
11. Hosgood SA, Nicholson ML. Normothermic kidney preservation. *Curr Opin Organ Tranplant* 2011; **16**: 169.
12. Bagul A, Hosgood SA, Kaushik M, Kay MD, Waller HL, Nicholson ML. Experimental renal preservation by normothermic resuscitation perfusion with autologous blood. *Br J Surg* 2008; **95**: 111.
13. Jochmans I, O'Callaghan JM, Pirenne J, Ploeg RJ. Hypothermic machine perfusion of kidneys retrieved from standard and high-risk donors. *Transpl Int* 2015; **28**: 665.
14. Moers C, Pirenne J, Paul A, Ploeg RJ. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* 2012; **366**: 770.
15. Zhong Z, Lan J, Ye S, *et al.* Outcome improvement for hypothermic machine perfusion versus cold storage for kidneys from cardiac death donors. *Artif Organs* 2017; **41**: 647.
16. Wight JP, Chilcott JB, Holmes MW, Brewer N. Pulsatile machine perfusion vs. cold storage of kidneys for transplantation: a rapid and systematic review. *Clin Transpl* 2003; **17**: 293.
17. De Deken J, Kocabayoglu P, Moers C. Hypothermic machine perfusion in kidney transplantation. *Curr Opin Organ Transplant* 2016; **21**: 294.
18. Thuong M, Ruiz A, Evrard P, *et al.* New classification of donation after circulatory death donors definitions and terminology. *Transpl Int* 2016; **29**: 749.
19. Ravaioli M, Grazi GL, Cescon M, *et al.* Liver transplantations with donors aged 60 years and above: the low liver damage strategy. *Transpl Int* 2009; **22**: 423.
20. Karpinski J, Lajoie G, Cattran D, *et al.* Outcome of kidney transplantation from high-risk donors is determined by both

structure and function. *Transplantation* 1999; **67**: 1162.
21. Remuzzi G, Grinyò J, Ruggenenti P, *et al.* Early experience with dual kidney transplantation in adults using expanded donor criteria. Double Kidney Transplant Group (DKG). *J Am Soc Nephrol* 1999; **10**: 2591.
22. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9**(Suppl 3): S1.
23. Humar A, Ramcharan T, Kandaswamy R, Gillingham K, Payne WD, Matas AJ. Risk factors for slow graft function after kidney transplants: a multivariate analysis. *Clin Transplant* 2002; **16**: 425.
24. Morrissey PE, Monaco AP. Donation after circulatory death: current practices, ongoing challenges, and potential improvements. *Transplantation* 2014; **97**: 258.
25. Hosgood SA, Shah K, Patel M, Nicholson ML. The effect of prolonged of warm ischaemic injury on renal function in an experimental *ex vivo* normothermic perfusion system. *J Transl Med* 2015; **13**: 207.
26. Portolés-Pérez J, Rubio-Muñoz JJ, Lafuente-Covarrubias O, *et al.* Development of a program for kidney transplants using organs donated from donors awaiting cardiac arrest (type III Maastricht). *Nefrologia* 2012; **32**: 754.
27. Alvarez J, del Barrio R, Arias J, *et al.* Non-heart-beating donors: estimated actual potential. *Transplant Proc* 2001; **33**: 1101.
28. Reid AW, Harper S, Jackson CH, *et al.* Expansion of the kidney donor pool by using cardiac death donors with prolonged time to cardiorespiratory arrest. *Am J Transplant* 2011; **11**: 995.
29. Yin M, Currin RT, Peng XX, Mekeel HE, Schoonhoven R, Lemasters JJ. Different patterns of renal cell killing after warm and cold ischemia. *Ren Fail* 2002; **24**: 147.
30. Wong G, Teixeira-Pinto A, Chapman JR, *et al.* The impact of total ischemic time, donor age and the pathway of donor death on graft outcomes after deceased donor kidney transplantation. *Transplantation* 2017; **101**: 1152.
31. Heylen L, Jochmans I, Samuel U, *et al.* The duration of asystolic ischemia determines the risk of graft failure after circulatory-dead donor kidney transplantation: a Eurotransplant cohort study. *Am J Transplant* 2018; **18**: 881.
32. De Carlis R, Di Sandro S, Lauterio A, *et al.* Successful donation after cardiac death liver transplants with prolonged warm ischemia time using normothermic regional perfusion. *Liver Transpl* 2017; **23**: 166.
33. Fan X, Chen Z, Nasralla D, *et al.* The organ preservation and enhancement of donation success ratio effect of extracorporeal membrane oxygenation in circulatory unstable brain death donor. *Clin Transplant* 2016; **30**: 1306.
34. Ravaioli M, Baldassare M, Vasuri F, *et al.* Strategies to restore ATP level after more than 20 hours of cold ischemia time in human marginal kidney grafts. *Ann Transpl* 2018; **23**: 34.
35. Watson CJ, Wells AC, Roberts RJ, *et al.* Cold machine perfusion versus static cold storage of kidneys donated after cardiac death: a UK multicenter randomized controlled trial. *Am J Transplant* 2010; **10**: 1991.
36. Rojas-Peña A, Sall LE, Gravel MT, *et al.* Donation after circulatory determination of death: the University of Michigan experience with extracorporeal support. *Transplantation* 2014; **98**: 328.
37. Farney AC, Hines MH, al-Geizawi S, Rogers J, Stratta RJ. Lessons learned from a single center's experience with 134 donation after cardiac death donor kidney transplants. *J Am Coll Surg* 2011; **212**: 440.
38. Kron P, Schlegel A, de Rougemont O, Oberkofler CE, Clavien PA, Dutkowski P. Short, cool, and well oxygenated – hope for kidney transplantation in a rodent model. *Ann Surg* 2016; **264**: 815.
39. Jochmans I, Moers C, Smits JM, *et al.* Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death: a multicenter, randomized, controlled trial. *Ann Surg* 2010; **252**: 756.
40. Bral M, Gala-Lopez B, Bigam D, *et al.* Preliminary single centre Canadian experience of human normothermic *ex vivo* liver perfusion: results of a clinical trial. *Am J Transplant* 2017; **17**: 1071.
41. Schlegel A, de Rougemont O, Graf R, Clavien PA, Dutkowski P. Protective mechanisms of end-ischemic cold machine perfusion in DCD liver grafts. *J Hepatol* 2013; **58**: 278.