

Cardiac transplantation with hearts from donors after circulatory declaration of death: haemodynamic and biochemical parameters at procurement predict recovery following cardioplegic storage in a rat model[†]

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Abstract

OBJECTIVES: Donation after circulatory declaration of death (DCDD) could significantly improve the number of cardiac grafts for transplantation. Graft evaluation is particularly important in the setting of DCDD given that conditions of cardio-circulatory arrest and warm ischaemia differ, leading to variable tissue injury. The aim of this study was to identify, at the time of heart procurement, means to predict contractile recovery following cardioplegic storage and reperfusion using an isolated rat heart model. Identification of reliable approaches to evaluate cardiac grafts is key in the development of protocols for heart transplantation with DCDD.

METHODS: Hearts isolated from anaesthetized male Wistar rats ($n = 34$) were exposed to various perfusion protocols. To simulate DCDD conditions, rats were exsanguinated and maintained at 37°C for 15–25 min (warm ischaemia). Isolated hearts were perfused with modified Krebs–Henseleit buffer for 10 min (unloaded), arrested with cardioplegia, stored for 3 h at 4°C and then reperfused for 120 min (unloaded for 60 min, then loaded for 60 min). Left ventricular (LV) function was assessed using an intraventricular micro-tip pressure catheter. Statistical significance was determined using the non-parametric Spearman rho correlation analysis.

RESULTS: After 120 min of reperfusion, recovery of LV work measured as developed pressure (DP)–heart rate (HR) product ranged from 0 to 15 ± 6.1 mmHg beats $\text{min}^{-1} 10^{-3}$ following warm ischaemia of 15–25 min. Several haemodynamic parameters measured during early, unloaded perfusion at the time of heart procurement, including HR and the peak systolic pressure–HR product, correlated significantly with contractile recovery after cardioplegic storage and 120 min of reperfusion ($P < 0.001$). Coronary flow, oxygen consumption and lactate dehydrogenase release also correlated significantly with contractile recovery following cardioplegic storage and 120 min of reperfusion ($P < 0.05$).

CONCLUSIONS: Haemodynamic and biochemical parameters measured at the time of organ procurement could serve as predictive indicators of contractile recovery. We believe that evaluation of graft suitability is feasible prior to transplantation with DCDD, and may, consequently, increase donor heart availability.

Keywords: Heart transplantation • Non-heart beating donors • Cardiac graft evaluation • Ischaemia-reperfusion • Isolated rat heart • Donation after cardiac death • Donation after circulatory declaration of death

INTRODUCTION

A major obstacle in heart transplantation is the lack of suitable donor organs. Indeed, it has been reported that ~15% of patients die while awaiting a donor heart [1]. Furthermore, the gap between donors and patients is growing and is expected to worsen in coming years [2]. One potential solution to increase donor heart availability is organ donation after circulatory

declaration of death (DCDD) [1, 3]. DCDD has been the subject of renewed interest in recent years, as a potential additional source of donor organs. Importantly, the use of hearts from DCDD is expected to increase the donor pool by 17% [1] and has recently been reported as clinically feasible in certain situations [4, 5].

Despite the considerable potential of DCDD, this donor population has not been adopted for heart procurement so far, mainly because of concerns regarding damage sustained following warm ischaemia. In the setting of DCDD, cardiac ischaemia may be initiated at the time of a precipitating event, such as trauma or

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withdrawal of life support, or at the time of cardiac arrest. Subsequently, an obligatory 'stand-off' period of ~10 min after cardiac arrest must be observed for ethical reasons, during which time no invasive intervention is permitted [6]. Initially, tissue damage is reversible, but becomes irreversible with increasing ischaemic severity or length [7]. Importantly, pre-clinical reports provide evidence that hearts are tolerant to warm ischaemia, if limited to a period of 20–30 min, when sufficient integrity for transplantation is maintained [8, 9]. Paradoxically, cardiac reperfusion itself causes further injury, mediated by generation of reactive oxygen species (ROS), calcium overload, rapid normalization of pH and inflammatory processes. As such, clinical methods to prolong ischaemic tolerance or reduce ischaemia-reperfusion damage are critical to promote the use of this donor pool.

In order to take advantage of the potential of cardiac grafts from DCDD, means to evaluate the suitability of hearts for transplantation are key in the development of clinical protocols. In the setting of DCDD, variability in tissue injury arising from differing severities of warm ischaemic damage and subsequent reperfusion injury are critical aspects that must be considered. Further complexity arises from the differing categories of DCDD, which have been defined as follows: dead on arrival at hospital (Maastricht category I; uncontrolled), unsuccessful resuscitation (Maastricht category II; uncontrolled), planned withdrawal of treatment (Maastricht category III; controlled) and brain-dead patients undergoing a cardiac arrest (Maastricht category IV; controlled) [10]. Importantly, these categories are likely to vary with respect to cardiac injury. Although the heart may temporarily continue to pump in an increasingly hypoxic environment prior to arrest in several of these donor categories, both the left and right ventricles are typically exposed to pressure and volume overload only in the setting of asphyxiation, such as might occur with planned withdrawal of treatment (Maastricht category III; controlled) [11]. The potential for recovery may thus differ in situations where death followed asphyxiation vs exsanguination, as suggested by a recent study performed in a porcine DCDD model [12]. To date, few reports have proposed evaluation methods to predict recovery of cardiac contractile function in the setting of DCDD. Potential approaches include the measurement of coronary flow (CF) [13], the use of an isolated myocardial perfusion system [14, 15] or fractional anisotropy [16]; however, the latter two necessitate somewhat lengthy procedures and specialized equipment. Importantly, we have recently reported that early reperfusion haemodynamic parameters correlate with cardiac recovery in the isolated rat heart when subjected to ischaemia followed by immediate reperfusion and recovery [17]. However, conditions used in this study were somewhat removed from the clinical situation; hearts were isolated prior to ischaemia, ischaemic temperature was 32°C, and contractile recovery was evaluated during reperfusion immediately following ischaemia; no period of cardioplegic storage was included.

In the current study, we aimed to identify means to evaluate cardiac graft suitability for transplantation using a newly developed isolated, working rat heart DCDD model. To do so, we assessed whether haemodynamic and biochemical parameters measured after a period of *in situ* warm ischaemia, at the time of heart procurement, are effective in predicting contractile recovery following subsequent periods of cardioplegic storage and reperfusion. We believe that evaluation of graft suitability is feasible prior to transplantation with DCDD, and may consequently increase donor heart availability.

METHODS

Materials

Albumin from bovine serum, palmitic acid, sodium-L-lactate, L-histidine, L-histidine monohydrochloride monohydrate, L-tryptophan and α -ketoglutaric acid potassium salt were obtained from Sigma-Aldrich (Buchs, Switzerland). Insulin was purchased from Nordisk Pharma (Actrapid[®] HM 100 IU/ml, Künsnacht, Switzerland) and dialysis membrane was obtained from Spectrum Labs (Spectra/por[®], Rancho Dominguez, CA, USA). All other chemicals were purchased from Merck (Darmstadt, Germany).

Ethics statement

All animal experimental procedures were approved by the Swiss animal welfare authorities and state veterinary office (Authorization number: 11/11), and were executed in compliance with the European Convention for Animal Care. Surgery was performed under anaesthesia and all efforts were made to minimize suffering.

Isolated heart preparation

A modified isolated working rat heart system was used, as previously described [18]. Briefly, adult male Wistar rats, fed with standard laboratory diet *ad libitum*, were anaesthetized using 100 mg/kg of ketamine (Narketan[®], Vetoquinol AG, Bern, Switzerland) and 10 mg/kg of xylazine (Xylapan[®], Vetoquinol AG, Bern, Switzerland) via an intraperitoneal injection. All hearts were excised and rapidly placed in ice-cold, modified Krebs–Henseleit bicarbonate buffer containing: 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄·7H₂O, 1.25 mM CaCl₂, 25 mM NaHCO₃ and 5.5 mM glucose (buffer A). Each heart was weighed, the aorta cannulated and retrograde perfusion started. Excess tissue was then removed for left atrial cannulation and introduction of the micro-tip pressure catheter (Millar instruments, Houston, TX, USA) into the left ventricle (LV). During all aerobic perfusions, buffers were equilibrated with 95% O₂/5% CO₂ and maintained at 37°C.

Experimental protocol

Animals were divided into six groups according to perfusion protocol (Fig. 1A–C).

Experimental groups. For the DCDD model, anaesthetized rats were heparinized via tail vein injection (60 units; Liquemin[®] 5000 IE/ml, Drossa Pharma AG, Basel, Switzerland) and then exsanguinated via the abdominal aorta and maintained at 37°C for various periods. In this manner, experimental groups with warm, *in situ*, global ischaemia for 15, 20 and 25 min were generated (Fig. 1A).

Immediately following ischaemia, hearts were isolated and aerobically perfused in a retrograde/Langendorff preparation with buffer A at a pressure of 60 mmHg for 10 min (procurement perfusion). During this time, a micro-tip pressure catheter was introduced in the LV and haemodynamic parameters, as well as CF, were recorded at 10 min.

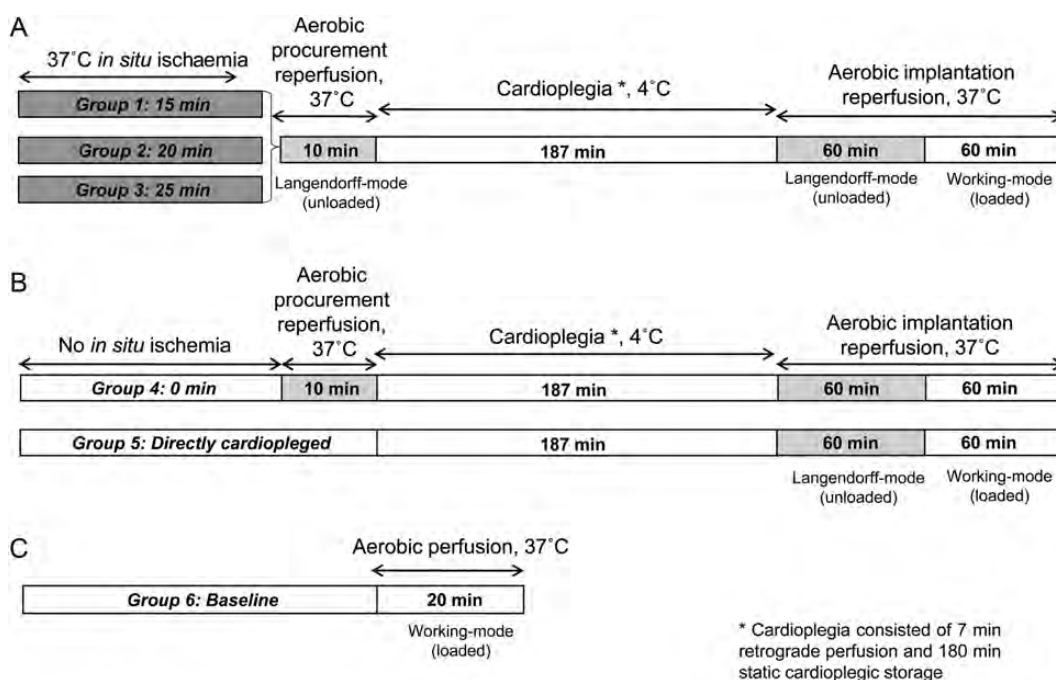


Figure 1: Perfusion protocols. Hearts ($n = 34$) were divided into six different perfusion groups. DCDD experimental groups are represented in (A) and include hearts that underwent *in situ* warm ischaemia followed by 10 min of procurement perfusion, cardioplegic perfusion and storage and implantation reperfusion for 120 min. Three different ischaemic periods were investigated: 15, 20, and 25 min. Control groups (B and C) comprised either 10 min of procurement perfusion, followed by cardioplegia and 120 min of reperfusion, direct cardioplegic perfusion and storage followed by 120 min of reperfusion, or 20 min of loaded aerobic perfusion.

Hearts were then arrested with cardioplegia (Custodiol) at 4°C for 7 min, delivered via Langendorff perfusion at a pressure of 65 mmHg. Cardioplegia contained: 150 mM NaCl, 90 mM KCl, 40 mM MgCl₂·6H₂O, 180 mM L-histidine monohydrochloride monohydrate, 1800 mM L-histidine, 20 mM L-tryptophan, 300 mM mannitol, 0.15 mM CaCl₂·2H₂O and 10 mM α -ketoglutaric acid potassium salt. Immediately following perfusion, hearts were immersed in cardioplegic solution at 4°C for 180 min.

Following cardioplegic storage, all hearts were aerobically reperfused for 120 min (implantation reperfusion). Hearts were initially perfused in an unloaded mode, with a constant aortic pressure of 60 mmHg for 60 min and then in loaded (working) mode, with a preload pressure of 11.5 mmHg and an afterload column of 80 mmHg for 60 min. The implantation reperfusion buffer contained: 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄·7H₂O, 1.25 mM CaCl₂, 25 mM NaHCO₃, 5.5 mM glucose, 100 μ IU/ml insulin, 3% albumin, 0.5 mM lactate and 1.2 mM palmitic acid (buffer B). High levels of fatty acids were chosen, as they are known to be elevated in humans undergoing cardiac surgery [19] and are detrimental to the recovery of contractile function after ischaemia [20]. Concentrations of glucose, insulin and lactate represent human physiological levels.

Control groups. Three separate control groups were used (Fig. 1B and C).

One control group was prepared identically to the DCDD experimental groups (described above), but was not subjected to warm ischaemia; immediately following exsanguination, the heart was isolated and perfused with buffer A. A second control group was generated by directly perfusing hearts with cold cardioplegic solution; these hearts were subjected neither to warm ischaemia nor any perfusion prior to cardioplegia. A third control, the baseline group, comprised hearts without any

pre-treatment (no heparinization, nor exsanguination) that were isolated and prepared as described above, and perfused in the aerobic, loaded mode for 20 min with buffer B.

Data collection

Possible predictive parameters including all haemodynamic and biochemical parameters were assessed at 10 min of procurement perfusion and implantation reperfusion. In addition, CF measured after 5 min of cardioplegic perfusion was considered a possible predictive parameter. Outcome parameters included all haemodynamic parameters described below, measured at 120 min of implantation reperfusion.

Haemodynamic parameters. A micro-tip pressure catheter coupled to a high-performance data acquisition system (PowerLab, ADInstruments, Spechbach, Germany) was continuously used to record LV pressure. CF and aortic flow (AF) were measured by timed collection of coronary effluent and used for the quantification of markers of necrosis and metabolism. These measurements were taken at 10 min of procurement perfusion and at 10 and 120 min of implantation reperfusion.

The following haemodynamic parameters were assessed:

- (i) heart rate [HR (beats min⁻¹)]
- (ii) peak systolic pressure [PSP (mmHg)]
- (iii) LV minimum pressure [P_{\min} (mmHg)]
- (iv) LV end-diastolic pressure [EDP (mmHg)]
- (v) LV developed pressure [DP (PSP- P_{\min} ; mmHg)]
- (vi) maximum and minimum first derivatives of LV pressure [dP/dt_{\max} and dP/dt_{\min} (mmHg s⁻¹)]
- (vii) coronary flow [CF (ml min⁻¹)]

(viii) cardiac output [CO (CF + AF; ml min⁻¹)

(ix) LV work

- (1) PSP-HR product (mmHg beats min⁻¹),
- (2) DP-HR product (mmHg beats min⁻¹),
- (3) DP-HR-CO product (mmHg beats ml min⁻²),
- (4) Triple product-PSP [TP (PSP) (PSP × dP/dt_{max} × HR; mmHg² beats min⁻¹ s⁻¹)] and
- (5) Triple product-DP [TP (DP) (DP × dP/dt_{max} × HR; mmHg² beats min⁻¹ s⁻¹)].

Markers of necrosis and metabolism. Lactate dehydrogenase release (LDH, mU min⁻¹ g wet⁻¹) was measured with a Roche MODULAR P800 analyser (Roche Diagnostics Corp., Indianapolis, IN, USA), and troponin T release (TnT, ng min⁻¹ g wet⁻¹) was measured with a Roche MODULAR E170 analyser (Roche, Basel, Switzerland) and an electro-chemiluminescence-immunoassay analyser (Roche, Basel, Switzerland). Markers of metabolism included oxygen consumption (O₂C, mmHg ml⁻¹ min⁻¹ g wet⁻¹), assessed using an iSTAT analyser (Abbott, Baar, Switzerland), and lactate release (μmol min⁻¹ g wet⁻¹), measured with a Roche/Hitachi MODULAR P analyser (Roche Diagnostics Corp., Mannheim, Germany).

Data analysis

Percentage recovery of each parameter for all hearts was assessed using baseline haemodynamic parameters. Unless stated otherwise, values are reported as mean ± SD. SPSS for Windows (version 17.0, SPSS, Inc., Chicago, IL, USA) was used for data analysis. A Kruskal-Wallis test was employed for an overview of differences between experimental groups. When significant overall results were observed, Mann-Whitney analysis was performed for comparisons between groups of particular interest at specific time points.

Among hearts exposed to 15–25 min of ischaemia, Spearman's rho analysis was used to investigate correlations between potential predictive parameters measured at 10 min of procurement perfusion or 5 min of cardioplegic perfusion and outcome parameters. In addition, correlations between potential predictive parameters measured at 10 min of implantation reperfusion and outcome parameters were analysed. The most effective approach for predicting the suitability/non-suitability of a heart for transplantation after a period of *in situ* warm ischaemia was investigated by comparing predictive abilities of parameters measured at 10 min of procurement perfusion with

outcome parameters. First, hearts were designated as those that recovered if percentage recovery of DP × HR product ≥40% baseline value or as those that did not recover if recovery of DP × HR product <40%. Hearts were then categorized as 'predicted to recover' or as 'predicted not to recover' according to threshold cut-off limits and verified against the actual measured recovery of DP × HR product after 120 min of implantation reperfusion. Correct positives (recovery) and negatives (no recovery), as well as false positives and negatives, were determined.

The sequential rejective Bonferroni procedure was used to correct for multiple comparisons and tests [21]. With this approach, families of parameters, rather than individual parameters, are considered for correcting *P*-values in order to prevent an overly severe correction [21]. To do so, three families of parameters were considered: baseline physiological parameters, haemodynamic parameters and biochemical parameters. All *P*-values were reported after correction. Corrected *P*-values < 0.05 were considered statistically significant.

RESULTS

An overview of the Kruskal-Wallis tests is provided in the [Supplementary Table](#).

Baseline characteristics

A total of 34 hearts were analysed in this study. Heart and body weights for each group are presented in Table 1. No difference was observed among groups for body weight. Heart weight for the 0-min ischaemic group was significantly lower than in the baseline group; that for 15- and 20-min ischaemic groups was higher than in the 0-min ischaemic group; and that for the 25-min ischaemic group was lower than in baseline and 15-min ischaemic groups (*P* < 0.05 for all comparisons).

Haemodynamic function

Haemodynamic function measured as DP × HR and CO at the end of the experimental protocol decreased with increasing length of warm ischaemia (Fig. 2A and B). Outcome parameters expressed as absolute value and as percent recovery are presented in Table 2.

Table 1: Baseline characteristics

	Groups						
	All hearts	Baseline	Directly cardiopleged	Ischaemic time			
				0 min	15 min	20 min	25 min
Number of hearts (<i>n</i>)	34	5	6	6	6	6	5
Body weight (g)	349.2 ± 52	382.6 ± 13.4	387.2 ± 33.5	305.2 ± 67.3	347.7 ± 26.2	339.0 ± 53.5	337.2 ± 62.5
Heart weight (g)	1.5 ± 0.2	1.7 ± 0.1	1.5 ± 0.3	1.3 ± 0.1 ^a	1.7 ± 0.2 ^b	1.6 ± 0.1 ^b	1.3 ± 0.2 ^{a,c}

Data are expressed as mean ± SD.

^a*P* < 0.05 vs baseline.

^b*P* < 0.05 vs 0 min of ischaemia.

^c*P* < 0.05 vs 15 min of ischaemia.

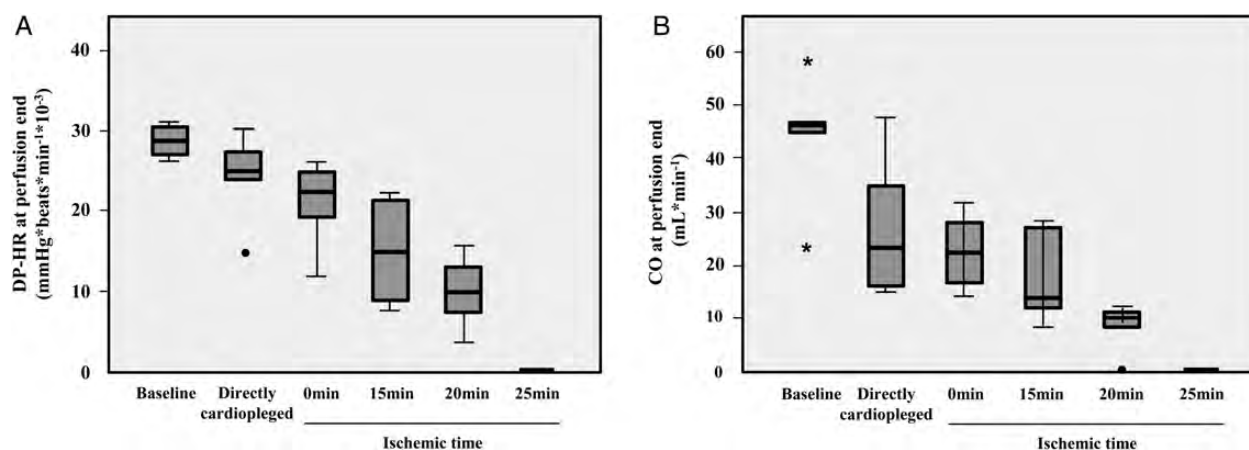


Figure 2: Haemodynamic recovery. Haemodynamic recovery measured at the end of the perfusion protocol for hearts subjected or not to *in situ* warm ischaemia (Groups 1–6) as HR–DP product (A) or as CO (B).

Predictive parameters

Predictive parameters measured at 10 min of procurement perfusion or at 5 min of cardioplegic perfusion are presented in Table 3, whereas predictive parameters measured at 10 min of implantation reperfusion are presented in Table 4.

Relationships between predictive and outcome parameters

Several parameters assessed at 10 min of procurement perfusion correlated significantly with outcome parameters measured during the subsequent loaded implantation reperfusion phase. An overview of these correlations is shown in Figure 3A. Of the parameters assessed at 10 min of procurement perfusion, DP × HR, PSP × HR and LDH release were among those that resulted in the greatest number of strong correlations ($P < 0.001$) and were the only three parameters that correlated strongly ($P < 0.001$) with all outcome measures of LV work. CF (at 10 min of procurement perfusion and at 5 min of cardioplegic perfusion) and oxygen consumption correlated with almost all outcome parameters.

An overview of the correlation between predictive parameters measured at 10 min of unloaded implantation reperfusion and outcome parameters is provided in Figure 3B. Similar to predictive parameters measured during the procurement perfusion, several haemodynamic parameters measured during the implantation reperfusion correlated with outcome parameters. CF and LDH release gave the greatest number of strong correlations ($P < 0.001$) with all outcome parameters, while CF gave the strongest correlation ($P < 0.001$) with outcome measures of LV work.

A comparison of the relationships between predictive parameters measured either at procurement or at implantation and outcome parameters revealed that most potential predictive parameters were associated with outcomes when measured at either time point (Fig. 3C). However, predictive parameters that gave the greatest number of strong correlations with outcome measures of LV work when measured during procurement perfusion were less strongly associated with outcome parameters when measured during implantation reperfusion. Interestingly, TnT release and CF demonstrate a greater number of

correlations with outcome parameters when evaluated at 10 min of implantation reperfusion vs 10 min of procurement perfusion (Fig. 3C).

Evaluation of predictive parameters

The ability of predictive parameters to correctly predict subsequent outcomes was evaluated (Table 5). HR, PSP × HR product and LDH release assessed during the initial 10 min of procurement perfusion appeared most effective for graft evaluation; compared with other predictive parameters, HR, PSP × HR product and LDH release could be used to best predict whether hearts would subsequently recover or not, without generating any false positives.

DISCUSSION

Increasing the pool of cardiac grafts is a major challenge in organ procurement for transplantation. We demonstrate that contractile and biochemical parameters assessed during an unloaded procurement perfusion correlate well with contractile recovery following cardioplegia and reperfusion. This study was performed in a new DCDD rat model, which includes a period of warm, global, *in situ* ischaemia and 3 h of cardioplegic storage. Our findings provide evidence that the assessment of haemodynamic and biochemical parameters in DCDD heart grafts at the time of procurement may facilitate evaluation of suitability for subsequent transplantation. This approach could be useful for cardiac graft assessment in general, and particularly in the context of transplantation with DCDD.

In order to simulate DCDD conditions, hearts were subjected to *in situ* warm ischaemia following exsanguination and cardioplegic storage. In a previous study, we proposed that haemodynamic parameters assessed during early, unloaded reperfusion may be useful to predict subsequent contractile heart recovery [17]. However, conditions used at this time were somewhat removed from the clinical situation; hearts were isolated prior to ischaemia, ischaemic temperature was 32°C and no period of cardioplegic storage was included. In the present study, we extend our findings by demonstrating that early reperfusion

Table 2: Haemodynamic parameters and CF at 120 min of reperfusion

	Groups										
	Baseline	Directly cardiopleged		Ischaemic time							
				0 min		15 min		20 min		25 min	
		Abs 120'	%	Abs 120'	%	Abs 120'	%	Abs 120'	%	Abs 120'	%
HR (beats min ⁻¹)	225 ± 38	230 ± 59	102 ± 26	196 ± 57	87 ± 25	169 ± 66	75 ± 29	153 ± 41	68 ± 18	0 ± 0 ^{a,b,c,d,e}	0 ± 0 ^{b,c,d,e}
PSP (mmHg)	128 ± 15	116 ± 10	89 ± 8.0	120 ± 17	92 ± 13	97 ± 12 ^{a,c}	75 ± 9.0 ^c	77 ± 15 ^{a,b,c}	59 ± 12 ^{b,c}	0 ± 0 ^{a,b,c,d,e}	0 ± 0 ^{b,c,d,e}
DP (mmHg)	130 ± 15	108 ± 9.9	83 ± 7.7	110 ± 15	85 ± 11	89 ± 13	68 ± 9.8	65 ± 23 ^{a,b,c}	50 ± 17 ^{b,c}	0 ± 0 ^{a,b,c,d,e}	0 ± 0 ^{b,c,d,e}
dP/dt _{min} (mmHg s ⁻¹ 10 ⁻²)	-30 ± 3.2	-26 ± 5.9	89 ± 20	-26 ± 4.5	88 ± 15	-19 ± 3.2 ^{a,b,c}	64 ± 11 ^{b,c}	-13 ± 4.1 ^{a,b,c}	44 ± 14 ^{b,c}	0 ± 0 ^{a,c}	0 ± 0 ^{b,c,d,e}
dP/dt _{max} (mmHg s ⁻¹ 10 ⁻²)	47 ± 15	32 ± 3.8	69 ± 8.0	29 ± 14	62 ± 30	26 ± 3.8 ^{a,b}	54 ± 8.1 ^b	19 ± 5.8 ^{a,b,d}	41 ± 12 ^b	0 ± 0 ^{a,b,c,d,e}	0 ± 0 ^{b,c,d,e}
PSP × HR (mmHg beats min ⁻¹ 10 ⁻³)	29 ± 2.1	26 ± 5.7	91 ± 20	23 ± 5.7	80 ± 20	16 ± 6.3 ^{a,b}	57 ± 22 ^b	12 ± 3.6 ^{a,b,c}	41 ± 13 ^{b,c}	0 ± 0 ^{a,b,c,d,e}	0 ± 0 ^{b,c,d,e}
DP × HR (mmHg beats min ⁻¹ 10 ⁻³)	29 ± 2.1	24 ± 5.2	85 ± 18	21 ± 5.1 ^a	74 ± 18	15 ± 6.1 ^{a,b}	52 ± 21 ^b	9.9 ± 4.1 ^{a,b,c}	34 ± 14 ^{b,c}	0 ± 0 ^{a,b,c,d,e}	0 ± 0 ^{b,c,d,e}
DP × HR × CO (mmHg beats ml min ⁻² 10 ⁻⁴)	127 ± 49	94 ± 36	74 ± 28	37 ± 19 ^{a,b}	29 ± 15 ^b	28 ± 20 ^{a,b}	22 ± 17 ^b	10 ± 6.4 ^{a,b,c}	8.0 ± 5.0 ^{b,c}	0 ± 0 ^{a,b,c,d,e}	0 ± 0 ^{b,c,d,e}
TP (PSP) (mmHg ² beats min ⁻¹ s ⁻¹ 10 ⁻⁶)	135 ± 36	85 ± 22	63 ± 16	66 ± 36 ^a	49 ± 27	43 ± 19 ^{a,b}	32 ± 14 ^b	23 ± 11 ^{a,b}	17 ± 8.0 ^b	0 ± 0 ^{a,b,c,d,e}	0 ± 0 ^{b,c,d,e}
TP (DP) (mmHg ² beats min ⁻¹ s ⁻¹ 10 ⁻⁶)	135 ± 36	79 ± 20 ^a	59 ± 15	61 ± 33 ^a	45 ± 25	40 ± 18 ^{a,b}	29 ± 13 ^b	20 ± 11 ^{a,b}	15 ± 8.1 ^b	0 ± 0 ^{a,b,c,d,e}	0 ± 0 ^{b,c,d,e}
CF (ml min ⁻¹)	17 ± 4.0	19 ± 9	109 ± 53	16 ± 2.4	90 ± 14	15 ± 5.0	86 ± 29	9.1 ± 4.2 ^{b,c}	52 ± 24 ^{b,c}	0 ± 0 ^{a,b,c,d,e}	0 ± 0 ^{b,c,d,e}
CO (ml min ⁻¹)	44 ± 13	27 ± 13	61 ± 29	23 ± 6.7	52 ± 15	17 ± 8.3	39 ± 19	9.1 ± 4.2 ^{a,b,c}	21 ± 9.5 ^{b,c}	0 ± 0 ^{a,b,c,d,e}	0 ± 0 ^{b,c,d,e}

Data are expressed as mean ± SD.

^aP < 0.05 vs baseline.

^bP < 0.05 vs directly cardiopleged.

^cP < 0.05 vs 0 min of ischaemia.

^dP < 0.05 vs 15 min of ischaemia.

^eP < 0.05 vs 20 min of ischaemia.

CF: coronary flow; CO: cardiac output; dP/dt_{min} and dP/dt_{max}: minimum and maximum first derivatives of LV pressure; DP: developed pressure; HR: heart rate; PSP: LV peak systolic pressure; TP (DP): triple product (DP)–DP × HR × dP/dt_{max}; TP (PSP): triple product (PSP)–PSP × HR × dP/dt_{max}

Table 3: Predictive parameters at 10 min of reperfusion (procurement perfusion) and at 5 min of cardioplegia

	Groups—ischemic time			
	0 min	15 min	20 min	25 min
HR (beats min ⁻¹)	236 ± 34	175 ± 79	142 ± 77	34 ± 62 ^a
P _{min} (mmHg)	-2.6 ± 4.5	2.8 ± 7.1	1.6 ± 9.3	NM
PSP (mmHg)	55 ± 17	61 ± 35	49 ± 19	19 ± 32
DP (mmHg)	56 ± 17	58 ± 32	48 ± 22	4.7 ± 6.8 ^{a,b,c}
dP/dt _{min} (mmHg s ⁻¹)	-1012 ± 320	-979 ± 405	-859 ± 325	-92 ± 127 ^{a,b,c}
dP/dt _{max} (mmHg s ⁻¹)	1466 ± 423	1320 ± 524	1351 ± 671	111 ± 152 ^{a,b,c}
PSP × HR (mmHg beats min ⁻¹ 10 ⁻³)	13 ± 4.1	8.8 ± 2.3	6.8 ± 4.3	1.1 ± 1.6 ^{a,b,c}
DP × HR (mmHg beats min ⁻¹ 10 ⁻³)	13 ± 3.9	8.6 ± 2.1	6.9 ± 4.4	0.5 ± 0.9 ^{a,b,c}
DP × HR × CF (mmHg beats ml min ⁻² 10 ⁻⁴)	11 ± 3.1	8.0 ± 2.7	8.0 ± 6.4	1.5 ± 2.8 ^{a,b,c}
TP (PSP) (mmHg ² beats min ⁻¹ s ⁻¹ 10 ⁻⁶)	20 ± 12	12 ± 7.1	11 ± 9.3	3.1 ± 4.4 ^{a,b,c}
TP (DP) (mmHg ² beats min ⁻¹ s ⁻¹ 10 ⁻⁶)	20 ± 12	12 ± 6.5	11 ± 9.0	1.3 ± 2.5 ^{a,b,c}
EDP (mmHg)	-1.5 ± 4.3	4.7 ± 7.5	3.3 ± 9.9	NM
CF (ml min ⁻¹)	8.3 ± 1.5	10 ± 1.1	10 ± 1.9	3.1 ± 1.3 ^a
Coronary cardioplegic flow (ml min ⁻¹)	10 ± 1.8	10 ± 1.7	7.9 ± 0.8 ^b	4.7 ± 1.6 ^a
Lactate (μmol min ⁻¹ g wet ⁻¹)	0.14 ± 0.1	0.31 ± 0.27	0.45 ± 0.20	0.39 ± 0.08
TnT (ng min ⁻¹ g wet ⁻¹)	0.15 ± 0.11	0.60 ± 0.34	0.93 ± 0.60	0.73 ± 0.45
LDH (mU min ⁻¹ g wet ⁻¹)	0.0 ± 0.0	46 ± 53	66 ± 56 ^a	795 ± 69 ^{a,c}
O ₂ C (mmHg ml ⁻¹ min ⁻¹ g wet ⁻¹)	1783 ± 581	1713 ± 130	1901 ± 487	634 ± 229

Data are expressed as mean ± SD. NM = not measurable.

^aP < 0.05 vs 0 min of ischaemia

^bP < 0.05 vs 15 min of ischaemia.

^cP < 0.05 vs 20 min of ischaemia.

CF: coronary flow; dP/dt_{min} and _{max}: minimum and maximum first derivatives of LV pressure; DP: developed pressure; EDP: LV end-diastolic pressure; HR: heart rate; LDH: lactate dehydrogenase; O₂C: oxygen consumption; P_{min}: LV minimum pressure; TnT: troponin-T; PSP: LV peak systolic pressure; TP (DP): triple product (DP)—DP × HR × dP/dt_{max}; TP (PSP): triple product (PSP)—PSP × HR × dP/dt_{max}.

Table 4: Predictive parameters at 10 min of reperfusion (implantation reperfusion)

	Groups				
	Directly cardiopleged	Ischaemic time			
		0 min	15 min	20 min	25 min
HR (beats min ⁻¹)	194 ± 30	213 ± 28	167 ± 51	175 ± 32	41 ± 56 ^{a,b,c,d}
P _{min} (mmHg)	1.2 ± 4.3	0.3 ± 6.9	1.3 ± 8.0	6.6 ± 8.9	NM
PSP (mmHg)	63 ± 6.1	64 ± 10	74 ± 9.1	59 ± 24	24 ± 34
DP (mmHg)	61 ± 3.7	62 ± 8.3	72 ± 9.4 ^a	52 ± 29	13.5 ± 22 ^{a,b,c}
dP/dt _{min} (mmHg s ⁻¹)	-1060 ± 101	-1120 ± 214	-1268 ± 323	-923 ± 489	-258 ± 353 ^{a,b,c}
dP/dt _{max} (mmHg s ⁻¹)	1425 ± 173	1238 ± 535	1701 ± 413	1292 ± 708	302 ± 414 ^{a,b,c}
PSP × HR (mmHg beats min ⁻¹ 10 ⁻³)	12 ± 2.6	13 ± 2.1	12 ± 4.1	11 ± 5.0	2.5 ± 3.4 ^{a,b,c}
DP × HR (mmHg beats min ⁻¹ 10 ⁻³)	12 ± 2.0	13 ± 2.3	12 ± 4.0	9.6 ± 5.7	1.3 ± 2.1 ^{a,b,c}
DP × HR × CF (mmHg beats ml min ⁻² 10 ⁻⁴)	9.2 ± 2.3	16 ± 3.6	12 ± 5.4	11 ± 6.7	0.3 ± 0.6 ^a
TP (PSP) (mmHg ² beats min ⁻¹ s ⁻¹ 10 ⁻⁶)	18 ± 5.4	17 ± 8.9	21 ± 11	17 ± 12	1.8 ± 2.6
TP (DP) (mmHg ² beats min ⁻¹ s ⁻¹ 10 ⁻⁶)	17 ± 4.4	17 ± 9.1	21 ± 11	16 ± 12	1.0 ± 1.6
EDP (mmHg)	2.5 ± 4.4	1.5 ± 6.8	3.3 ± 7.6	8.1 ± 9.2	NM
CF (ml min ⁻¹)	15 ± 2.1	13 ± 3.6	11 ± 1.3 ^a	9.9 ± 2.5 ^a	5.5 ± 2.2 ^{a,b}
Lactate (μmol min ⁻¹ g wet ⁻¹)	0.20 ± 0.34	0.50 ± 0.88	0.27 ± 0.18	0.27 ± 0.56	0.17 ± 0.40
TnT (ng min ⁻¹ g wet ⁻¹)	3.68 ± 2.61	3.18 ± 2.47	4.33 ± 1.72	7.56 ± 6.78	52.34 ± 69.24
LDH (mU min ⁻¹ g wet ⁻¹)	223 ± 98	247 ± 249	582 ± 187 ^a	729 ± 501	3491 ± 3265 ^{a,b,d}
O ₂ C (mmHg ml ⁻¹ min ⁻¹ g wet ⁻¹)	2587 ± 447	2283 ± 785	1604 ± 262 ^a	1566 ± 567 ^a	999 ± 421 ^{a,b}

Data are expressed as mean ± SD.

^aP < 0.05 vs directly cardiopleged.

^bP < 0.05 vs 0 min of ischaemia.

^cP < 0.05 vs 15 min of ischaemia.

^dP < 0.05 vs 20 min of ischaemia.

CF: coronary flow; dP/dt_{min} and _{max}: minimum and maximum first derivatives of LV pressure; DP: developed pressure; EDP: LV end-diastolic pressure; HR: heart rate; LDH: lactate dehydrogenase; O₂C: oxygen consumption; P_{min}: LV minimum pressure; TnT: troponin-T; PSP: LV peak systolic pressure; TP (DP): triple product (DP)—DP × HR × dP/dt_{max}; TP (PSP): triple product (PSP)—PSP × HR × dP/dt_{max}; NM = not measurable.

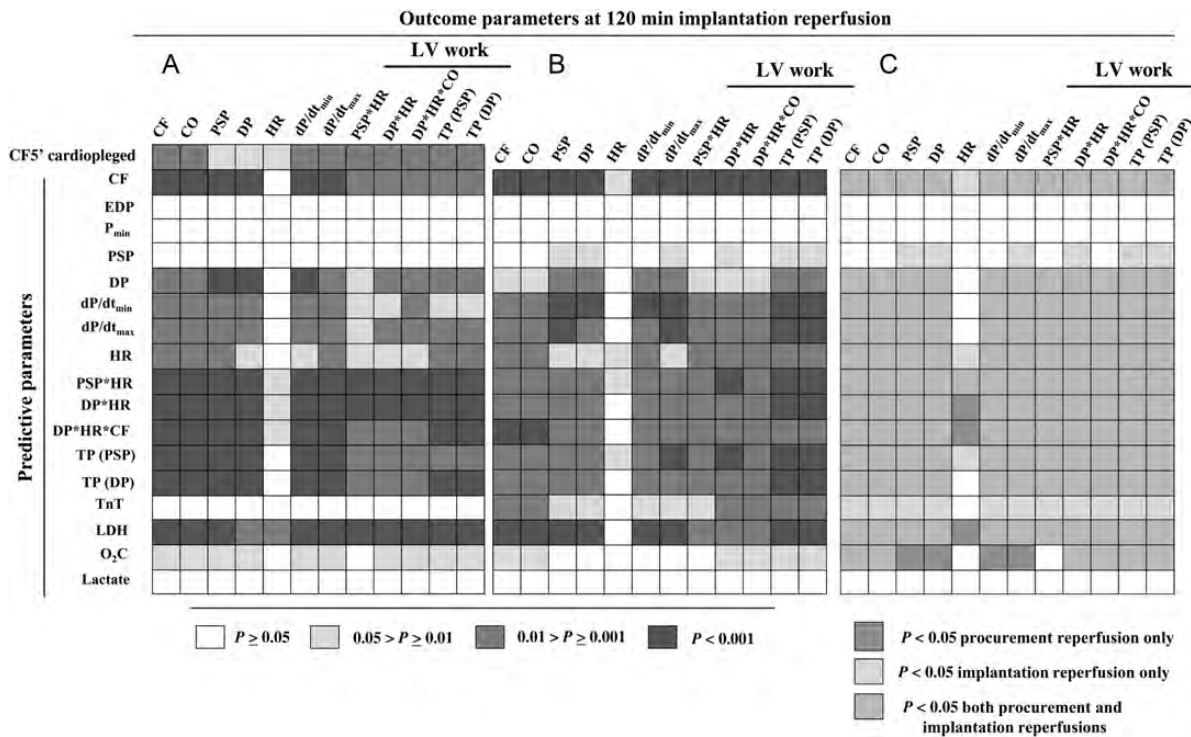


Figure 3: Spearman correlations between predictive and outcome parameters. Spearman's rho analysis was used to investigate relationships between potential predictive and outcome parameters: overview of correlations between predictive parameters measured prior to cardioplegic storage and outcome parameters (A); overview of correlations between predictive parameters measured immediately following cardioplegic storage and outcome parameters (B); comparison of relationships between predictive parameters measured at different time points and outcome parameters (C).

predictive parameters are indeed effective in a model that is closer to the clinical setting of DCDD. Specifically, hearts were subjected to an *in situ* ischaemia at 37°C and a second period of ischaemia in which cardioplegia was included. In addition, rats were not given any pre-treatment, with the exception of heparin, which, according to local regulations, may or may not be permitted in the clinical setting. Recovery of ~52% (measured as DP × HR relative to baseline) was found after 15 min of warm *in situ* ischaemia and 3 h of cold cardioplegia (Table 2). These findings are in agreement with previous reports of heart recovery in other DCDD models [8, 9]. Importantly, no difference in recovery was found between the directly cardiopleged group and the 0-min ischaemic group, which suggests that, under these conditions, a brief, unloaded reperfusion prior to cardioplegia is well tolerated by the heart. Furthermore, it is noteworthy that this brief, unloaded reperfusion provides a window of opportunity, not only to assess heart suitability after a stand-off period in the setting of DCDD as we describe here, but also to potentially treat hearts, for example, with anti-oedemic agents, scavengers of oxygen radicals or calcium-channel blockers, so that subsequent recovery is optimized.

Several early reperfusion predictive parameters strongly correlated with outcome parameters. In agreement with our previous findings, measures of left ventricular work (DP × HR, PSp × HR or as TP (DP) and TP (PSP)) correlated well with outcome parameters. However, in contrast to our previous work, P_{min} and EDP during early reperfusion did not correlate with outcomes. Importantly, the current study, which represents a more 'realistic' model of transplantation, provides evidence that P_{min} and EDP are less attractive candidates than measures of LV work for the prediction of graft suitability. Furthermore, we demonstrate that, immediately following ischaemia, early procurement perfusion measures of oxygen

consumption and release of LDH correlated with most outcome parameters including all measures of LV work. Interestingly, TnT release was more closely associated with outcome parameters when measured during early implantation reperfusion compared with procurement perfusion. This finding most probably results from the delay in the release of TnT following cardiac damage and suggests that assessment of LDH is preferable to TnT for the evaluation of cardiac grafts at the time of procurement.

In the current study, exsanguination has been used to induce cardiac death. It is, however, critical to note that this approach is not necessarily representative of all candidates for DCDD. Importantly, multiple aspects of transplantation with DCDD remain under intense discussion and current practices vary from country to country [22, 23]. In particular, it is unclear whether heart transplantation with DCDD will become widespread and whether all categories of donors will be considered acceptable for heart donation. On the one hand, it would seem logical that patients undergoing planned withdrawal of treatment (Maastricht category III) may become the donor of preference, as the conditions surrounding the cardiac arrest can be closely monitored. On the other hand, it may be that donors who have undergone cardiac arrest subsequent to exsanguination, i.e. exposed to less damaging conditions for the heart when compared with asphyxiation, will be preferred [12]. In the case of our current study, the primary aim was to determine whether parameters measured at the time of procurement could provide an indication of post-cardioplegic recovery, and thus conditions surrounding donor death were chosen to generate three groups of reproducible, but distinct, levels of recovery. Obviously, further studies are required to investigate the validity of the identified predictive parameters in other DCDD models, including models in which death is induced by asphyxiation.

Table 5: Evaluation of predictive parameters

	Threshold cut-off limits	Number of hearts (absolute values/percentage)		
		False negatives	False positives	Correct positives and negatives
HR (beats min ⁻¹)	225	2/12	0/0	15/88
DP (mmHg)	30	0/0	5/29	12/71
dP/dt _{min} (mmHg s ⁻¹)	-700	0/0	5/29	12/71
dP/dt _{max} (mmHg s ⁻¹)	700	0/0	5/29	12/71
PSP × HR (mmHg beats min ⁻¹ 10 ⁻³)	9	2/12	0/0	15/88
DP × HR (mmHg beats min ⁻¹ 10 ⁻³)	8	1/6	1/6	15/88
DP × HR × CF (mmHg beats ml min ⁻¹ 10 ⁻⁴)	8	1/6	1/6	15/88
TP (PSP) (mmHg ² beats min ⁻¹ s ⁻¹ 10 ⁻⁶)	10	2/12	2/12	13/76
TP (DP) (mmHg ² beats min ⁻¹ s ⁻¹ 10 ⁻⁶)	11	2/12	1/6	14/82
CF (ml min ⁻¹)	10.3	1/6	2/12	14/82
Coronary cardioplegic flow (ml min ⁻¹)	7.9	0/0	4/24	13/76
LDH (mU min ⁻¹ g wet ⁻¹)	20	1/6	0/0	16/94
O ₂ C (mmHg ml ⁻¹ min ⁻¹ g wet ⁻¹)	1400	0/0	5/29	12/71

Data are expressed as number of hearts (absolute value/percentage). Hearts were then categorized as 'predicted to recover' or as 'predicted not to recover' according to threshold cut-off limits and verified against the actual measured recovery of DP × HR product after 120 min of reperfusion (implantation reperfusion). Correct positives and negatives, as well as false positives and negatives, were determined.

CF: coronary flow; dP/dt_{min} and dP/dt_{max}: minimum and maximum first derivatives of LV pressure; DP: developed pressure; HR: heart rate; LDH: lactate dehydrogenase; O₂C: oxygen consumption; PSP: LV peak systolic pressure; TP (DP): triple product (DP)–DP × HR × dP/dt_{max}; TP (PSP): triple product (PSP)–PSP × HR × dP/dt_{max}.

For eventual translation to clinical practice, predictive parameters must provide rapid and reliable information to support the 'yes or no' decision to proceed with transplantation following organ procurement. Furthermore, predictive parameters must demonstrate a low rate of false positives (wrongly classified as suitable for transplantation) because the use of such an organ as a cardiac graft would have fatal consequences. Under our experimental conditions, we demonstrate that LDH release, HR and PSP × HR were the most effective parameters for graft evaluation as they could be used to most accurately predict which hearts would subsequently recover (or not) without generating false positives. Similar to our previous findings, parameters associated with cardiac recovery reported by other groups, such as lactate release [24] and CF [13], appear to be less promising than other biochemical or haemodynamic measures in our model. Taken together, LDH release, HR and HR × PSP appear to be the most promising parameters to be pursued in the development of eventual clinical protocols.

Summary

We believe that evaluation of graft suitability is feasible prior to transplantation with DCDD and may consequently increase

donor heart availability. All cardiac grafts used for transplantation are exposed to damaging situations, not only as a result of warm ischaemia with DCDD, but also as a result of neurological and haemodynamic instability associated with brain death in conventional donors [25]. As such, evaluation of cardiac graft suitability is a critical aspect in transplantation. We report that haemodynamic and biochemical analysis may facilitate evaluation of cardiac grafts at the time of procurement, during a brief, unloaded reperfusion prior to cardioplegia. This analysis could be performed in an *ex vivo* preparation and does not require specialized equipment. Furthermore, in clinical practice, this technique could be adopted in the setting of heart transplantation to screen all donors. Since not only ischaemia, but also reperfusion, causes cardiac damage, graft evaluation after reperfusion, as proposed here, may be advantageous. Importantly, our approach could rapidly provide information permitting a 'yes or no' decision with respect to suitability for transplant.

LIMITATIONS

This work demonstrates potential approaches that may be used towards the development of a clinical protocol for the evaluation of cardiac grafts from DCDD. Clearly, further investigations are required prior to the translation of this approach to the clinic. For example, pre-clinical experiments will be necessary to validate potential predictive parameters in a larger heart model. In addition, given that differing DCDD categories, as well as reperfusion strategies, are likely to affect post-transplant contractile recovery, predictive parameters must be validated under various conditions in order to identify the most robust parameters for cardiac graft evaluation. Finally, heart recovery was assessed at 120 min of reperfusion following cardioplegia in the absence of blood. As such, the validity of our predictive parameters remains to be investigated with longer-term recovery, as well as with blood reperfusion.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

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APPENDIX. CONFERENCE DISCUSSION

Dr J. Dinkhuysen (São Paulo, Brazil): I want to say that I published a paper last year in *Revista Brasileira de Cirurgia Cardiovascular* on the application of the non-working-beating-heart in humans. In 20 patients the ischaemic time was reduced to 45-60 minutes. Using normothermia, the first anastomosis we construct is the aortic, which immediately reperfuses the coronary system, and the heart is made to beat with a counter-shock. The last anastomoses are made with the heart beating and there were no problems with the implant. I think this clinical application is a new step in transplantation because the ischaemia time is considerably reduced.

Dr T. Wahlers (Cologne, Germany): Your paper presents relevant experience in the field of experimental heart transplantation from non-heart-beating donors. Numerous modified protocols regarding the suitability of potential marginal cardiac donors have been published over the past 25 years, as we have recently published in a manuscript in *Transplant International*.

The practice of using non-heart-beating donors as the "ultimate" marginal donor has increasingly become a part of transplant programmes worldwide, even becoming an option in thoracic organ transplantation. Currently, non-heart-beating donors are clinically used, especially in pulmonary transplantation, in several countries around the world.

Your manuscript is based on a sophisticated small animal model. It gives further evidence that even heart transplantation could be performed using these donors. Identification of clinically applicable parameters for evaluation of graft cardiac function have been presented by your group. Also, this experimental concept might not be directly transferable to clinical standard practice. I want to ask you two questions.

Which parameters should be used in approaching non-heart-beating donors in the clinical field based on your experience, and since your group also has extensive experience in clinical heart transplantation, what should be the next step in order to approach a large animal model? And do you think the same parameters will work in the big animal models, and do you also think that perhaps reperfusion time should be increased?

Dr Dornbierer: Concerning your first question about which parameter was probably best, to really have no false-positive cases, we propose that LDH heart rate and peak systolic pressure-heart rate product probably were the best or most effective and the safest. But, of course, this needs to be further validated in another model, including a large animal model.

As to what needs to be done before we can move to the clinic or a larger animal model, we are in the process of setting up an isolated working system for pig hearts, but it is quite tricky. We will use this to validate the predictive parameters. And, of course, we hope to use the same predictive parameters but we will probably have to consider many more factors as well.

Dr Wahlers: My personal feeling would be that since small hearts and big hearts are different, perhaps heart rate might be not as sensitive as it is in your pig model or even in humans. So, therefore, I have a little doubt about the transfer of all parameters.

Dr Dornbierer: Yes, definitely; we will see.

Dr J. Dark (Newcastle Upon Tyne, UK): Can I question your method of inducing death? Exsanguination is very far removed from what clinically happens during DCD donation. I suspect it may be setting up these animals in a very different fashion. Cardiac distension probably occurs in the clinical setting and may give you a completely different set of measurements. Would you like to comment on that?

Dr Dornbierer: For us exsanguination was quite straightforward to use in our model and we thought for this study that it was also best to compare the hearts of the rats that underwent the same process of dying. I would say it was the easiest approach for us. Of course, the most used categories for non-heart-beating donors may be category III where there is withdrawal of life support and expected cardiac death. This was technically more difficult to induce in our model, which is why we chose exsanguination. Although it is not the only type of non-heart-beating donor, we thought it was a good model for this study.

Dr Dark: I appreciate that, but I think if you are going to the cost and expense and complexity of a large animal model, you should simulate what happens in clinical reality where there is always an agonal phase. There may be catecholamine released during that phase, and I think it may be having a very profound effect on these donor hearts.

Dr Dornbierer: Yes, that is true.