Heparin does not improve graft function in uncontrolled non-heart-beating lung donation: an experimental study in pigs

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Abstract

OBJECTIVES: Non-heart-beating donation (NHBD) has the potential to increase the number of patients treated with lung transplantation. Our study investigated, in a simulated clinical situation in the uncontrolled NHBD setting, whether or not heparin administration after death affects the donor lung function.

METHODS: Twelve Swedish domestic pigs underwent ventricular fibrillation and were left untouched for 7 min followed by cardiopulmonary resuscitation with mechanical compressions for 20 min. The animals were declared dead after a 'hands-off' period of 10 min and randomized to heparin (300 IU/kg) or placebo given into a central venous catheter. In the animals receiving heparin, 2 more minutes of chest compression followed. Intrapleural cooling was initiated 1 h after death, and prevailed for 2 h. Ex vivo lung perfusion (EVLP) was performed with the Vivoline® system. Lung function was evaluated with blood gases at different oxygen levels, pulmonary vascular resistance (PVR), wet/dry weight ratio, macroscopic appearance and histology.

RESULTS: During EVLP, there were no significant differences between groups in PaO2 or PVR at any investigated FiO2 level (1.0, 0.5 or 0.21). At FiO2 1.0 the PaO2 in the heparin group was 64 ± 2 (range 57–73) kPa and in the non-heparin group 63 ± 4 (range 51–71) kPa. The values for PVR were 592 ± 90 (range 402–1044), respectively. There was no significant difference between groups in wet/dry ratio or histology.

CONCLUSIONS: The use of heparin is of no obvious benefit to the donor lungs in the uncontrolled NHBD setting. The exclusion of heparin will simplify lung donation from NHBDs.

Keywords: Lung transplantation • Ex vivo lung perfusion • Heparin • Non-heart beating donor • Donation after cardiac death

INTRODUCTION

Non-heart-beating donation (NHBD), also referred to as donation after cardiac death (DCD), has the potential to increase the number of patients treated with lung transplantation since multi-organ donors suitable for lung donation are very limited. Depending on the circumstances at the time of death, a potential NHBD is referred to as uncontrolled or controlled. Uncontrolled donors are dead on arrival (Category I) or have had an unsuccessful resuscitation (Category II). Controlled donors have a cardiac arrest awaited (Category III) or develop cardiac arrest after brain death (Category IV) [1]. Successful clinical lung transplantation from an uncontrolled NHBD was first reported in 2001 by Steen et al. [2], and more recently by de Antonio et al. [3]. However, lung transplantation from uncontrolled NHBDs might be associated with a higher degree of graft dysfunction than otherwise observed [3]. To improve the outcome, evaluation with ex vivo lung perfusion (EVLP) may be essential [4]. In lung transplantation from controlled NHBDs, which are performed routinely in a handful of centres worldwide [5–7], intravenous (IV) heparin is often administered to the potential donor before the controlled cardiac arrest. In the uncontrolled NHBD situation Steen et al. [1,8] used a preservation method of the potential graft including IV heparin and chest compressions post mortem. Any active intervention, such as giving IV heparin and chest compressions, after a failed resuscitation may be ethically disputable and impractical and make hospital staff feel uncomfortable, thereby decreasing their willingness to initiate the donation process [9]. However, the optimal preservation method for lungs from uncontrolled NHBD is under debate. Our study investigated, in a pig model, with a clear effort to simulate the clinical situation in the uncontrolled
NHBD setting, whether or not heparin, administered after death, affects the donor lung function.

MATERIALS AND METHODS

Animal model

Twelve Swedish domestic pigs with a mean weight of 41.2 ± 4.5 kg were used. The animals received care in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986) and the ‘Principles of Laboratory Animal Care’ formulated by the National Society for Medical Research and the ‘Guide for the Care and Use of Laboratory Animals’ (National Institute of Health publications 85–23, revised 1996). The Ethical Committee of the University of Lund approved the study that was performed as a collaboration between the Sahlgrenska Academy, University of Gothenburg and the University of Lund.

All animals received premedication with an intramuscular injection of Ketamin 15 mg/kg body weight (Ketalar Pfizer AB, Sollentuna, Sweden) and xylazin 0.2 mg/kg (Rompun vet Bayer, Leverkusen, Germany) in the stables. An IV catheter was placed in the ear and atropine sulphate 25 mg/kg (Atropin Mylan, Leverkusen, Germany) was given to prevent excessive airway secretion together with Penthotal Natrium 250 mg (Hospira Enterprises B.V, Hoofddorp, Netherlands). The pig was transferred to the operating room and placed in a supine position. Tracheal intubation was performed after IV administration of 4 mg Pancuronium bromide (Pavulon N.V Organon, Oss, The Netherlands). Ventilation was maintained at a volume-controlled, pressure-regulated setting at a rate of 20/min with a minute volume of 200 ml/kg, maximum inspiratory pressure of 30 mmHg, positive end expiratory pressure (PEEP) of 5 mmHg and FiO2 of 0.5 using a Servo Ventilator (Servo Ventilator 300A, Siemens-Elema AB, Solna, Sweden). Anaesthesia was maintained during the experiment with a solution of 8 g ketamin and 240 mg Pancuronium bromide mixed in 500 ml of sodium chloride given at a rate of 10 ml/h. A cut down in the neck allowed the placement of a central venous catheter into the right atrium through the right internal jugular vein and an arterial catheter into the aorta through the right carotid artery. A temperature probe was placed rectally. Baseline values of blood gases (ABL 725 Radiometer, Copenhagen, Denmark) and active coagulation probe was placed rectally. Baseline values of blood gases (ABL 725 Radiometer, Copenhagen, Denmark) and active coagulation time (ACT) (Hepcon HMS PLUS, Medtronic, Minneapolis, MN, USA) were measured and recorded.

Non-heart-beating donor model

Ventricular fibrillation was induced electrically with a needle perforating through the chest wall to the heart surface. The tracheal tube was disconnected from the ventilator when circulatory arrest was confirmed and the animal was left untouched for 7 min. Cardiopulmonary resuscitation (CPR) was then started with LUCAS 2 (Jolife, Lund, Sweden) at a rate of 100 compressions per minute. Ventilation with 10 breaths a minute by means of a Ruben's bag connected to a tube with 15 l/min of O2 was manually performed. CPR was ended after 20 min. The animals were declared dead after an additional 10 min ‘hands-off’ period and at the very end of this period, randomized to heparin (300 IE/kg) or placebo (sodium chloride) in equivalent volume. The drug or placebo was injected in the central venous catheter. In order to distribute heparin to the lung circulation, an additional 2 min of CPR with mechanical compression and ventilation was now followed in the heparin group but not in the placebo/no heparin group. Blood was drawn from the arterial line 90 s after administration of heparin for ACT analysis. The tracheal tube was once again disconnected and a temperature probe was inserted deep into the bronchus through the tracheal tube.

One hour after the declaration of death, intrapleural topical cooling with cold Perfadex (Vitrolife AB, Gothenburg, Sweden) with added calcium chloride (1 mmol/l) was given through two bilateral pleural chest tubes as described previously by Steen et al. [8]. Cold ischaemia prevailed for 2 h followed by lung harvesting (Table 1).

Lung harvesting

After topical cooling, a median sternotomy was performed. The left atrial appendage was opened and visible clots removed. The pulmonary artery was visualized through a 4 cm-long longitudinal incision in the right ventricle and clots were removed when present. The pulmonary artery was cannulated via the right ventricle with a 28 F cannula secured with a purse string suture placed in the annulus of the pulmonary valve. The lungs were perfused antegrade with 2 l of cold Perfadex with added isotonic trometamol 1.0 ml (Addex-THAM 3.3 mmol/ml; Fresenius Kabi AB Uppsala, Sweden), calcium chloride 2 ml (0.45 mmol/ml) and nitro-glycerine 3 ml (5 mg/ml; BMM Pharma AB, Stockholm, Sweden) distributed at low pressure (<20 mmHg). The cannula was removed from the pulmonary artery and inserted in the left atrium through the opening of the atrial appendage and secured with a purse string suture. The pulmonary valve was made insufficient and an additional litre of cold Perfadex with additives as above was infused retrogradely. The heart and lungs were harvested en bloc in a standard fashion.

Table 1: Experimental design

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Event</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>Ventricular fibrillation</td>
</tr>
<tr>
<td>7</td>
<td>External mechanical chest compressions</td>
</tr>
<tr>
<td>27</td>
<td>Ventilation with FiO2 1.0 and respiratory rate 10/min</td>
</tr>
<tr>
<td></td>
<td>Hands-off period</td>
</tr>
<tr>
<td>37</td>
<td>Tracheal tube disconnected and open to air</td>
</tr>
<tr>
<td>39</td>
<td>Declaration of death</td>
</tr>
<tr>
<td></td>
<td>Heparin or placebo administration by central venous line</td>
</tr>
<tr>
<td>220</td>
<td>Chest compressions and ventilation for 2 min in heparin but not in control group</td>
</tr>
<tr>
<td>250</td>
<td>Harvesting of lung enbloc following ante- and retrograde flush of Perfadex</td>
</tr>
<tr>
<td></td>
<td>Lungs connected to EVLP machine and start of reconditioning followed by evaluation of lungs</td>
</tr>
</tbody>
</table>
The heart was separated from the lungs, which were weighed and thereafter connected to the EVLP unit (Fig. 1).

**Ex vivo lung perfusion**

EVLP was performed with the CE-marked Vivoline LS1 (Vivoline Medical AB, Lund, Sweden). The system was primed with 2.0 l of Steen Solution (Vitrolife AB, Gothenburg, Sweden) and mixed with washed concentrated leucocyte depleted packed red blood cells to a haematocrit of 10-15%. Separate pigs were used as blood donors and their blood was matched by blood group and x-match to the blood of the individual lung donor. Imipenem 500 mg/Cilastatin 500 mg (Fresenius Kabi AB Uppsala, Sweden) and 100.00 IU Heparin (LEO Pharmaceutical Products Ballerup, Copenhagen, Denmark) were also added. The pH of the solution, measured after 15 min of circulation in the system, was corrected for every unit below zero in base excess with 1 ml of isotonic trometamol (Addex-THAM, Fresenius Kabi AB, Uppsala, Sweden) in order to achieve a pH of 7.35–7.45 before the lungs were connected to the EVLP unit.

A custom-fitted cannula was placed in the main pulmonary artery and secured with the suture previously placed in the pulmonary outflow tract at harvesting and reinforced with an additional cotton band. The cannula was then connected to the outflow line from the EVLP unit. A silicon tube, size-matched for the trachea, was secured in place with a cotton band and connected to the servo ventilator. Figure 1 illustrates the set up of the lung in the EVLP system. The remnant of the left atrium was opened widely to prevent pulmonary vein outflow obstruction and also for assuring a left atrial pressure near 0 mmHg. A temperature probe was sutured inside the left atrium. Arterial pressure was continuously measured in the main pulmonary artery and the pressure limit was set to 15 mmHg. Zero pressure is calibrated to the bottom of the lung evaluation box. The Vivoline LS1-machine is automated and has one phase for lung reconditioning and one for evaluation of lung function. During reconditioning, the oxygenator was supplied with a gas mixture: nitrogen 74%, oxygen 21% and carbon dioxide 5%. In the reconditioning phase, pulmonary artery flow was not allowed to exceed 70 ml/kg/min. Perfusion was started and warming initiated with the target temperature set to 37°C. The temperature difference between lung in- and outflow was not allowed to exceed 8°C. The shunt from the inflow cannula was closed after assuring that the perfusate was completely de-aired. If perfusion was uneventful, the arterial pressure limit was increased to 20 mmHg. When the temperature in the left atrium reached 32°C, ventilation was started at a minute-volume of 1.5 times the pulmonary artery flow, PEEP of 5 mmHg, frequency of 12/min and FiO2 of 0.5. If uncomplicated, ventilation was increased to 100 ml/kg/min of the donor animal. At 36°C, the procedure was shifted to the evaluation phase. The oxygen supply to the oxygenator was disconnected, and subsequently the oxygenator was only used to deoxygenate the perfusate in the EVLP system with a gas mixture of 93% nitrogen and 7% carbon dioxide. After 10 min blood gases were drawn from the left atrium for evaluation of lung performance but also from the blood entering the lung to ensure adequate deoxygenation. FiO2 was now increased to 1.0 and after another 10-min period new blood gases were drawn in the same manner. FiO2 was finally set to 0.21 and blood gases once again analysed. The lungs were finally ventilated with a FiO2 of 100% for 5 min before a collapse-test was performed in conjunction with the disconnection of the tracheal tube at the end of inspiration. Lung collapse was classified as normal or impaired. The same investigator judged all lungs subjectively.

**Histology**

The lungs were disconnected from the EVLP system and weighed. The arteries were opened as distally as possible and examined for thrombus material in all lungs. Tissue for microscopic examination was excised from the base of the lower lobe and from the apico-medial aspect of the upper lobe of both lungs. Samples were fixed in 10% buffered formalin solution, embedded in paraffin, sectioned in 4 µm thickness and stained using haematoxylin and eosin. A blinded pathologist evaluated the sections under a light microscope assessing lung injury using the following parameters: vascular thrombosis, haemorrhage, necrosis, interstitial oedema, intra alveolar enema, intra alveolar fibrin deposition, arteriolar thickening, cell infiltration, peribronchial enema and cell infiltrate. The severity of these changes was graded as previously described by Inci et al. [10] and as follows: 0 as absent; 1 as mild; 2 as moderate; and 3 as severe.

**Wet/dry ratio**

The left lung was prepared as for transplantation with trimming of the vessels and the main bronchus. It was then placed in a 60°C oven and weighed daily until no further weight loss could be recorded.

**Statistics**

Continuous data are presented as mean and SEM or as median and range. Groups were compared with non-parametric tests using the Mann–Whitney method or when appropriate the Wilcoxon signed-rank test (Instat, vers. 3.0, GraphPad Software Inc., La Jolla, CA, USA).
RESULTS

Exclusion

No animal met the predefined exclusion criteria of anatomical anomalies, confirmed malignancies at autopsy or technical problems affecting study results. One animal in the no-heparin group showed signs of swelling of the abdomen during CPR, high PVR during EVLP, inferior blood gas values and very high wet/dry ratio, and an extensive pulmonary embolism was found when examined post mortem. One animal had signs of pericarditis. One had pleuritis with a minor lung abscess. None of these animals were excluded.

Donor animal characteristics

No significant differences were observed for animal weight, pre-mortem oxygenation capacity, ACT before drug administration, warm ischaemic time, cold ischaemic time, temperature after 60 min of cooling or lowest cooling temperature between groups. As expected, there was a highly significant difference in ACT between groups. For further details on baseline parameters (see Table 2).

All animals in the no-heparin group, but none in the heparin group, had organized thrombi extracted from the pulmonary arteries. There was no signif-

cant difference in ACT at any FiO2 level (1.0, P = 0.49; 0.5, P = 0.39; 0.21, P = 0.31).

Ex vivo lung perfusion

The mean time in the reconditioning phase, i.e. from when perfusion of the lungs was started until the oxygenator was disconnected and evaluation began, was exactly the same (66 min, P = 1.0) in both groups.

Lung gas function

Details regarding blood gas parameters are listed in Table 3. During EVLP, there were no significant differences in PaO2 between groups at any FiO2 level (1.0, P = 0.82; 0.5, P = 0.59; 0.21, P = 0.82). There were no significant differences in PaCO2 between groups during EVLP at any FiO2 level (1.0, P = 0.49; 0.5, P = 0.39; 0.21, P = 0.31).

Haemodynamic data

Haemodynamic measurements were continuously performed regarding flow and pulmonary artery pressure. PVR values were registered at four occasions during EVLP: at the three oxygenation levels during the evaluation and at 36°C, when the oxygenator still supplied oxygen. There was no significant difference in PVR at these occasions. PVR parameters are listed in detail in Table 3.

Macroscopic appearance and histology

A collapse test gave an impaired recoil in the basal parts of the lungs overall, and no difference was observed between groups. Two lungs in the heparin group developed large subpleural haematomas. Severe pulmonary enema developed in two cases, one in each group. Atelectases were, to some extent, present in all lungs. The lung with suspected donor infection in the control group had a small (<2 cm) venous thrombosis. In the remaining three specimens, two from the heparin group and one from the no-heparin group, there were signs of minor embolic deposition (Fig. 3). The emboli were assessed as originating from bone marrow and muscle tissue, as often seen in conjunction with trauma. Findings of lung injury were few and did not differ between groups according to the grading system previously mentioned.

Wet/dry ratio

There was no significant difference between groups in wet/dry ratio. Wet/dry ratio results are shown in Table 3.

Table 2: Animal characteristics

<table>
<thead>
<tr>
<th></th>
<th>Heparin</th>
<th>No heparin</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal weight (kg)</td>
<td>40.2 ± 1.7</td>
<td>42.2 ± 2.0</td>
<td>0.59</td>
</tr>
<tr>
<td>ACT baseline</td>
<td>88 ± 5</td>
<td>86 ± 3</td>
<td>0.94</td>
</tr>
<tr>
<td>ACT after heparin administration (PaO2/FiO2)</td>
<td>962 ± 37</td>
<td>81 ± 4</td>
<td>0.002</td>
</tr>
<tr>
<td>In vivo oxygenation capacity (PaO2/FiO2)</td>
<td>66.2 ± 3.2</td>
<td>66.0 ± 1.6</td>
<td>0.59</td>
</tr>
<tr>
<td>Warm ischaemia (min)</td>
<td>98 ± 0.5</td>
<td>97 ± 0.8</td>
<td>0.24</td>
</tr>
<tr>
<td>Cold ischaemia (min)</td>
<td>215 ± 6</td>
<td>203 ± 5</td>
<td>0.24</td>
</tr>
<tr>
<td>Temperature after 60 min cooling</td>
<td>15.1 ± 1.0</td>
<td>14.3 ± 0.9</td>
<td>0.94</td>
</tr>
<tr>
<td>Lowest temperature during cold ischaemia (°C)</td>
<td>13.2 ± 0.4</td>
<td>13.2 ± 0.4</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Donor animal characteristics expressed as mean ± SEM. ACT could not be measured beyond 999 s.

Table 3: EVLP data

<table>
<thead>
<tr>
<th></th>
<th>Heparin Median</th>
<th>Heparin Range</th>
<th>No heparin Median</th>
<th>No heparin Range</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2 (kPa)</td>
<td>22.3</td>
<td>14.8-28.0</td>
<td>23.3</td>
<td>8.2-33.5</td>
<td>0.59</td>
</tr>
<tr>
<td>PaCO2 (kPa)</td>
<td>4.2</td>
<td>3.8-5.4</td>
<td>4.8</td>
<td>3.7-5.8</td>
<td>0.39</td>
</tr>
<tr>
<td>PaO2-0.5</td>
<td>12.7</td>
<td>10.3-16.3</td>
<td>12.5</td>
<td>9.5-14.5</td>
<td>0.82</td>
</tr>
<tr>
<td>FiO2-1.0</td>
<td>63.7</td>
<td>57.3-73.1</td>
<td>62.8</td>
<td>51.3-70.9</td>
<td>0.82</td>
</tr>
<tr>
<td>FiO2-0.21</td>
<td>12.7</td>
<td>10.3-16.3</td>
<td>12.5</td>
<td>9.5-14.5</td>
<td>0.82</td>
</tr>
<tr>
<td>PVR [(dynes × sec)/cm5]</td>
<td>1340</td>
<td>0.21</td>
<td>1044</td>
<td>0.70</td>
<td>0.82</td>
</tr>
<tr>
<td>Wet/dry ratio</td>
<td>5.9</td>
<td>5.1-6.5</td>
<td>5.8</td>
<td>5.3-7.3</td>
<td>0.70</td>
</tr>
</tbody>
</table>
difference was demonstrated between groups in our study in comparison with Inokawa et al. In another study, Rega et al. [13] have demonstrated excellent graft function from non-heparinized NHBD lungs after 90 min of warm ischaemia and 24 h of cold storage. The same group have, in two different studies, stated the superiority of in situ topical cooling and confirmed that 1 h of warm ischaemia does not affect pulmonary graft function [14, 15]. In accordance with our study, an additional finding in both these studies was that graft performance in NHBDs without added heparin was equally good as in a heart beating donor control group.

With a clear effort to simulate the clinical situation, we opted to use the LUCAS 2 system for resuscitation, which has proved to be an adequate and reproducible method for chest compressions [16]. Previously, 1 h of warm ischaemia has been proved safe from a lung function perspective [12, 17–19] and was therefore chosen. This hour provides time in the clinical setting to inquire about the patient’s position regarding donation from relatives or registries. The lung-cooling procedure was designed to be simple, without compromising the donor lung function, thereby allowing NHBD lung donation even in the smaller hospital settings. Intrapleural cooling with Perfadex was chosen since it has been shown to be the method of choice for in situ lung preservation [20, 21]. Blood gases were deemed the most important and easily obtained end-point parameters in the clinical situation in lung donation. The implication of these methods makes us believe that our NHBD model is more realistic from a clinical perspective than previous studies of the heparin effect in the uncontrolled NHBD [11, 22]. Cypel et al. [23] recently demonstrated that EVLP is a confident method to predict graft function in the recipient after transplantation. Therefore, we do not think that our results necessarily are impaired by not transplanting the lungs, although we realize that the optimal test of lung function is the recipient performance after transplantation. We observed that, within the heparin group, two animals developed intrapulmonary haematoma during EVLP. These findings may be interpreted as related not only to heparin but also to the chest compressions that followed the administration of heparin. In addition, chest compressions after cardiac death are hypothesized to cause lung contusion injuries and may also transport embolic material into the lung circulation. In our setting, the formation of a thrombus occurred in the main pulmonary artery when heparin was not administered; however, larger thrombi were easily removed manually from the pulmonary artery and the left atrium. Smaller clots, which we have occasionally observed, seem to be eliminated during a pulmonary flush. Moreover, the lung has an excellent thrombolytic capacity to dissolve thrombi. Despite the formation of thrombi, problems related to thrombotic occlusions, such as inferior oxygenation, elevated PVR or areas of visible hypo-perfusion did not appear in the no-heparin group. However, in the clinical setting, one should be aware of this potential complication. Since heparin does not seem to be essential in our NHBD model, it can be avoided as well as chest compressions after death, further simplifying the care of the donor.

Independent of the chest compression method used, lungs may become injured by the trauma caused by resuscitation. Furthermore, the long period of warm ischaemia, nearly 100 min, adds even more stress to the lung tissue, and taken together, our model is potentially as destructive and challenging for the lungs as the reality would be. However, animals seemed to react individually to the trauma of resuscitation, preservation...
and surgery. Even if there was no difference between the groups in our study, there was a marked variability within the groups in terms of lung function during the EVLP and W/D ratio. Some of this variability may be related to individual differences, but we think that the most plausible reason is the trauma generated by the model itself. As described above, we have had lungs that, after evaluation *ex vivo*, should have been deemed unsuitable for transplantation, not primarily due to inferior blood gases but rather parameters such as large haematoma or suspected lung infections. We experienced three animals with on-going subclinical infection indicated by inferior blood gases, pleuritis and pericarditis. One of these animals had signs of a more extensive formation of thrombi likely due to infection-induced hypercoagulability. In our opinion, this is congruent with the real-world situation with differences in donor lung function before cardiac arrest and varying degrees of lung injury during resuscitation that inevitably will translate into not all NHBD lungs being transplantable. However, the potential pathology induced by resuscitation or infection was easily revealed during the EVLP, despite normal blood gases, which overall supports the use of EVLP in lung donation of NHBD donors. Therefore, in our opinion, lung evaluation with EVLP is a necessity in the uncontrolled NHBD situation. This is also indirectly supported by the findings of de Antonio *et al.* showing a less than ideal outcome after a clinical NHBD lung transplantation without the use of EVLP [3]. The likely explanation for the poor outcome following cardiac death in the uncontrolled donor may well be that concomitant diseases and inferior lung function are not detected in the short time available for decision making prior to transplantation. Moreover, the EVLP system increases the actual time period available for additional investigations as well as methods to treat and optimize the lungs further. EVLP provides an excellent tool to unmask lung pathology and evaluate lung function prior to decision making regarding lung transplantation.

In conclusion, we have shown that the use of heparin is of no obvious benefit to the donor lungs in the uncontrolled NHBD situation since there is no significant difference between groups in terms of gas exchange, PVR, W/D ratio and no difference in gross, or microscopic, embolic material after EVLP. In order to launch a donation programme with uncontrolled NHBD lungs, the first and highest priority is to simplify the donation procedure and minimize donor interventions without compromising organ quality. The implication of this study is that heparin administration to uncontrolled NHBDs seems unnecessary and that the evaluation of lung function in uncontrolled NHBDs requires an EVLP system.

**LIMITATIONS**

This study was performed with non-blinded randomization to heparin or placebo. Variations between animals regarding healed or on-going subclinical infections may also skew the data, however, randomization seems to have corrected for this since there were minor outliers in both groups.

**ACKNOWLEDGEMENTS**

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**Conflict of interest:** Authors have no conflict of interest with the exception of Stig Steen who is the inventor of Steen Solution and of the Vivoline system.

**REFERENCES**

The study by Wallinder et al. in this issue [1] of the journal demonstrates in a large animal model that heparinization is not a critical requirement in the setting of uncontrolled non-heart beating donors (NHBD). The avoidance of heparin would simplify the donation process and diminish ethical concerns related to interventions prior to family consent for donation in this specific category of donors [2, 3]. These results have also been corroborated by a recent clinical study by Erasmus et al. [4] using category III NHBD. However, in uncontrolled donation, previous studies using large animal lung transplantation models have demonstrated the importance of heparin administration within 30 min of cardiac arrest to avoid severe graft dysfunction [5, 6]. In the current report, both study groups appeared to have similar function during 1 h of ex vivo evaluation, however, organs were not transplanted, therefore the assurance of organ quality in both groups cannot be fully determined and more pre-clinical studies are necessary before safely moving this concept to the clinical arena. The importance of the ‘transplantation test’ becomes more evident in studies like this where microthrombosis and release of inflammatory mediators leading to endothelial injury might only become evident after exposure to immune cells from the recipient. Furthermore, in our experience, only 1 h of ex vivo assessment is insufficient time to determine the ultimate lung quality. We have shown that 3–4 h of stable lung functional parameters during ex vivo lung perfusion (EVLP) are required to achieve predictable post-transplant outcomes in humans [7].

While the routine use of EVLP in controlled NHBD lung transplantation is still somewhat controversial, we fully agree that EVLP should be mandatory in the setting of uncontrolled NHBD. To that end, the Spanish experience demonstrates a significant improvement in post-transplant outcomes, including a decreased incidence of primary graft dysfunction, after they adopted 4 h of EVLP testing (as opposed to a single pass donor blood assessment) as a standard practice for this type of donation [8].

Whereas the current research on avoidance of the use of heparin for NHBD lung donation seems appealing, the question of heparinization or not in uncontrolled NHBD might become a less-relevant topic in the near future when programmes for multi-organ donation from uncontrolled NHBD are established. Microvascular thrombosis seems to be a more significant problem for liver and kidneys in the setting of NHBD. In fact, many liver transplant programmes are now using fibrinolitics for controlled NHBD with the aim to improve their outcomes [9]. Current activities in the well-established Spanish and French programmes using uncontrolled NHBD include the initiation of venoarterial extracorporeal membrane oxygenation as soon as death is declared and obtaining legal permission in order to preserve abdominal organs [8]. In this case, the use of heparin seems to be mandatory.

Finally, we should not forget that only 15% of organs from brain death donors and <10% from controlled NHBD are currently utilized by transplant programmes in North America and Europe [10]. While research should continue with uncontrolled NHBD as a promising source of donation, we should continue to direct efforts towards optimizing utilization from the currently available donor pool. Both improvements in donor management and organ assessment, and ex vivo treatment of injured organs using EVLP as a platform aim to bring the utilization of donor lungs to at least 50% in the coming years.

**Keywords:** Lung transplantation • Non-heart beating donors • Heparin • Ex vivo lung perfusion