

Long-term Preservation With Interim Evaluation of Lungs From a Non-Heart-Beating Donor After a Warm Ischemic Interval of 90 Minutes

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Objective: To investigate the value of in situ preservation and ex vivo evaluation of lungs from a non-heart-beating donor (NHBD) prior to long-term cold storage.

Summary Background Data: The use of pulmonary grafts from NHBD might alleviate the organ shortage. However, viability testing of these grafts is mandatory to transplant only those lungs with excellent function.

Methods: Pigs were divided into two groups. In the control group, lungs were flushed, explanted, and stored for 4 hours (4°C). In the study group, pigs were killed and left untouched for 90 minutes. Thereafter, the lungs were cooled for 150 minutes via chest drains. Graft function of the left lung in both groups was assessed in an isolated ventilation and reperfusion circuit 4 hours after death. The lung was then cooled and stored. Twenty-four hours after death, the pulmonary graft was reassessed in the same model.

Results: We did not observe a statistical significant difference between the two groups in pulmonary vascular resistance, mean airway pressure, and partial oxygen tension at each time point. There was also no statistical significant difference in wet-to-dry weight ratio. Finally, no statistical difference was found within both groups comparing the assessment at 24 hours with the interim evaluation at 4 hours.

Conclusions: These data demonstrate that: 1) 90 minutes of warm ischemia and 150 minutes of intrapleural cooling do not affect pulmonary graft function; and 2) NHBD lungs can be safely preserved up to 24 hours. Finally, we have demonstrated that interim ex

vivo evaluation of NHBD lungs is a valid and safe method to assess graft function.

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Lung transplantation, like other forms of solid organ transplantation, is limited by the number of good donor organs. It is estimated that fewer than 25% of all available multiorgan donors have lungs suitable for transplantation.¹ There is a growing interest in increasing the potential donor pool by turning to alternative sources such as the use of marginal donors,² lobar or split transplants,³ living-related donors,⁴ or organs from circulation-arrested or so called non-heart-beating donors (NHBDs).⁵

Many groups^{6–12} have now reported data supporting the hypothesis formulated in 1991 by Egan et al (Chapel Hill, NC)⁵ that transplantation of lungs from NHBD might be an interesting strategy to resolve the problem of organ shortage. Also, in our laboratory we have been interested for many years in exploring the possibility of using lungs from these donors. In previous rabbit studies, we have investigated the effect of postmortem cadaver lung inflation, ventilation, and cooling^{13–16} on the catabolism of adenine nucleotides,^{17,18} on pulmonary cell viability,¹⁵ and on graft function.¹⁹

In the NHBD, the period of tolerable warm ischemia following cardiac arrest is estimated to be 1 hour.^{5,7,9,19} After this period, lungs should be protected against (further) tissue degradation to extend the time interval necessary to obtain family consent for organ donation and to organize organ retrieval.

Different opinions exist regarding the ideal technique to protect warm ischemic lungs. Some groups^{13,14,20–22} are convinced that postmortem ventilation or inflation is the preferred method of protection. Others believe that topical cooling of the lungs inside the cadaver is the best technique to preserve lung viability.^{8,23} We have recently demonstrated that postmortem topical cooling is more effective than ventilation to protect the lung from ischemic damage after an initial 1-hour warm ischemic interval.²⁴ In a further study, we have shown that this technique may be safely extended up to

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7 hours postmortem, thereby creating a sufficient interval to organize organ retrieval (Rega F, Neyrinck A, Verleden G, et al How long can we preserve the pulmonary graft inside the non-heart-beating donor? Presented at the 39th Annual Meeting of the Society of Thoracic Surgeons, January 29–February 2, San Diego, CA).

In the uncontrolled NHBDs (category I, death on arrival; and category II, failed resuscitation),²⁵ it is impossible to evaluate the quality of the lung prior to death. To accept only those lungs with adequate function, pretransplant graft assessment is mandatory in order not to jeopardize the life of the recipient. We have developed an *ex vivo* evaluation model based on our own experience with isolated reperfusion of rabbit lungs¹⁹ and on the experience of Steen et al (Lund, Sweden). His group performed and described the first clinical case of successful single lung transplantation from an NHBD²⁶ after a warm ischemic interval of 65 minutes.

In this animal study, we have investigated the value of *in situ* preservation, including 90 minutes of warm ischemia and 150 minutes of topical cooling followed by *ex vivo* evaluation of lungs from NHBD prior to long-term cold storage up to 24 hours.

MATERIALS AND METHODS

Experimental Groups

Ten domestic pigs (31.3 ± 0.9 kg) were randomly assigned to two groups (Fig. 1). In the control group, lungs

from heart-beating donors (HBDs) were flushed with cold Perfadex (Vitrolife, Göteborg, Sweden), explanted, and stored in the same solution (4°C) for 4 hours ($n = 5$). In the study group, pigs were killed and left untouched for 90 minutes (NHBD, $n = 5$). Thereafter, both lungs were cooled via two intrapleural drains. Topical cooling was continued for 150 minutes. The left lung in both groups was then assessed in an isolated reperfusion model prior to cold storage. Lungs were reevaluated in the same model after 24 hours.

Animal Preparation

All animals received human care in compliance with 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” published by the National Institutes of Health (NIH Publication No. 86–23, revised 1985).

Animals were premedicated with an intramuscular injection of 2.5 mL Zolazepam/Tiletamine (Zoletil 100, Virbac s.a., Carros, France) and 2.5 mL Xylazine (Xyl-M 2%, V.M.D. nv/sa, Arendonk, Belgium). The animals were intubated with an endotracheal tube no. 7.5 (Portex Tracheal Tube 7.5, SIMS Portex limited, Hythe, Kent, UK) and the lungs were ventilated (Titus, Dräger, Germany) with an inspired oxygen fraction (FiO_2) of 0.5, a tidal volume of 0.450 L, a positive end-expiratory pressure of 5 cm H_2O , and

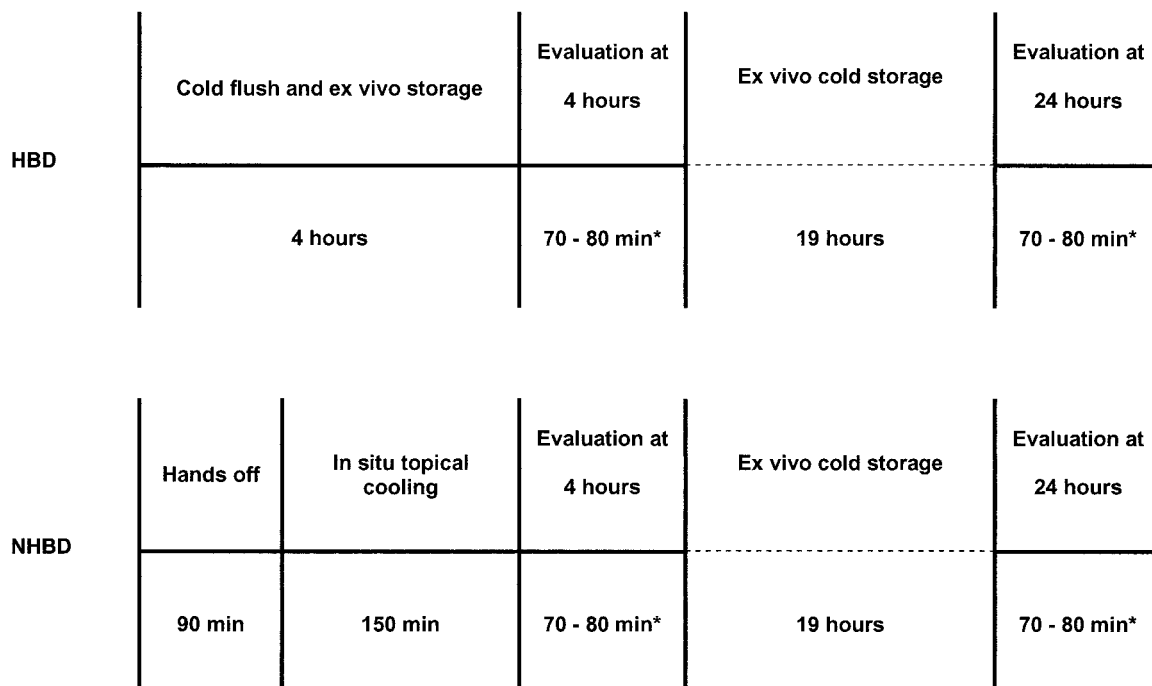


FIGURE 1. Schematic overview of different time periods in both experimental groups starting at death of the animals. HBD, heart-beating donor, NHBD, non-heart-beating donor. *Including time to prepare heart-lung block, warming up of the heart-lung block and 10 minutes assessment.

a frequency of 14 breaths/min. Anesthesia was maintained with 0.6 to 1% isoflurane (Forene, Abbott laboratories Ltd, Queensborough, Kent, UK). Muscle relaxation was controlled with an intermittent bolus of pancuronium bromide (Pavulon, 2 mg/mL, Organon Teknika, Boxtel, The Netherlands). A 7.5-Fr thermodilution catheter (Swan-Ganz Thermodilution Paceport Catheter, 931HF75, Baxter, Baxter Healthcare Corporation, Irvine, CA) was positioned in the pulmonary artery via the right cephalic vein and connected to a cardiac output computer (Com-1, American Edwards Laboratories, Irvine, CA). Baseline ventilatory and hemodynamic parameters were recorded and are presented in Table 1. Venous and arterial blood gases were taken via two catheters (Secalon T, 14G, Becton Dickinson, Singapore) positioned via a cervicotomy in the right internal jugular vein and common carotid artery, respectively.

Preservation of the HBD Lungs

After median sternotomy thymic tissue was excised. The pleural cavities and the pericardium were opened. The superior and inferior caval veins, the ascending aorta, and the pulmonary artery trunk were encircled. Sodium heparin 10,000 IU (Heparine Rorer, 5000 IU/mL, Rhône-Poulenc Rorer, Brussels, Belgium) was administered. The main pulmonary artery was cannulated with a 24-Fr catheter (DLP inc, Grand Rapids, MI) and secured by a purse-string in the right ventricular outflow tract. The ascending aorta was ligated and the pulmonary artery was isolated from the right ventricle by ligature around the tip of the catheter just distal to the pulmonary valve, creating pulmonary ischemia. All remaining tourniquets were tightened, the left atrial appendage was incised for venting, and the lungs were flushed with cold 50 mL/kg Perfadex (4°C) with addition of the buffer solution

Trometamol (0.3 mL/L, 2 g/5 mL, Addex-THAM, Kabi, Sweden) and CaCl₂ (1.2 mL/L, 11 mEq/g). After this flush, catheters were removed and the deflated heart-lung block was excised and immersed for 4 hours in Perfadex (4°C) adjusted in the same manner as described above.

Preservation of the NHBD Lungs

The pigs in the NHBD group were killed by myocardial fibrillation induced with a subxyphoid needle puncture using a square pulse generator (amplitude ranged between +15 to -15 volts, currency not more than 300 mAmp, and a frequency of 50 Hz). Blood pressure dropped immediately below 20 mm Hg, and the animals were declared death after 5 minutes. The endotracheal tube was disconnected from the ventilator and left open to the air. A temperature probe was introduced via the tube deeply into the lung to measure decline in endobronchial temperature. The animals were left untouched for 90 minutes at room temperature (21°C). After this in situ warm ischemic period, chest drains (16-Fr Trocar catheter, Argyle, Sherwood Medical, Tullamore, Ireland) were inserted in the thoracic cavity,²⁶ two in each pleural space (one for inflow, one for outflow). Via these chest drains, cold (6°C) saline solution was infused and continuously recirculated with a roller-pump from a reservoir placed in an ice basket. The system was filled with approximately 6 L of saline. To be sure that the lungs were adequately immersed in cold saline, a 5 cm H₂O overflow system was connected to the outflow drains. This so-called topical cooling was continued for 150 minutes to create the same length of ischemic interval (4 hours) identical to the HBD group. After this in situ preservation period, sternotomy was performed and the heart-lung block was excised.

TABLE 1. Hemodynamic and Aerodynamic Parameters and Blood Gas Analysis in the Donor Animals and During the Ex Vivo Assessment of the Left Lung at 4 Hours in HBD and NHBD

	In Vivo Both Lungs (Left Lung) (n = 10)	Ex Vivo Left Lung (HBD + NHBD, n = 10)	P
Total cardiac output (CO) (L/min)	3.9 ± 0.18		
Pulmonary artery flow (PAF) (L/min)	(1.31 ± 0.11*)	1.36 ± 0.03	(0.65*)
Pulmonary vascular resistance (PVR) (Dynes × sec × cm ⁻⁵)	270.2 ± 29.93 (811 ± 164 [†])	1149.7 ± 45.5	0.0002 (0.004**)
Mean airway pressure (mAwP) (5 cm H ₂ O PEEP) (mm Hg)	8.7 ± 0.29	7.7 ± 0.18	0.02
Partial oxygen tension (pO ₂) (FiO ₂ 0.5) (mm Hg)	261.0 ± 2.93	267.8 ± 4.9	0.17
Partial carbon dioxide tension (pCO ₂) (mm Hg)	41.0 ± 0.59	41.6 ± 0.41	0.07
End-tidal CO ₂ (EtCO ₂) (mm Hg)	40.3 ± 0.88	41.5 ± 0.26	0.09

*The flow over the left lung in domestic pigs is estimated to be one third of the total cardiac output or 1.31 ± 0.11 L/min.

[†]PVR in the left lung can then be estimated to be PVR × 3 or 811 ± 164 dynes × sec × cm⁻⁵.

Preparation of the Heart-Lung Block

After the 4-hour ischemic interval, the heart-lung block in both groups was prepared for evaluation in an isolated reperfusion circuit. The right lung was extracted to evaluate the left lung only. The pulmonary artery trunk was cannulated through the right ventricular outflow tract using a 36-Fr catheter after careful removal of all clots. The ascending aorta was ligated and the pulmonary artery was isolated from the right ventricle by ligature around the tip of the catheter just distal to the pulmonary valve. The left atrium was cannulated with a 36-Fr catheter through the apex of the left ventricle and secured by a purse-string. Finally, a tube no. 6.5 was placed in the trachea and tightened.

Preparation of the Perfusate

Autologous blood was drawn premortem from the donor animal via the catheter in the right internal jugular vein and collected in an empty sterile bag (1000 mL NaCl 0.9%, Baxter, Lessines, Belgium) containing 2 mL of heparin (2 mL/L, 5000 IU/mL). Blood was centrifuged for at least 10 minutes at 5600 rpm using a Cell Saver (Sequestra 1000, Medtronic Inc, Parker, CO). Remaining leukocytes were removed using a leukocyte filter (Imugard III-RC, Terumo Europe N.V., Haasrode, Belgium) (Table 2). The red blood cell concentrate (300 mL) was then diluted to a hematocrit of approximately 15% (Table 2) with a low potassium dextran solution (Perfadex) and albumin (Albumine 20%, CAF-DCF,

Leuven, Belgium). Thirty minutes prior to reperfusion, the perfusate was finalized by adding CaCl₂ (1.2 mL/L, 11mEq/g), heparin (2 mL/L, 5000 IU/mL), nitroglycerine (Nitro-Pohl, 2 mL/L, 50 mg/50 mL), and sodium bicarbonate (4.5 mL/L, 0.8 mol/L, Baxter, Lessines, Belgium).

Isolated Reperfusion Circuit

The perfusate was placed in a hardshell reservoir (Vision, GISH Biomedical Inc, Irvine, CA) and circulated using a centrifugal pump (Bio-Medicus, Medtronic, Minneapolis, MN) (Fig. 2). After passing an in-line leukocyte filter (Pall LeukoGuard, Pall Biomedical Products Co, East Hills, NY), the perfusate was sent through an adult gas exchanger (Vision, GISH Biomedical Inc). The heating element of the gas exchanger was connected to a heater/cooler (Bio-Cal, Medtronic, Minneapolis, MN). Before entering the pulmonary circulation, pulmonary artery flow (FF 100T 10 mm probe connected to the MFV-3200 electromagnetic flow meter; Nihon Kohden, Tokyo, Japan), pulmonary artery pressure (PAP) (Uniflow type 43-600F; Baxter, Uden, The Netherlands connected to a monitor type 78534A, Hewlett Packard) and temperature of the perfusate were recorded online on the inflow cannula.

Evaluation 4 Hours After Death

PAP and temperature of the oxygenated inflowing perfusate were gradually increased. This is the so-called warm-

TABLE 2. Characteristics During Isolated Reperfusion

	HBD	NHBD	P
Evaluation at 4 hours			
Timing			
Time to prepare heart-lung block (min)	32 ± 0.55	34.8 ± 2.18	0.54
Time to warm up the heart-lung block (min)	36.2 ± 1.07	39.4 ± 1.63	0.09
Reperfusion solution			
Hematocrit	0.14 ± 0.01	0.15 ± 0.01	0.22
White blood cell count (× 10 ⁹ L)	0.0 ± 0.0	0.02 ± 0.02	0.69
pH	7.36 ± 0.02	7.36 ± 0.01	0.55
pCO ₂ (mm Hg)	45.04 ± 1.72	44.24 ± 1.64	>0.99
pO ₂ (mm Hg)	57.66 ± 1.71	60.76 ± 5.03	0.31
Evaluation at 24 hours			
Timing			
Time to prepare of heart-lung block (min)	19.8 ± 1.16	18.0 ± 1.14	0.22
Time to warm up the heart-lung block (min)	37.6 ± 1.03	37.2 ± 1.36	>0.99
Reperfusion solution			
Hematocrit	0.14 ± 0.01	0.15 ± 0.01	0.55
White blood cell count	0.0 ± 0.0	0.04 ± 0.02	0.3
pH	7.35 ± 0.02	7.36 ± 0.02	0.84
pCO ₂	46.2 ± 1.91	46.4 ± 1.83	>0.99
pO ₂	63.22 ± 3.06	58.84 ± 2.14	0.15

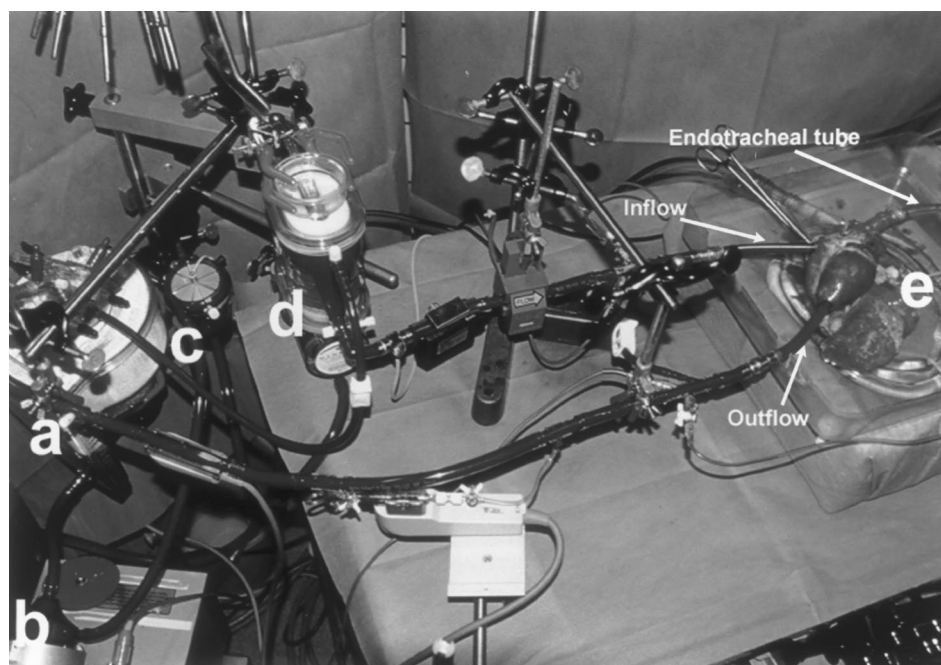


FIGURE 2. Isolated reperfusion circuit for ex vivo evaluation of pulmonary grafts. From the hard shell reservoir (a) the perfusate was recirculated by a centrifugal pump (b) passing a leukocyte filter (c) and an adult gas exchanger (d) before entering the pulmonary vasculature (e, heart-lung block). An inflow catheter was positioned in the pulmonary artery and an outflow catheter in the left atrium via the apex of the left ventricle. The lungs were ventilated via an endotracheal tube.

ing up interval during reperfusion (Table 2). PAP was increased following a strict protocol. During the first 15 minutes of the reperfusion, PAP pressure was kept between 5 and 10 mm Hg. Thereafter the pressure was increased with 1 mm Hg per minute and this to a maximum of 20 mm Hg at 25 minutes. Ventilation was not started until the temperature of the effluent had reached 32°C. The oxygen flow (0.4 L/min) through the gas exchanger was switched at that moment to a mixture of CO₂ (8%), O₂ (6%), and N₂ (86%), thereby deoxygenating the perfusate. Tidal volume and positive end-expiratory pressure were slowly increased up to 0.140 L and 5 cm H₂O, respectively, and this at a frequency of 12 to 14 breaths per minute (FiO₂ = 0.5). Mean airway pressure was continuously monitored. When PAP and the temperature of the lung had reached 20 mm Hg and 37.5°C, respectively, functional assessment was performed during 10 minutes (Table 1).

Graft Parameters

During this 10-minute interval, three consecutive measurements of pulmonary artery flow (L/min), PAP (mm Hg), LAP (mm Hg), and mean airway pressure (cm H₂O) were recorded and three blood gas samples were taken from the outflowing perfusate to analyze pO₂ (ABL 4 Radiometer A/S, Copenhagen, Denmark). Mean values from the three measurements were calculated for all parameters.

Pulmonary vascular resistance (PVR) was calculated using the formula $PVR = (PAP - LAP) \times 80 / \text{pulmonary artery flow}$ and was expressed in $\text{dynes} \times \text{sec} \times \text{cm}^{-5}$.

Cooling, Storage, and Evaluation 24 Hours After Death

After this first assessment, the temperature of the lung was lowered to approximately 20°C by decreasing the temperature of the perfusate. A plastic bag containing the heart-lung block was filled with 400 mL of perfusate with addition of 500 mg of Imipenem (Tienam monoval 500, Merck Sharp & Dohme, Whitehouse Station, NJ). The lung was then stored in an ice basket (4°C). Twenty-four hours after death, a second assessment was performed in the same manner using a clean circuit and fresh perfusate. The same parameters were measured.

At the end of the isolated reperfusion, the left lung was excised from the heart, weighed, and dried in an oven (model HT 600; Heraeus, Hanau, Germany) at 150°C overnight to constant weight. The wet-to-dry weight ratio was calculated as an estimate of the amount of lung edema.

Statistics

Data analysis was performed using the software package Statistica 6.0 (Statsoft Inc., Tulsa, OK). All data are expressed as mean \pm SEM. A Mann-Whitney *U* test was

used to compare both groups at each time point (4–24 hours) or to compare values from the ex vivo evaluation at 4 hours with in vivo registered values. Using difference scores between the two time points, a Mann-Whitney *U* test was used to verify if both groups differed in their evolution. A Wilcoxon matched pairs test was used to verify if there was a difference between both time points irrespective the group. Values of $P < 0.05$ were accepted as significant.

RESULTS

Animal Parameters

No statistical significant difference was observed between HBD and NHBD in animal weight (31.6 ± 1.09 kg vs. 31.0 ± 1.5 kg; $P = 0.84$) and premortem rectal temperature ($37.7 \pm 0.5^\circ\text{C}$ vs. $37.4 \pm 0.4^\circ\text{C}$; $P = 0.7$). The rectal temperature after 90 minutes in the NHBD group was $35 \pm 0.6^\circ\text{C}$. The decline in endobronchial temperature in NHBD during topical cooling inside the cadaver is depicted in Figure 3. Four hours after death, the temperature had reached $7.3 \pm 0.23^\circ\text{C}$.

Perfusion Parameters

Timing and composition of the solution during isolated reperfusion are shown in Table 2. There were no statistical significant differences in preparation time ($P = 0.54$) and warming up time of the heart-lung block ($P = 0.09$). Also, no statistical significant difference was found in hematocrit concentration, white blood cell count, and pH, $p\text{O}_2$ and $p\text{CO}_2$ in the reperfusion solution between the two study groups at both evaluations.

PVR

PVR is presented in Figure 4. No significant difference was observed between HBD and NHBD during the assessment at 4 hours (1181 ± 37 dyne \times sec \times cm⁻⁵ vs. 1119 ± 86 dyne \times sec \times cm⁻⁵, respectively; $P = 0.83$) and at 24 hours (1235 ± 57 dyne \times sec \times cm⁻⁵ vs. 1233 ± 89 dyne \times sec \times cm⁻⁵, respectively; $P = 0.59$). There was no statistical significant time effect between both groups ($P = 0.62$). There was also no significant time effect irrespective the group ($P = 0.07$).

Mean Airway Pressure

Mean airway pressure is presented in Figure 5. No significant difference was observed between HBD and NHBD during the assessment at 4 hours (7.7 ± 0.3 cm H₂O vs. 7.6 ± 0.24 cm H₂O, respectively; $P = 0.73$) and at 24 hours (8.0 ± 0.0 cm H₂O vs. 7.8 ± 0.37 cm H₂O, respectively; $P = 0.52$). There was no statistical significant time effect between both groups ($P = 0.92$). There was also no significant time effect irrespective the group ($P = 0.15$).

Partial Oxygen Tension

$p\text{O}_2$ is presented in Figure 6. No significant difference was observed between HBD and NHBD during the assessment at 4 hours (263.4 ± 7.6 mm Hg vs. 272.2 ± 6.6 mm Hg, respectively; $P = 0.12$) and at 24 hours (243.9 ± 21 mm Hg vs. 257.1 ± 10.3 mm Hg, respectively; $P = 0.92$). There was no statistical significant time effect between both groups ($P = 0.46$). There was also no significant time effect irrespective the group ($P = 0.39$).

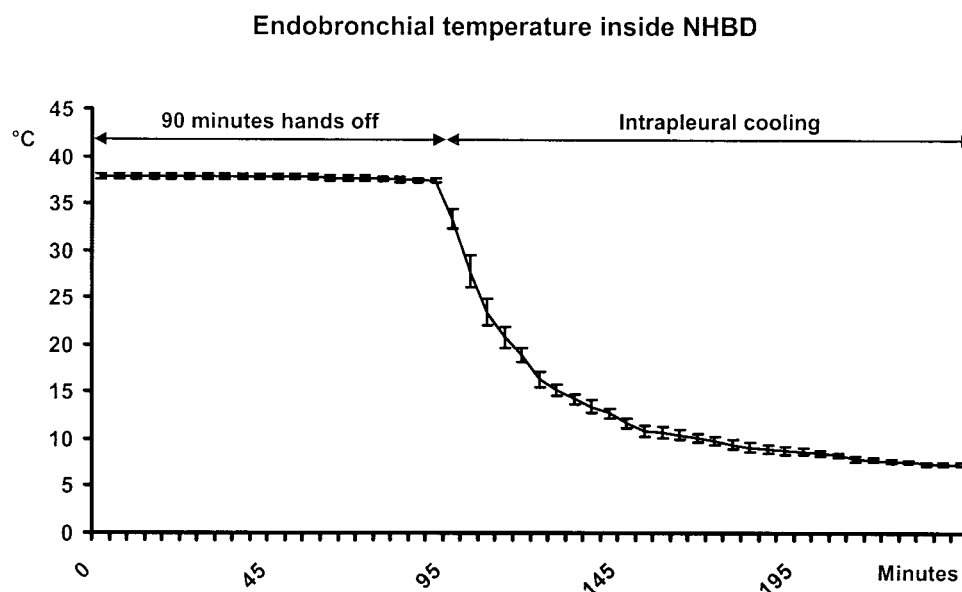


FIGURE 3. Decline in endobronchial temperature during lung preservation inside the cadaver in the NHBD group ($n = 5$).

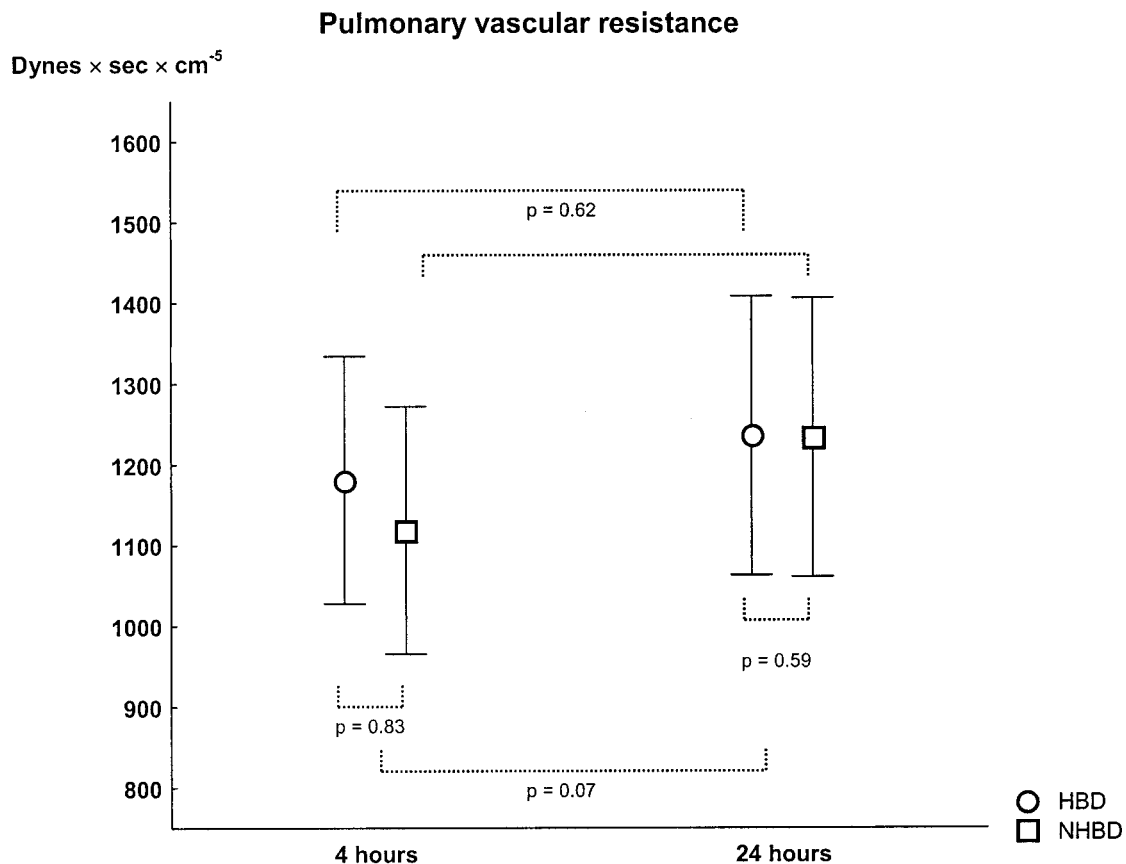


FIGURE 4. Pulmonary vascular resistance in the two groups (n = 5 in each group). HBD versus NHBD at 4 hours, *P* = 0.83; HBD versus NHBD at 24 hours, *P* = 0.59; HBD versus NHBD within time, *P* = 0.62. Time effect irrespective groups, *P* = 0.07. Vertical bars represent 95% confidence intervals.

Wet-to-Dry Weight Ratio

The wet-to-dry weight ratio is presented in Figure 7. No significant difference was observed between HBD and NHBD at the end of the second evaluation after 24 hours (4.94 ± 0.09 vs. 4.88 ± 0.07, respectively; *P* = 0.6).

In Vivo Versus Ex Vivo Assessment

Mean values for all parameters recorded during ex vivo assessment of the left lung at 4 hours in both groups (HBD + NHBD, n = 10) are listed in Table 1 and compared with premortem values measured in all donor animals (n = 10), reflecting the in vivo function of both left and right lung. No statistical significant difference was observed in partial oxygen tension (*P* = 0.17), partial carbon dioxide tension (*P* = 0.07), and end-tidal CO₂ (*P* = 0.09). PVR was significantly lower in vivo (left and right lung) when compared with ex vivo (*P* = 0.0002). Mean airway pressure was statistically higher in vivo when compared with ex vivo (*P* = 0.02).

DISCUSSION

Over the recent years, there has been an increasing interest in the use of lungs from NHBDs since Egan reintro-

duced the concept in 1991 in a number of lung transplant experiments in dogs.^{5,12,27} This concept was based on the fact that pulmonary tissue may remain viable after circulatory arrest for a considerable time. Based on research from our own laboratory¹⁹ and other groups,^{5,7,9} we know that 1 hour of warm ischemia is not deleterious for the pulmonary graft. From the present study, we now believe that the warm ischemic interval might be safely extended from 60 to 90 minutes. This interval should be long enough to consider NHBD lung donation and to arrange everything for further graft protection inside the cadaver. An efficacious technique of organ preservation inside the cadaver will also help to extend the pre-explantation interval, thereby allowing the transplant team to organize family consent and organ retrieval. Different opinions exist regarding the preferred preservation protocol for lungs from NHBD. Some authors suggested that postmortem ventilation^{9,12,21,22,27} is the preferred technique to preserve the graft inside the NHBD; others are more in favor of topical cooling.^{8,11,23,26} Steen et al²⁶ proposed a very elegant method of topical cooling via chest drains in the pleural cavities. This technique can be per-

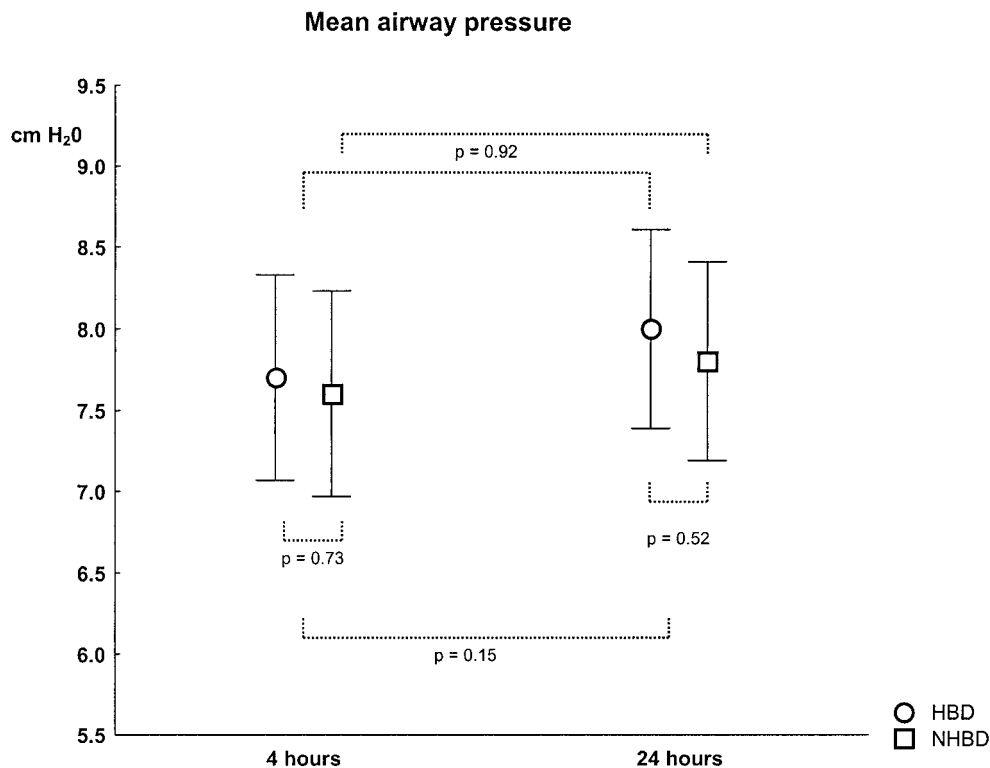


FIGURE 5. Mean airway pressure in the two groups ($n = 5$ in each group). HBD versus NHBD at 4 hours, $P = 0.73$; HBD versus NHBD at 24 hours, $P = 0.52$; HBD versus NHBD within time, $P = 0.92$. Time effect irrespective of groups, $P = 0.15$. Vertical bars represent 95% confidence intervals.

formed by any trained physician working in an emergency department or intensive care unit. In a recent study performed in our laboratory, we have demonstrated that topical cooling is more efficacious than postmortem ventilation in protecting the pulmonary graft from NHBD.²⁴ In another recent study, we have demonstrated that intrapleural cooling initiated 1 hour postmortem can be continued for at least 6 hours without damaging the pulmonary graft (Rega F, Neyrinck A, Verleden G, et al. How long can we preserve the pulmonary graft inside the non-heart-beating donor? Presented at the 39th Annual Meeting of the Society of Thoracic Surgeons, January 29–February 2, San Diego, CA). This long interval will facilitate distant organ retrieval, optimal donor-recipient matching, and organ exchange.

We used saline solution to cool the lungs inside the cadaver because of the short ischemic interval. For longer cold ischemic intervals, it has been suggested that a low potassium dextran solution (Perfadex[®]) might be a better solution because of reduced inflammatory changes on the outer surface of the lung.²⁸

In the present study, the combination of 90 minutes of warm ischemia followed by 150 minutes of topical cooling inside the cadaver did not affect the viability of the pulmonary graft. Partial oxygen tension ($P = 0.12$), mean airway

pressure ($P = 0.73$), and PVR ($P = 0.83$) did not differ between HBD and NHBD at the 4-hour evaluation. These three parameters give us a good idea of the extent of ischemia-reperfusion injury. With fixed ventilatory settings (FiO₂, frequency and tidal volume), pO₂ is a sensitive parameter to assess damage at the level of the alveolo-arterial barrier. Mean airway pressure and wet-to-dry weight ratio are two parameters that reflect the extent of pulmonary edema. Finally, PVR is a reliable parameter to evaluate endothelial damage and microvascular obstruction.

In our NHBD group, we did not administer heparin prior to death as it will not be clinically possible in the uncontrolled NHBD.²⁵ We believe that category I and II donors will offer the transplant community enough organs of good quality to decrease the still existing mortality on the waiting list. These categories include patients with fatal craniocerebral trauma or bleeding, cardiac arrest after myocardial infarction, or ruptured aortic aneurism. We have noticed that there is nearly no clotting in the microvasculature of the lung after circulatory arrest. The endothelial cells of the pulmonary vasculature might produce anticoagulant factors that prevent clotting, although we did not find any arguments in the literature to support this hypothesis.

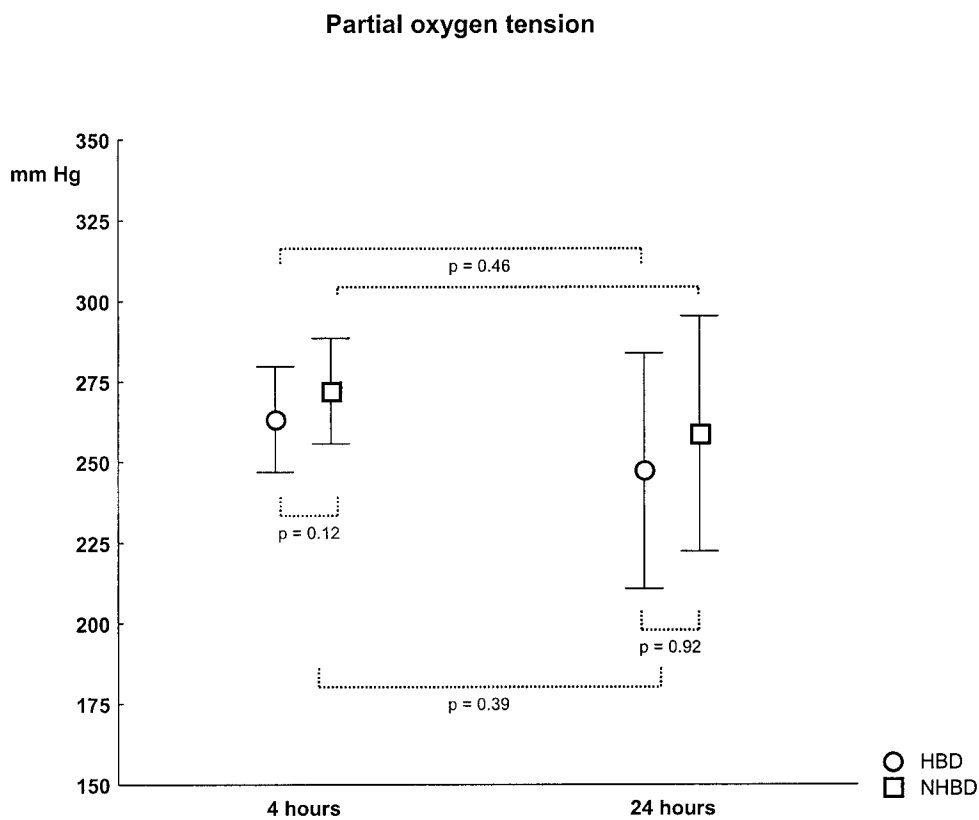


FIGURE 6. Partial oxygen tension in the two groups ($n = 5$ in each group). HBD versus NHBD at 4 hours, $P = 0.12$; HBD versus NHBD at 24 hours, $P = 0.92$; HBD versus NHBD within time, $P = 0.46$. Time effect irrespective groups, $P = 0.39$. Vertical bars represent 95% confidence intervals.

Not many papers have been published so far on ex vivo evaluation of lungs retrieved from NHBDs. Such pretransplant evaluation is mandatory to minimize the risk for the recipient. Aitchison et al²⁹ and Steen et al²⁶ recently described an isolated ventilation and perfusion technique to evaluate lungs from a NHBD.

Ex vivo assessment will enable the transplant surgeon to carefully palpate the lungs to exclude the presence of tumors, areas of contusion and infection, bullae, or interstitial parenchymal pathology. This technique will also facilitate care for the dead body and will allow the family much earlier to bid farewell to their beloved one. The purpose of ex vivo evaluation is to assess pulmonary function. We have correlated the findings of the evaluation with values measured in vivo (Table 1). In our reperfusion model, we only assessed the left lung. The right lung was extracted for morphologic and biochemical analysis. So far these data have not been processed completely. When one considers pO_2 to be the most important indicator of pulmonary graft function, we did not find a difference between the value registered in vivo and the value recorded at the 4 hours evaluation ($P = 0.17$) (Table 1). PVR, measured in vivo over both lungs, was

significantly lower compared with ex vivo ($P = 0.0002$) (Table 1). Even if we take into account that in a pig the flow over the left lung is about one third of the total cardiac output, PVR during ex vivo evaluation was still higher than the adjusted PVR ($P = 0.004$) (Table 1). An explanation might be that in our reperfusion model a continuous flow is sent through the pulmonary circulation instead of a more physiologic pulsatile flow.

The mean airway pressure was slightly lower ex vivo ($P = 0.02$) (Table 1) because of the absence of the chest wall that may influence respiratory mechanics.

By simply decreasing the temperature of the perfusate at the end of the assessment, the lung can be cooled to approximately 20°C and then further stored at 4°C. We did not find a statistically significant difference between the evaluation at 4 hours and 24 hours. This illustrates that an interim evaluation on itself is not damaging the pulmonary graft and that our proposed method to preserve lungs in the reperfusion medium after the first evaluation is favorable for long-term preservation, even up to 24 hours. This long-term preservation interval would facilitate optimal planning of the transplantation procedure. We believe that two key elements

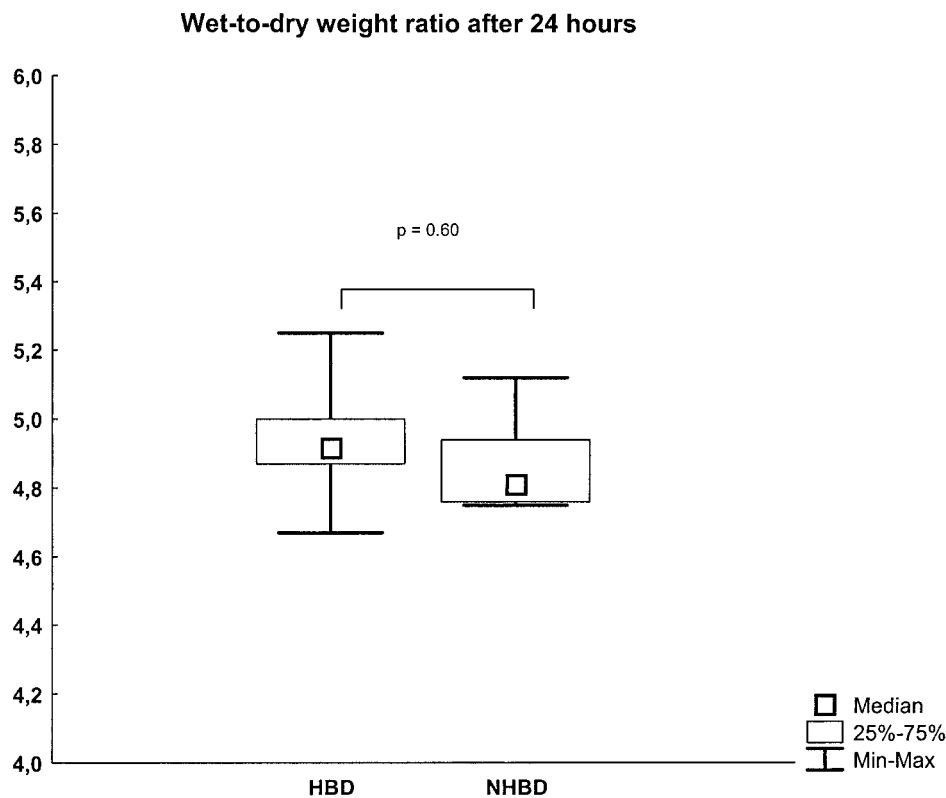


FIGURE 7. Wet-to-dry weight ratio in the two groups after 24 hours (n = 5 in each group). HBD versus NHBD, $P = 0.6$.

for successful ex vivo evaluation are the use of the optimal reperfusion solution (Steen solution, Vitrolife, Göteborg, Sweden) and the technique of controlled reperfusion.³⁰

Leukocytes have been recognized to play an important role in ischemia reperfusion injury.³¹ Rigorous leukocyte filtration is therefore of critical importance, and this not only during preparation of the reperfusion solution. During reperfusion many resident leukocytes are mobilized from the pulmonary vasculature. We therefore inserted an inline leukocyte filter to continuously sequester the major part of the circulating leukocytes, although the first 50 mL of effluent was already collected for biochemical analysis.

CONCLUSION

We have found that: 1) 90 minutes of warm ischemia does not affect pulmonary graft function upon reperfusion; 2) in situ NHBD lung preservation by intrapleural cooling during 150 minutes is as protective as immediate cold flush; and 3) NHBD lungs can be safely preserved up to 24 hours. Finally, we have also demonstrated that the use of an ex vivo reperfusion model for interim evaluation of NHBD lungs is a valid and safe method to assess graft function after additional cold storage inside the cadaver.

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REFERENCES

- Egan TM, Boychuk JE, Rosato K, et al. Whence the lungs? A study to assess suitability of donor lungs for transplantation. *Transplantation*. 1992;53:420–422.
- Pierre AF, Sekine Y, Hutcheon MA, et al. Marginal donor lungs: a reassessment. *J Thorac Cardiovasc Surg*. 2002;123:421–427.
- Artemiou O, Birsan T, Taghavi S, et al. Bilateral lobar transplantation with the split lung technique. *J Thorac Cardiovasc Surg*. 1999;118:369–370.
- Cohen RG, Starnes VA. Living donor lung transplantation. *World J Surg*. 2001;25:244–250.
- Egan TM, Lambert CJ Jr, Reddick R, et al. A strategy to increase the donor pool: use of cadaver lungs for transplantation. *Ann Thorac Surg*. 1991;52:1113–1120.
- Buchanan SA, DeLima NF, Binns OA, et al. Pulmonary function after non-heart-beating lung donation in a survival model. *Ann Thorac Surg*. 1995;60:38–44.
- Loeche F, Mueller C, Annecke T, et al. Pulmonary graft function after long-term preservation of non-heart-beating donor lungs. *Ann Thorac Surg*. 2000;69:1556–1562.

8. Shennib H, Kuang JQ, Giaid A. Successful retrieval and function of lungs from non-heart-beating donors. *Ann Thorac Surg.* 2001;71:458–461.
9. Greco R, Cordovilla G, Sanz E, et al. Warm ischemic time tolerance after ventilated non-heart-beating lung donation in piglets. *Eur J Cardiothorac Surg.* 1998;14:319–325.
10. Shimada K, Kondo T, Handa M, et al. The possibility of lung transplantation from non-heart-beating donors: experimental study in a canine model. *Transplant Proc.* 1994;26:880–881.
11. Steen S, Sjoberg T, Ingemansson R, et al. Efficacy of topical cooling in lung preservation: is a reappraisal due? *Ann Thorac Surg.* 1994;58:1657–1663.
12. Ulicny KS Jr, Egan TM, Lambert CJ Jr, et al. Cadaver lung donors: effect of preharvest ventilation on graft function. *Ann Thorac Surg.* 1993;55:1185–1191.
13. Van Raemdonck DE, Jannis NC, Rega FR, et al. Extended preservation of ischemic pulmonary graft by postmortem alveolar expansion. *Ann Thorac Surg.* 1997;64:801–808.
14. Van Raemdonck DE, Jannis NC, De Leyn PR, et al. Alveolar expansion itself but not continuous oxygen supply enhances postmortem preservation of pulmonary grafts. *Eur J Cardiothorac Surg.* 1998;13:431–440.
15. Kuang JQ, Van Raemdonck DE, Jannis NC, et al. Pulmonary cell death in warm ischemic rabbit lung is related to the alveolar oxygen reserve. *J Heart Lung Transplant.* 1998;17:406–414.
16. Van Raemdonck DE, Jannis NC, Rega FR, et al. External cooling of warm ischemic rabbit lungs after death. *Ann Thorac Surg.* 1996;62:331–337.
17. Van Raemdonck DE, Jannis NC, Rega FR, et al. Delay of adenosine triphosphate depletion and hypoxanthine formation in rabbit lung after death. *Ann Thorac Surg.* 1996;62:233–240.
18. De Leyn PR, Lerut TE, Schreinemakers HH, et al. Effect of inflation on adenosine triphosphate catabolism and lactate production during normothermic lung ischemia. *Ann Thorac Surg.* 1993;55:1073–1078.
19. Van Raemdonck DE, Jannis NC, De Leyn PR, et al. Warm ischemic tolerance in collapsed pulmonary grafts is limited to 1 hour. *Ann Surg.* 1998;228:788–796.
20. D'Armini AM, Roberts CS, Griffith PK, et al. When does the lung die? I. Histochemical evidence of pulmonary viability after 'death'. *J Heart Lung Transplant.* 1994;13:741–747.
21. Greco R, Benito J, Gonzalez M, et al. Lung transplantation from ventilated non-heart-beating donors: experimental study in a neonatal swine model. *J Pediatr Surg.* 1999;34:360–366.
22. Hennington MH, D'Armini AM, Lemasters JJ, et al. Cadaver lungs for transplantation: effect of ventilation with alveolar gas. *Transplantation.* 1996;61:1009–1014.
23. Steen S, Ingemansson R, Budrikis A, et al. Successful transplantation of lungs topically cooled in the non-heart-beating donor for 6 hours. *Ann Thorac Surg.* 1997;63:345–351.
24. Rega FR, Jannis NC, Verleden GM, et al. Should we ventilate or cool the pulmonary graft inside the non-heart-beating donor? *J Heart Lung Transplant.* 2003;22:1226–1233.
25. Kootstra G, Daemen JH, Oomen AP. Categories of non-heart-beating donors. *Transplant Proc.* 1995;27:2893–2894.
26. Steen S, Sjoberg T, Pierre L, et al. Transplantation of lungs from a non-heart-beating donor. *Lancet.* 2001;357:825–829.
27. Roberts CS, D'Armini AM, Egan TM. Canine double-lung transplantation with cadaveric donors. *J Thorac Cardiovasc Surg.* 1996;112:577–583.
28. Steen S. Preservation of the endothelium in cardiovascular surgery: some practical suggestions—a review. *Scand Cardiovasc J.* 2001;35:297–301.
29. Aitchison JD, Orr HE, Flecknell PA, et al. Functional assessment of non-heart-beating donor lungs: prediction of post-transplant function. *Eur J Cardiothorac Surg.* 2001;20:187–194.
30. Bhabra MS, Hopkinson DN, Shaw TE, et al. Controlled reperfusion protects lung grafts during a transient early increase in permeability. *Ann Thorac Surg.* 1998;65:187–192.
31. Welbourn CR, Goldman G, Paterson IS, et al. Pathophysiology of ischaemia reperfusion injury: central role of the neutrophil. *Br J Surg.* 1991;78:651–655.

Discussion

DR. P.A. CLAVIEN: I would like to thank the organizing committee for the kind invitation to discuss this paper and Dr. Rega for the privilege of receiving the manuscript. This is an elegant study in a pig model of non-heart-beating donor (NHBD) lung transplantation, pointing out that in situ topical cooling of the pleural cavity might be a suitable technique to increase the lung donor pool. The authors should be praised for taking the clinical observation by Steen et al on the potential benefit of in situ flush preservation in the pleural cavity in the NHBD situation back to the animal model, and for comparing this approach with the standard immediate cold flush of the lungs in heart-beating donor. As many might appreciate lung transplant is not my forte, and I will leave the erudite questions to the thoracic surgeons.

I have three questions. First, you describe that assessment of the graft viability was possible by slow reperfusion of the lungs with a red cell-based solution after 24 hours of cold preservation. Leukocytes and platelets were not included in your perfusate, and as these blood elements may significantly contribute to reperfusion injury, I was wondering whether you are not missing an important part of the injury. On the same token, may I ask if you correlated the results of this preimplantation assessment of graft function with the outcome of the graft after transplantation? My second question relies on the absence of an important control group. You compared your NHBD protocol with standard in situ flushing with cold preservation in a heart-beating model. To assess the benefit of topical cooling in the pleural space in the NHBD model, should you not compare your results with the immediate use of the lungs without cooling? Finally, the wide use of NHBD has been limited by ethical issues. We recently reported in the *New England Journal of Medicine* (2002;347:248–255) our experience with NHBD kidney transplantation and pointed out that the main factor favoring acceptance throughout the medical and general population relies on the absence of any manipulation of the potential donor prior to death. Thus, my question is: how do you plan to apply this strategy in patients, particularly regarding the insertion of large thoracic drains in the pleural cavity for rapid cooling after death? I enjoyed your paper very much. Like any fine studies, this work has generated further important questions.

DR. F. REGA: Thank you very much for your questions and comments.

Regarding your first question on leukocytes and their role in ischemia/reperfusion injury. As we all know from a number of publications, leukocytes indeed play a very important role in ischemia/reperfusion injury. We therefore de-leukocytized our setting at three levels: first, at the level of the reperfusion solution, which was completely de-leuko-

cyted; second, at the level of the first effluent, which we discarded since it contained an enormous amount of resident leukocytes; and finally at a third level where we continuously filtered the reperfusion solution from remaining leukocytes. So far, we did not look at what would happen if we would not implement these three levels of leukocyte depletion.

We know from a number of publications that leukocytes are important. We have a first phase response induced by donor leukocytes and a second phase response induced by receptor leukocytes. During the initial phase of reperfusion, the so-called pro-inflammatory cascade is activated, which causes the so-called early reperfusion injury. It's obvious we need to perform an evaluation without damaging the pulmonary graft. Removal of the leukocytes is therefore mandatory.

Regarding your second question, we believe that a heart-beating donor control is an ideal control since everyone is familiar with the good outcome of organs from heart-beating donors preserved after a flush through the pulmonary artery. We are aware of data from one group that preserved organs by simply opening the thorax and by cooling down the lungs with ice sludge. This seemed to be as protective as immediate flush; they also used heart-beating controls.

Your third question on the ethical issue is very important. As Steen stated in his article in the *Lancet*, he put a lot of efforts in trying to sensitize the Swedish public to accept the concept of non-heart-beating donors. In Belgium, we have the advantage of an opting-out law. Everyone is considered to be a donor, unless registered not being a donor. We have a protocol approved by the ethical committee of our hospital to start, for example, placement of a femoral catheter to preserve kidneys from non-heart-beating donors. We believe that the simple insertion of thoracic drain might not be a problem, but indeed we need to inform our own population carefully.

SIR P.J. MORRIS: I have one question: have you retained any of the histologic specimens? (The question about leukocytes Professor Clavien asked could also be answered indirectly by morphologic analysis.)

DR. F. REGA: We have been doing quite some work in the histologic field, and the problem we encountered was the species, namely, we used specimens from pigs. Apparently, there are no pig antisera available to perform immunohistochemical analysis. We are now considering repeating our experiments with smaller animals, such as rats, simply for the histochemical analysis. We did some electron microscopy, and there we found that there was no damage.

DR. R.J. PLOEG: Oxygenation is a double-edged sword in organ preservation, even at low temperatures. On one hand, you want to have the oxygen at time of reperfusion for revitalization; on the other hand, you can cause injury due to

reactive oxygen species. I wonder: have you studied in this experiment in both groups whether the surplus of oxygen, which is abundantly available in the lung during preservation, causes injury by determination of melonaldehyde or increase of TBARS?

DR. F. REGA: In these experiments we did not look at that. Also glutathione was not measured. Our reperfusion and ventilation are very controlled, so we are not giving an immediate burst of oxygen directly, the lung is heated up slowly so all the metabolism is coming awake, so to say, very slowly and apparently as you can see, this is not damaging the lung.

DR. R.J. PLOEG: The point I am trying to make is as follows: during the 90 minutes and during the 4 hours of preservation, when you have oxygen in the lung, does it cause injury in both groups, and are you sure that you were able to detect it and distinguish between the groups? As we have noticed that also at 0–4°C oxygen-free radicals are formed and can induce local damage, which is accelerated during reperfusion. But maybe you did not have a look at it.

DR. F. REGA: No, we did not.

DR. P.J. FRIEND: I want to pick up on the point that Professor Clavien made in relation to ischemia reperfusion. You would not really see ischemia reperfusion until you reperfuse the blood with all the immunologic effects and that necessarily includes leukocytes. My question is whether you have seen anything to suggest a severe ischemia reperfusion effect in the transplant experiments you tell us you have now started.

DR. F. REGA: So far, yes, this was probably related to the fact that we preserved our organs for more than 20 hours. We only use lungs from heart-beating controls. We did not implement the protocol of 90 minutes of warm ischemia and 150 minutes of topical cooling in our transplantation experiments. To be honest, I cannot answer your question completely with only six transplantation experiments performed up to now.

DR. P.J. FRIEND: Just very briefly: it seems obvious to validate your observations with transplant experiments. Have you done that, or do you plan to do that? It seems to me that it is necessary before you go to the clinical practice that you do this kind of experiment. So what is the status of such experiments?

DR. F. REGA: I fully agree, and that is the reason that we are putting a lot of work in it right now.