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Overcoming the Limits of Reconditioning: Seventeen Hours of Ex-Vivo Lung Perfusion (EVLP) with Successful Transplantation from Uncontrolled Circulatory Death Donor

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Abbreviations:

bpm, breaths per minute

CIT, cold ischemic time

DCD, donation after circulatory death

EVLP, ex-vivo lung perfusion

FEV1, forced expiratory volume in one second

FiO_2, fraction of inspired oxygenNRP, normothermic regional perfusion

PaO_2, partial pressure of oxygen

PEEP, positive end-expiratory pressure

PRBC, packed red blood cell

PVR, pulmonary vascular resistances

RR, respiratory rate

rTPA, recombinant tissue plasminogen activator

T-LGL, T-large granular lymphocyte leukaemia

TV, tidal volume

uDCD, uncontrolled donation after circulatory death

Abstract

Background:

Uncontrolled donors after circulatory death (DCD) are an extraordinary resource to increase the number of lungs available for transplantation. However, the risk of the warm ischemia resulting from cardiac arrest to irreversibly damage the organs is considerable. Moreover, graft preservation issues and organizational problems often worsen the dangerous effects of warm ischemia. Ex-Vivo Lung Perfusion (EVLP) enables to evaluate and recondition lungs whose functionality is doubtful, as well as to overcome the difficulties related to time and logistics.

Methods:

We report the case of uncontrolled DCD lungs successfully treated with an exceptionally prolonged EVLP. Since the donor's blood count and liver biopsy showed signs of a possible leukaemia, EVLP was protracted up to seventeen hours while waiting for immunohistochemical analyses to rule out this diagnosis; eventually, the results came back negative and lungs were judged suitable for transplantation.

Results:

The recipient was a 32-year old male with cystic fibrosis, colonized by *Pandoraea pnomenusa.* Bilateral transplantation required central extracorporeal membrane oxygenation. The patient was extubated after 36 hours and was discharged 21 days after the operation. Despite early re-colonization by *Pandoraea pnomenusa* and airway complications requiring pneumatic dilatation, he is alive and has a satisfactory respiratory function 15 months after transplantation.

Conclusions:

Uncontrolled DCD represents a challenge due to both logistical issues and the complexity of grafts evaluation before procurement: EVLP with cellular perfusate could be a valuable tool to overcome these limits. Nonetheless, caution should be exercised when interpreting the effects of this technique on airway healing.

INTRODUCTION

Lung procurement from donation after circulatory death (DCD) donors is currently under development for increasing the organ pool[1]. In this scenario, the uncontrolled (u-) setting is the most promising for its potential. Nevertheless, the challenges to overcome organizational problems and those related to graft preservation and evaluation are undeniable[2,3,4]. In 2014 our Lung-DCD project started, including *in-situ* preservation with normothermic open-lung approach and *ex-situ* assessment with ex-vivo lung perfusion (EVLP)[5]. We present a case that required an extraordinary long period of EVLP to overcome the unpredictability of the uDCD setting.

MATERIALS AND METHODS

This study was approved by Ethics committee of Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico of Milan. Ref. n° 181 (24/01/2017). A 57-year-old non-smoking male had a witnessed cardiac arrest at home and received immediately the basic life support by bystanders. Brought to the San Gerardo Hospital (Monza), after 78 minutes of cardiopulmonary resuscitation (3 shocks, 12 adrenaline boluses, 69 minutes of manual chest compressions, and 9 minutes of mechanical chest compressions), the patient was declared dead. The only known disease in his medical history was systemic hypertension. The local team considered the subject as a possible lung donor and started the *in-situ* protocol preservation[5]. Briefly, after the clinical diagnosis of death, a recruitment manoeuvre was performed and continuous positive end-expiratory pressure (10 cmH_2O, 100% FiO_2) was applied until death confirmation (20 minutes of flat electrocardiogram). After the consent from the relatives, heparin was given (10000 U endovenous), a new recruitment manoeuvre performed and the *in-situ* preservation with protective ventilation (tidal volume, TV 6 ml/kg ideal body weight, PEEP 8 cmH_2O, respiratory rate, RR of 4 breaths per minute, bpm, FiO_2 100%) completed until the arrival of the retrieval team from the Milan lung transplant centre. The chest radiograph showed right upper lobe opacity (Figure S1 http://links.lww.com/TP/C111); bronchoscopy revealed abundant frothy

secretions. At inspection, the lungs were judged suitable for EVLP. The main pulmonary artery was cannulated, 500 mcg of prostaglandins E1 administered. Anterograde cold flush with low-molecular dextran solution with 15 mg of rTPA was started together with topical cooling. *In-situ* retrograde flushing was completed, and the lung block was stored on ice, after usual retrieval. In the meantime, the first results available showed a B blood group and an inversion of the CD4+ to CD8+ lymphocyte ratio; upon suspicion of a previously unknown lymphoproliferative disease, with the features of T-large granular lymphocyte leukaemia (T-LGL), the retrieval team obtained lymphnode, spleen, hepatic and renal biopsies. Grafts were transported to our centre for the usual evaluation[6,7]. In short, our EVLP system consists of a custom-made circuit. The lungs are accommodated in a dedicated organ chamber from which the perfusate is drained by gravity in a venous reservoir and then drawn by a centrifugal pump and pushed into a de-oxygenator connected to a heat-exchanger. The perfusate then reaches a pulmonary cannula fastened to the pulmonary artery, crosses the lung vasculature, and is drained from the left atrium left open to return to the organ chamber. All the circuit elements are connected by 0.375-inch heparin-coated polyvinyl tubing system. EVLP lasts 4 hours, including a perfusate flow of 40% of donor cardiac output with target pulmonary artery pressure of less than 15 mmHg, an open atrium and a cellular perfusate. The ventilatory setting provides for a TV of 7 ml/kg (donor ideal body weight) with a RR of 7 bpm (FiO_2 21%). The pulmonary functional assessment is made hourly at FiO_2 of 100%. The first lung evaluation after one hour showed a good lung function in terms of oxygenation, lung mechanics and hemodynamic: PaO_2/FiO_2 407 mmHg, static lung compliance 115 ml/cmH_2O, pulmonary vascular resistances (PVR) 187 dyn*sec/cm^5. Simultaneously, however, the detection of a mononuclear cell infiltrate in the portal spaces but, most notably, in the sinusoids of the donor's liver (Figure 1) supported the suspect of a T-LGL, which could be excluded only upon immunohistochemical analysis, the results of which would have only been available on the next morning. At this point, we were faced with a difficult choice: wait several hours for the

definitive histological diagnosis, suspend the EVLP and prolong the cold ischemic time (CIT), or discard the grafts. The partial results supporting the suspicion of a T-LGL came when the EVLP was already underway. At that point, stopping the EVLP would have meant a very prolonged cold storage, the first CIT of 3 h 46 minutes plus the second CIT of around 13 h waiting for anatomopathological definitive results. Even though experimental[8] and clinical[9] studies reported successful transplantation after prolonged preservation time, in this case a total CIT of almost 25 hours for the left lung and 28 hours for the right lung, would have been added to the 223 minutes of warm ischemic time of an uncontrolled DCD. We believed that it would have been too great a risk. Moreover, in this peculiar scenario of prolonged CIT in a uDCD, for extreme caution, we would have reassessed the lungs through a second EVLP before transplant. The option of a double EVLP and a triple CIT appeared to be even less predictable and unexplored than the one we adopted. We decided to continue the EVLP beyond our standard 4-hour protocol. The circuit was primed with 2000 ml of STEEN solution plus 2 units of packed red blood cell (PRBC). Assessment of respiratory, hemodynamic, and metabolic parameters was performed throughout the entire EVLP duration (Figure 2). Blood gas analyses were collected hourly for the first 4 hours and then every 2-3 hours. Before any evaluation, recruitment manoeuvres were performed. PaO_2/FiO_2 ratio improved steadily from 407 to 577 mmHg; the static lung compliance maintained good stable values (106±11 ml/cmH_2O) during all 17 hours. PVR remained constantly below 200 dyn*sec/cm^5. Careful perfusate changes were made by monitoring sodium, glucose, and lactate levels. Regular replacements of 250 ml of perfusate every hour for the first 4 hours and subsequently every three hours were performed, while 1 unit of PRBC was added around every 4 hours for a total of 4500 ml of STEEN solution and 1800 ml of PRBC (i.e. 6 units) throughout the whole EVLP perfusion. The haematocrit of the perfusate was maintained within 10 and 15% for the entire EVLP but at the 17th hour, when we recorded 17% of haematocrit. Thamesol 3,6% was given in repeated boluses for a total of 120 ml and continuous infusion of free water started from the seventh hour of the EVLP (500

ml total). Supplements of antibiotics and steroids were made for a total of 2 g of Imipenem and 750 mg of Methylprednisolone. At 8:30 am, the pathologist reported a global picture consistent with a reactive lymphocyte expansion. The final lung function evaluation showed a PaO_2/FiO_2 of 577 mmHg, a static lung compliance of 96 ml/cmH_2O and 75 dyn*sec/cm^5 of PVR. Sodium, glucose and lactate levels were respectively 152 mmol/L, 162 mg/dl and 13 mmol/L. There was no sign of oedema at macroscopic visual and palpatory assessment, the X-rays showed the resolution of the right upper lobe infiltrate (Figure S2 http://links.lww.com/TP/C111). The bronchoscopy showed no secretions, bleeding, or plasmorrhea. After a collective discussion, the lungs were judged suitable for transplantation. EVLP duration was 17 hours and 19 minutes. The recipient was a 32-year-old patient with cystic fibrosis, identical blood group, on waiting list for 13 months; his last lung allocation score was 37.12. The patient was on 24/24 hours oxygen supply and nocturnal non-invasive ventilatory support, recovering from recent pulmonary exacerbation. His lower airways were colonized by *Pandoraea pnomenusa*. Our immunosuppressive regimen consists of triple drug therapy (calcineurin inhibitor, antiproliferative agent, corticosteroids) without induction therapy. The bilateral transplantation required a central extracorporeal membrane oxygenation support before the first pneumonectomy, which was ceased after the reperfusion of the second graft. Airway anastomoses were covered with vital recipient tissue. Seven units of PRBC and eight units of fresh frozen plasma were administered. At the end of surgery, the patient was ventilated with a TV of 7 ml/kg with a RR of 15 bpm. He had a PaO_2/FiO_2 of 212 mmHg, a $PaCO_2$ of 44 mmHg and a static lung compliance of 39 ml/cmH_2O. Stable hemodynamic was recorded with a minimum vasoconstrictor support (Noradrenaline 0.06 mcg/kg/min). Figure 3 depicts the timeline of the process.

RESULTS

The patient was extubated after 36 hours and discharged from the intensive care unit on the third postoperative day. Primary graft dysfunction at 24, 48 and 72 hours was scored to 2, 1, and 1, respectively[10]. The postoperative period was uneventful, and the patient was discharged at home after 21 days from transplantation with a FEV1 of 51%. After three months, he was hospitalized for acute respiratory failure; chest radiograph revealed left basal pneumonia; bronchoalveolar lavage culture confirmed the early airway re-colonization by *Pandoraea pnomenusa*. At the same time, bronchoscopy showed expulsion of suture fragments within the bronchial lumen as well as a non-suture-related stenosis of the bronchus intermedius (extent a) and superior left lobar bronchial stenoses distal to bronchial suture (extent b)[11]. He was successfully treated with an aggressive course of antibiotics (carbapenems, cotrimoxazole, ciprofloxacin), high flow nasal cannula oxygen therapy and he underwent pneumatic dilatation. The patient was successfully weaned from oxygen supply and adapted to non-invasive ventilation. Two months after, a second pneumatic dilatation was performed. Surveillance CT scans and transbronchial biopsies at 6 and 12 months did not show any sign of lung allograft dysfunction (Figure S3 http://links.lww.com/TP/C111). The last bronchoscopy revealed a satisfactory patency of bronchial tree. The patient is currently (15 months after surgery) eupnoeic on room air, with good exercise tolerance; last FEV1 is 50% of predicted (best FEV1 54%) and the distance covered at 6-minutes walking test is 628 meters on room air. He is currently struggling to find a suitable job and practices soccer twice a week.

DISCUSSION

In the presented case of transplantation from uDCD, extending the duration of the EVLP up to 17 hours proved feasible, once granted the necessary time to provide the correct answer to the clinical question. The procedure showed positive outcomes in the short-mid term. Lungs from DCD donors suffer less from the sympathetic and inflammatory cytokine storm, which is typical of donations after brain death[12-14]; in addition, if kept ventilated, lungs well tolerate

warm ischemia[15,16]. In a recently published case series, patients transplanted with lungs from uDCD donors, showed excellent long-term outcomes, comparable to those achieved with a DBD donation[17]. These findings should encourage clinical utilization of lungs from DCD donors. Currently, there are roughly 350000 cases of cardiopulmonary resuscitation per year in Europe, 40% of which results in recoveries[18]. The opportunity provided by the great number of potential uDCD donors available each day is not adequately seized to date. In this context, lungs procurement from uDCD donors poses more serious logistical and safety challenges, when compared to standard procedures. We have been working on this issue for several years, managing preclinical research as well as logistical organization; the Welfare Direction of Regione Lombardia has actively endorsed our efforts. Our program includes lung procurement from different scenarios; a Full-DCD program for uncontrolled and controlled DCD donor combined with abdominal organs (normothermic regional perfusion, NRP) and a Lung-DCD program for uncontrolled DCD donor for isolated lung[5,19]. This last setting proves very promising since it does not require any extracorporeal program, thus it can be applied virtually in all hospitals, as recently confirmed[20]. Our first reported clinical case is today in good condition, showing no sign of chronic rejection (FEV1: 117%) and experiencing a self-reported high quality of life almost five years after transplantation[5]. In the case we are now reporting we stressed our Lung-DCD protocol by extending lung reconditioning to a previously unreported limit. EVLP was essential to increase the graft preservation time up to more than 36 hours. In an uDCD setting, gaining time is of paramount importance to enhance the donor evaluation, optimize the logistics and organization of the transplant, and, possibly, improve the graft reconditioning. The present report is meaningful: without the opportunity to extend the lungs preservation through normothermic perfusion, the graft would have been discarded due to high oncological risk. To the best of the authors' knowledge, human EVLP of similar duration has been reported only by the Padua group[21]. It is worth mentioning how both protocols, the Padua Organ Care System and our Milan EVLP technique, employed perfusate

enriched with erythrocytes. Few preclinical studies on prolonged EVLP are available, and those reporting extended EVLP time beyond 12 hours are even fewer. The available results after 24 hours of EVLP were produced by only two research groups[22,23]. The studies suggest that cellular normothermic EVLP, within a specific experimental design, may effectively extend lung preservation up to 24 hours. However, they do not address the uDCD scenario with its own peculiarities and present experimental models with limited warm ischemic times. Finally, pulmonary functional parameters at the end of the 24 hours EVLP or after subsequent transplant showed values at the limits of standard practice. Therefore, up to now, both preclinical and clinical data suggest that to prolong EVLP beyond 4-6 hours is challenging. In the presented case, we were particularly concerned about the large bronchi; in fact, if the functionality of the parenchyma had been adequately evaluated during the EVLP, no information would have been available on the bronchi viability and bronchial artery circulation[24]. Actually, our patient has developed bilateral airway stenosis. We can speculate on the potential role of re-colonization by *Pandoraea pnomenusa*, a multidrug-resistant bacterium, which is emerging in the context of cystic fibrosis. Also, the findings related to the expulsion of suture fragments within the bronchial lumen are difficult to interpret. Moreover, the use of alpha agonists as noradrenaline in the perioperative time could have played a role in the development of airway complications, even though the overall duration and the average dosage used in this case were very low. Furthermore, since our surgical technique does not include bronchial circulation sparing, norepinephrine could not have theoretically reached the airway vascularization. Anyway, it is undeniable that the bronchi experienced extended low perfusion, therefore caution with very prolonged EVLP especially with open atrium should be taken. In conclusion, we reported the first account of successful transplantation from uDCD donor employing lungs preserved with EVLP for 17 hours. Uncontrolled DCD is frequently

perceived as a challenge for increased logistical requirements and limited pre-procurement assessment; in our experience, prolonged EVLP with cellular perfusate showed that those limits could possibly be overcome. Great caution is mandatory in interpreting the effect of prolonged EVLP on airway healing.

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Figure legends:

Figure 1. Frozen section of the donor liver showing, at low magnification (a. Haematoxylin/eosin, 4x) a preserved lobular architecture with mild macrovescicular steatosis, absence of fibrosis and a moderate small lymphocyte infiltrate in the portal spaces, which is also noted in the sinusoids (b. Haematoxylin/eosin, 20x), together with sparse granulocytes.

Figure 2. Trends during 17 hours of EVLP. Panel A: PaO_2/FiO_2; Panel B: Static lung compliance; Panel C: Pulmonary vascular resistances; Panel D: Na^+ concentration.

Figure 3. Ischemic and preservation times from donor to EVLP and during recipient management. WIT, warm ischemic time; CIT, cold ischemic time

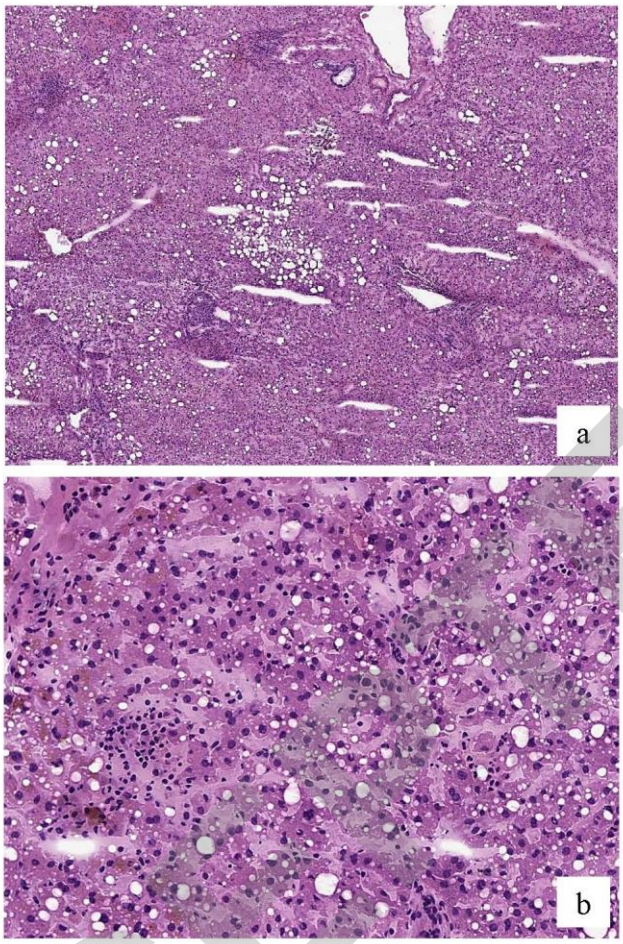

Figure 1.

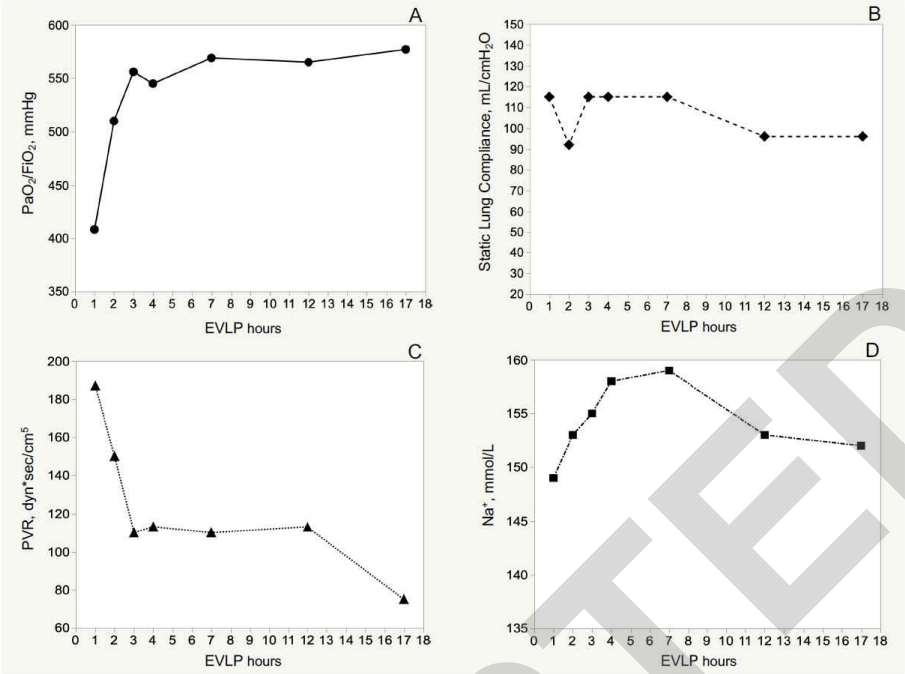

Figure 2.

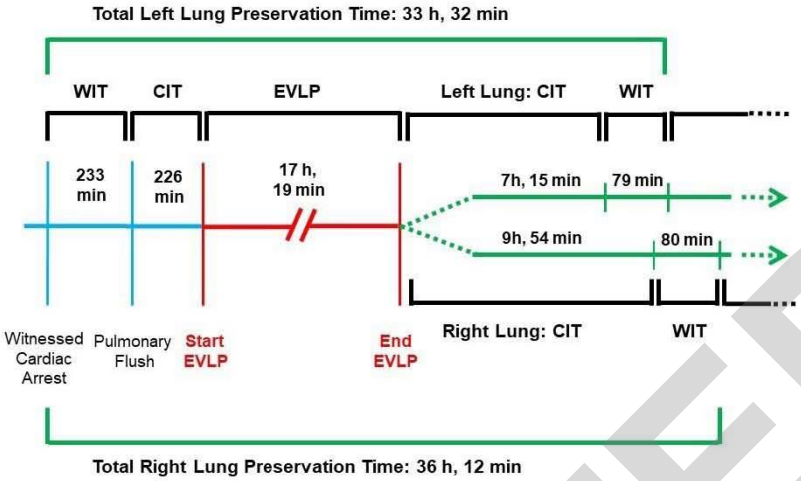

Figure 2: Ischemic and preservation times from donor to EVLP and recipient management

Figure 3.