Successful Transplantation of Lungs From an Uncontrolled Donor After Circulatory Death Preserved In Situ by Alveolar Recruitment Maneuvers and Assessed by Ex Vivo Lung Perfusion

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We developed a protocol to procure lungs from uncontrolled donors after circulatory determination of death (NCT02061462). Subjects with cardiovascular collapse, treated on scene by a resuscitation team and transferred to the emergency room, are considered potential donors once declared dead. Exclusion criteria include unwitnessed collapse, no-flow period of >15 min and low flow >60 min. After death, lung preservation with recruitment maneuvers, continuous positive airway pressure, and protective mechanical ventilation is applied to the donor. After procurement, ex vivo lung perfusion (EVLP) is performed. From November 2014, 10 subjects were considered potential donors; one of these underwent the full process of procurement, EVLP, and transplantation. The donor was a 46-year-old male who died because of thoracic aortic dissection. Lungs were procured 4 h and 48 min after death, and deemed suitable for transplantation after EVLP. Lungs were then offered to a rapidly deteriorating recipient with cystic fibrosis (lung allocation score [LAS] 46) who consented to the transplant in this experimental setting. Six months after transplantation, the recipient is in good condition (forced expiratory volume in 1 s 85%) with no signs of rejection. This protocol allowed procurement of lungs from an uncontrolled donor after circulatory determination of death following an extended period of warm ischemia.

Abbreviations: CPAP, continuous positive airway pressure; CPR, cardiopulmonary resuscitation; DCDD, circulatory determination of death; EVLP, ex vivo lung perfusion; PEEP, positive end-expiratory pressure; uDCDD, uncontrolled DCDD

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Introduction

As a measure to increase the number of organs available for transplantation, many countries worldwide have adopted organ procurement from donors after circulatory death (1–3). The majority of organs so far have been procured from controlled donors, according to the Maas-tricht definition (4,5), mainly in Belgium, The Netherlands, the United Kingdom, Australia, and the United States. However, despite being an indisputable source of organs, as emphasized by the World Health Organization (6), this procedure has been considered with caution. Indeed, the possibility that a number of potential donors after brain death (DBD) turn into donors after circulatory determination of death (DCDD) within the controlled category of donors has caused skepticism (7). If true, this
would eventually lead to a lesser number of organs procured, since the numbers of organs procured from DCDD are significantly lower than those procured from DBD (8). Conversely, recovery of organs from uncontrolled DCDD (uDCDD) would increase the pool of organs available for transplantation (9). However, donation from this category adds logistical, ethical, and legal complexity to the donation process in many countries (10). In fact, far fewer organs have been recovered from uncontrolled donors, mainly in France and Spain (11).

Preclinical data show that lungs have the potential to better tolerate warm ischemia relative to other solid organs (12–15). This could make the uDCDD process safer in the lungs compared to other solid organs. However, although deceased donors are considered a valuable strategy to procure lungs (16), there are few reports on lung donation from uDCDD (17–19).

We are currently investigating the safety/efficacy of a clinical protocol designed to procure lungs from uncontrolled donors after determination of death with circulatory criteria. In this report, we present the first case recruited, and discuss some peculiarities of our protocol.

Materials and Methods

The trial was approved by the Ethics Committee of the Fondazione IRCCS Ca’ Granda and of the San Gerardo Hospital (NCT02061462).

Subjects with cardiovascular collapse, treated by an advanced life support crew on scene first, then transferred to the emergency room (San Gerardo Hospital, Monza), are considered to be potential donors if declared dead after advanced cardiac life support attempts failed. Unwitnessed collapse, no-flow period of >15 min, or low flow >60 min are among exclusion criteria. After clinical diagnosis of death (5 min of no touch), a recruitment maneuver (RM) is performed (RM: positive end-expiratory pressure, PEEP 5; inspiratory/expiratory [I/E] ratio 1:1; respiratory rate, recruitment maneuver (RM) is performed and ventilation is started (respiratory rate 4/min, tidal volume 6 mL/kg, PEEP 8 cmH2O, fraction of inspired oxygen [FiO2] 100%, I/E ratio 1:1). If chest radiograph is negative, the subject is transferred because of cardiocirculatory arrest and transferred to the emergency room of the San Gerardo Hospital. However, all of them arrived beyond recruitment time and were not considered potential lung donors. On November 1, 2014 recruitment was activated on a 24-h-a-day, 7-days-a-week basis. Since then, the potential donor recruitment system has been activated on a 24-h-a-day, 7-days-a-week basis. Recruitment was interrupted for logistical reasons during the summer (from August 1, 2014 to September 15). During this first period, five subjects aged <65 years were treated because of cardiocirculatory arrest and transferred to the emergency room of the San Gerardo Hospital. However, all of them arrived beyond recruitment time and were not considered potential lung donors. On November 1, 2014 recruitment was activated on a 24-h-a-day, 7-days-a-week basis. Since then, the potential donor recruitment system has been activated for 10 subjects. Details are shown in Table 1.

Subject number 1 in Table 1 was the only one with lungs procured that had EVLP. These lungs were transplanted. He was a 46-year-old male who had thoracic pain and, soon after arrival of the emergency team, collapsed. CPR was started immediately (0 min no flow); a first return of spontaneous circulation revealed ST-elevated myocardial infarction, but pulseless electrical activity developed soon after. The subject was transferred to the emergency room while automated chest compression (LUCAS™, Jolife AB/Physio-Control, Lund, Sweden) was ongoing. After the diagnosis of aortic dissection, the possibility of receiving extracorporeal life support (VA-ECMO) was excluded and the medical team decided to withdraw

Results

A pilot phase of potential donor’s recruitment active from 8 am to 4 pm, 7 days a week started on May 12, 2014. Recruitment was interrupted for logistical reasons during the summer (from August 1, 2014 to September 15). During this first period, five subjects aged <65 years were treated because of cardiocirculatory arrest and transferred to the emergency room of the San Gerardo Hospital. However, all of them arrived beyond recruitment time and were not considered potential lung donors. On November 1, 2014 recruitment was activated on a 24-h-a-day, 7-days-a-week basis. Since then, the potential donor recruitment system has been activated for 10 subjects. Details are shown in Table 1.
further treatment. The subject was declared dead after a total low-flow time of 45 min. Recruitment maneuvers and CPAP were applied and death was confirmed. Chest radiograph showed reduced lung volumes and a wide mediastinum consistent with the diagnosis of dissection (Figure 2). Consent for donation was obtained 2 h after death. At that time, heparin was given and ventilation started. The donor was then transferred to the operating room where lungs were procured (4 h and 48 min after death) and cold stored on ice. Upon arrival to the Fondazione Ca’ Granda, EVLP was run for a total of 6 h, after which lungs were deemed suitable for transplantation (Table 2) and cooled down. Time flow of the donation process is shown in Table 3. Lungs were offered to a rapidly deteriorating recipient with cystic fibrosis (LAS 46) who had been hospitalized for 4 months. The patient was on noninvasive ventilation 24 h a day and consented to the transplant in this experimental setting. Surgery was complicated by cardiogenic shock and need of VA-ECMO support with massive bleeding. Intensive care unit stay (19 days) was initially characterized by distributive-hypovolemic shock. Primary graft dysfunction at 72 h was grade 2; lung function was proper throughout the following days. Weaning from mechanical ventilation was difficult because of muscle fatigue due to preoperative deconditioning. Hospital length of stay was 39 days. Six months after transplantation, the recipient is at home, in good condition (forced expiratory volume in 1 s 85%). Three- and 6-month surveillance lung biopsy were both negative.

Discussion

The present case report confirms that lung procurement from uDCDD is feasible. The protocol implemented allowed procurement of lungs even after an extended period of warm ischemia.

Preclinical investigations show that lungs may be preserved in the non-heart-beating donor with lung inflation and ventilation (13,15). Lungs are anatomically open to air and can receive oxygen through diffusion. Consequently, they better tolerate the absence of blood. Moreover, as many as 60 min of total warm ischemia time is considered clinically safe according to UK criteria for DCDD organ procurement (7). Taking advantage of this background, and after preclinical investigations, we developed an in situ preservation strategy to procure lungs from uDCDD. The procedure consisted of lung recruitment maneuvers, CPAP, and protective mechanical ventilation.

Lung recruitment maneuvers are of crucial importance to fully open up the lung at the beginning of in situ preservation in order to facilitate oxygen diffusion to distal alveoli. Recruitment maneuvers, together with chest radiograph (Figure 2), can also provide important information on lung function at early stages of the donation process. As keeping the lung open over time is imperative, in our protocol CPAP is applied at the outset. This maintains the lung fully open during the 20-min ECG recordings required by Italian legislation, time possibly needed in other countries to procure organs. Thereafter, low tidal volume–high PEEP ventilation is applied with a low respiratory rate to avoid the harm of hypocapnia (21). Using this strategy, in our donor respiratory mechanics were stable over 4 h (Figure 3).

The preservation strategy we adopted differs from that described by Steen et al. (22). Indeed, whereas they applied a technique of topical lung cooling via chest tubes, we used in situ preservation with lung recruitment maneuvers, CPAP, and mechanical ventilation between declaration of death and cold flush and storage. It is possible that topical cooling allows longer in situ preservation time (23,24), relative to our strategy, aimed at gaining time for procurement. However, this case report indicates that lungs recovered from uDCDDs can be suitable for transplant after >4 h of total warm ischemic time, in line with results obtained from preclinical investigations (15). The recipient’s postoperative course, likely caused by massive blood loss that required intraoperative ECMO, might have also been related to the use of uDCDD lungs. However, the 6-month clinical outcome proves the feasibility of this preservation strategy.

The validity of machine perfusion when solid organs are procured from uDCDD donors has been suggested (25,26). Because of the impossibility to obtain PaO2/FiO2 for lung evaluation after cardiac arrest, we decided to include EVLP in our protocol as Steen mentions in his seminal article (22).

During EVLP, pulmonary vascular resistances were higher than in DBD donors, as previously shown (27). Assessment of vascular resistance is of great relevance.
## Table 1: Potential lung donors

| Subject | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   |
|---------|------|------|------|------|------|------|------|------|------|------|
| Sex     | M    | M    | M    | M    | M    | M    | M    | M    | M    | M    |
| Birth   | September 01, 1968 | June 07, 1981 | November 30, 1969 | October 24, 1957 | July 29, 1965 | February 10, 1957 | October 11, 1957 | October 16, 1949 | April 15, 1966 | March 15, 1983 |
| Age     | 46   | 33   | 45   | 57   | 49   | 49   | 57   | 65   | 48   | 32   |
| Clinical events | Date | December 11, 2014 | December 25, 2014 | December 12, 2014 | November 24, 2014 | December 11, 2014 | January 01, 2015 | January 31, 2015 | February 19, 2015 | March 15, 2015 |
| CCA     | 10:15 | 15:20 | 17:50 | 15:53 | 9:13 | 11:38 | 18:45 | 17:48 | 22:10 |
| CPR     | Y    | Y    | N    | Y    | Y    | Y    | Y    | Y    | Y    | Y    |
| BLS     | 10:15 | 16:19 | 15:32 | 18:15 | 16:00 | 9:23 | 11:53 | 18:56 | 17:58 | 22:25 |
| Rhythm  | PEA  | Asystole | Asystole | PEA  | VF   | Asystole | VF   | Asystole | Asystole | Asystole |
| ALS     | 10:15 | –     | 15:35 | 18:17 | 16:05 | 9:29 | 11:53 | 18:56 | 18:04 | 22:32 |
| ER      | 10:50 | 16:56 | 16:06 | 19:07 | 17:06 | 10:18 | 12:48 | 19:47 | 18:48 | 23:15 |
| Exitus  | 11:00 | 17:07 | 16:23 | 19:08 | 17:30 | 10:38 | 13:29 | 19:55 | 19:00 | 23:24 |
| Exclusion criteria | Witness | Y    | N    | N    | Y    | Y    | Y    | Y    | Y    | Y    |
| No Flow | 0:00 | –    | 0:12 | 0:25 | 0:07 | 0:10 | 0:15 | 0:11 | 0:10 | 0:15 |
| Low Flow | 0:45 | 0:48 | 0:51 | 0:53 | 1:30 | 1:15 | 0:36 | 0:59 | 0:02 | 0:59 |
| Other   | –    | Smoking | LMA | Smoking | Aspiration | – | Smoking | Aspiration | – | N    |
| Consent | Y    | –    | Y    | N    | –    | –    | –    | –    | N    | –    |

CCA, cardiocirculatory arrest; CPR, cardiopulmonary resuscitation; BLS, basic life support; PEA, pulseless electrical activity; VF, ventricular fibrillation; ALS, advanced life support; ER, emergency room; Smoking, active smoking of >20 cigarettes/day or history of >20 packs/year; LMA, laryngeal mask airway.
when dealing with DCDD lungs to exclude clot formation after circulation has stopped. In our protocol, heparin was added only after consent to donation was obtained, but lungs were treated with rTPA before flushing with the preservation solution. In fact, fibrinolytic treatment improves the quality of DCDD when applied during EVLP (27). Importantly, at this time of the process, response of the lung to vasculature flushing is used to decide whether to proceed with EVLP or not (see Figure 1, procurement). Indeed, in a rat model the time to flush the lungs with a constant volume of preservation solution correlates with the development of lung edema (28). This first “successful case” supports the validity of our protocol. However, while it allows the assumption that lung procurement is feasible even after an extended warm ischemia time, efficacy and safety remain to be more extensively proven. Nevertheless, our case may add to the discussion on the relevance of lungs procurement from uDCDD. In fact, the present protocol has a number of potential advantages. There are virtually no costs; indeed, most of the subjects are intubated at the time of death after CPR withdrawal, and only a ventilator is needed to preserve the lungs. Procedures are not invasive: at the time of donor’s death, relatives face an intact body, apart from endotracheal intubation. Clearly, impossibility to procure organs other than the lungs is a major weakness of the present protocol. However, tissues may be procured. Moreover, as the lung preservation strategy is simple, if proven safe and efficacious, many emergency rooms that do not have the possibility of setting up ECMO technology might be actively involved in lung and tissue procurement in a hub-and-spoke model as the one we have described. As recently pointed out by Egan and Reqaurd, a number of ethical issues come with uDCDD programs (19). A clear separation of treatment from procurement is one of these. For this reason, the regional (AREU 118) and local (San Gerardo Hospital) emergency teams treated the subject until death diagnosis. Thereafter, a separate team of neuro-intensivists (San Gerardo Hospital), eager in the process of donation, took the responsibility upon the arrival of the procurement team (Fondazione Ca’ Granda). As in the protocol of Egan et al (19), we are committed to build a multidisciplinary team. Moreover, a continuous program of medical and paramedical staff education is active.
During the first 6 months of activity, there were two denials of consent out of five requests. In a situation such as sudden death, this can be expected, particularly if, as in Egan’s protocol (19), witness to the cardiac arrest is not necessarily the next of kin. In this regard, the possibility to extend the time from death to organ consent offered by the *in situ* preservation strategy is of great interest.

Lung preservation procedures, including heparin, ventilation, and bronchoscopy, were all applied postmortem. Only blood was withdrawn just before hands-off. This decision might be considered a weakness of our protocol, particularly in countries where, unlike Italy, there is a general education about this kind of donation. We elected to use this strategy to make this novel donation process easier to accept.

In conclusion, we have confirmed previous findings on the feasibility of lung donation from uDCDD. We also provided evidence that *in situ* lung preservation with recruitment maneuvers, CPAP, and protective ventilation followed by EVLP after procurement allows lung transplantation after extended periods of warm ischemia.

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### Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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