ORIGINAL ARTICLE

Early pulmonary function and mid-term outcome in lung transplantation after *ex-vivo* lung perfusion – a single-center, retrospective, observational, cohort study

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SUMMARY

Outcomes after transplantation of lungs (LuTX) treated with *ex-vivo* lung perfusion (EVLP) are debated. In a single-center 8 years of retrospective analysis, we compared: donors' and recipients' characteristics, gas exchange and lung mechanics at ICU admission, 3, 6, and 12 months, and patients' survival of LuTX from standard donors compared with EVLP-treated grafts. A total of 193 LuTX were performed. Thirty-one LuTX, out of 50 EVLP procedures, were carried out: 7 from nonheart beating and 24 from extended criteria brain-dead donors. Recipients' characteristics were similar. At ICU admission, compared with standard donors, EVLP patients had worse PaO_2/FiO_2 [276 (206; 374) vs. 204 (133; 245) mmHg, $P < 0.05$], more frequent extracorporeal support (18% vs. 32%, $P = 0.053$) and longer mechanical ventilation duration [28 days of ventilator-free days: 27 (24; 28) vs. 26 (19; 27), $P < 0.05$]. ICU length of stay [4 (2; 9) vs. 6 (3; 12) days, $P = 0.208$], 28-day survival (99% vs. 97%, $P = 0.735$), and 1-year respiratory function were similar between groups. Log-rank analysis (median follow-up 2.5 years) demonstrated similar patients' survival ($P = 0.439$) and time free of chronic lung allograft disease ($P = 0.484$). The EVLP program increased by 16% the number of LuTX. Compared to standard donors, EVLP patients had worse respiratory function immediately after LuTX but similar early and mid-term outcomes.

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Introduction

Lung transplantation (LuTX) represents the only therapeutic option in many life-shortening lung conditions [1]. Unfortunately, the available pool of donors is limited, leading to 1-year waiting list mortality of patients enlisted for LuTX as high as 10–20% [2,3]. *Ex-vivo* lung perfusion (EVLP) is a strategy to evaluate and recondition extended criteria donor lungs and, eventually, increase the number of organs available for transplantation [4].

Many centers around the world adopted an EVLP program [5-11] reporting acceptance rates of extended criteria donor lungs after EVLP of about 80–90% and thus allowing a 15–20% increase in the total number of transplantations. To date, there is no universally recognized clinical standard for EVLP. Indeed, different criteria for selection of lungs candidate to the EVLP procedure have been described, leading to its application in (i) lungs from standard donors; (ii) lungs from brain-dead donors (DBD) with poor gas exchange and/or chest X-ray abnormalities; and iii) lungs from donors after cardiac death (DCD) donors, in whom evaluation of gas exchange is suboptimal. Similarly, different protocols [12-14] and technologies (i.e., XVIVO Perfusion™ and Organ Care™ Systems-OCS) for EVLP procedures are currently available for clinical application.

Despite this heterogeneity in indications and *ex-vivo* perfusion procedures, multiple studies reported that the early clinical outcomes of recipients of standard [15,16] and extended criteria [5-6,8,10,11] donor lungs undergoing EVLP are comparable to those of recipients of standard donor lungs preserved with static cold storage [17]. However, the actual impact of EVLP treatment on early postoperative and late graft function and on long-term patient outcome remains a matter of debate. In this scenario, a recent prospective observational trial questioned the safety and the efficacy of the EVLP technique [18]. The authors reported a low (<50%) acceptance rate of lungs after the EVLP treatment, and most importantly, they described a higher requirement of postoperative ventilatory and extracorporeal support and lower 1-year survival. Conversely, the Toronto group reported an acceptance rate >80% applying EVLP in both extended criteria DBD and DCD donors [6]. Recovery of graft function early after LuTX, mid-term graft function, indicators of patients' quality of life [19], and long-term patients' survival [20] are similar between recipients of conventional and EVLP-treated grafts.

The aim of the present study was to investigate retrospectively early and mid-term pulmonary function and patients' survival after LuTX from a mixed population of extended criteria DBD and DCD donors treated with EVLP compared with standard donor lungs undergoing static cold storage.

Methods

This single-center, retrospective, observational, cohort study was authorized by the Institutional Review Board (Comitato Etico Milano Area B—determina #181-2017). All patients candidate to LuTX at our Institution at the time of enlistment gave their written informed consent for data utilization and consented to receive extended criteria donor lungs treated with EVLP. The latter consent is renewed at the time of organ availability for transplantation.

Patients

Since January 2011, an EVLP program was started at the Milan Lung Transplant Center—Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. Data from all donors and recipients of LuTX performed between January 2011 and December 2018 at our Institution were included in this single-center observational study. Exclusion criteria were as follows: (i) LuTX recipient age <16 years old and (ii) DBD lungs undergoing normothermic *ex-vivo* perfusion due only to expected preservation time longer than 6 h. We analyzed lung donor characteristics, recipients' early and long-term respiratory function, and patients' survival comparing lungs from standard donors undergoing static cold storage (standard group) with lungs from either extended criteria DBD or DCD treated with EVLP (EVLP group).

Intervention

The Nord Italian Transplant program (NITp), the local organ procurement organization, allocates lungs to potential recipients based on blood group match. Until February 2016, lungs were offered in rotation to the different transplant centers that could accept or refuse the organ. Since March 2016, the evaluation of patients' Lung Allocation Score (LAS) was introduced in order to assess recipient's priority to LuTX [21]. Urgent cases take priority and are offered any compatible lung.

Standard group

After NITp allocation according to standard criteria, the thoracic surgeons procuring the lungs make the final decision about lung suitability for transplantation. After

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aortic cross-clamping and atrial venting, the lungs undergo anterograde flush with Perfadex™ solution, en-block recover, further retrograde flush, and finally stored in ice.

EVLP group

In our center, EVLP is employed in the following cases: (i) lungs from DBD with PaO_2/FiO_2 <300 mmHg on a positive end-expiratory pressure (PEEP) of 5 cmH_2O and/or with chest X-ray abnormalities after optimization of mechanical ventilation; (ii) lungs from DBD undergoing venoarterial ECMO (extra corporeal membrane oxygenation) for cardiocirculatory support during brain-dead observation, whose evaluation of gas exchange is suboptimal; and (iii) lungs from uncontrolled DCD II and controlled DCD III and IV donors according to the Maastricht classification [22,23]. Donors with massive lung contusion, history of aspiration of gastric content, pneumonia, or sepsis are excluded. Whether the preservation time is expected to last longer than 6 h because of logistical problems, the donor lungs undergo OCS treatment [16].

The DCD program applied at our Institution consists of a nonrapid normothermic open-lung procurement protocol, namely without pleural topical cooling before the start of pneumoplegia. The peculiarity of the Italian legislation is a 20 min of flat electrocardiogram observation to obtain a cardiac death diagnosis. In order to maintain lung oxygenation in the absence of blood flow, either mechanical ventilation or CPAP are ensured throughout the whole cardiac death observation and lung procurement procedures (Appendix S1 for DCD protocol details).

All grafts candidate to EVLP are transported to our Institution cold stored in ice. Once the graft arrives at the lung transplant center operating room, normothermic *ex-vivo* perfusion is performed with an open atrium technique, Steen solution added with red blood cells to obtain a low hematocrit (5–10%) is used as a perfusion solution, the target perfusate flow is 40% of the estimated donor's cardiac output, and the duration of the procedure is 4 h, as previously described elsewhere [24]. Lungs are considered unsuitable for transplantation if deterioration of lung mechanics, pulmonary vascular resistance, X-ray imaging, and fibrobronchoscopy is observed along the EVLP procedure and if the pulmonary venous PaO_2/FiO_2 after completion of EVLP is <450 mmHg.

Lung transplantation surgery, anesthesia management, the requirement of intraoperative extracorporeal membrane oxygenation (ECMO) support, and postoperative care are similar in the standard and EVLP groups (Appendix S1). The evaluation of graft function was performed at 3, 6, and 12 months after LuTX or whenever clinical signs of deterioration were observed [25].

Data source

Information regarding lung donors was obtained by reviewing the NITp database and the data recorded by the thoracic surgeons performing organ procurement. Postoperative data were collected from the medical and nursing records of the patients' ICU and hospital stay. Actual survival was determined by accessing the last updated follow-up visits.

Measurements

Donors' demographic characteristics, cause of death, and lung function were collected at the time of the lungs' offer. The last PaO_2/FiO_2 value available from donors' lung was also recorded. The Oto lung donor score [26] was calculated for all patients, except for DBD on extracorporeal support and all DCD. In both groups, total preservation time was calculated from pulmonary flush on the donor site to lung positioning into the recipient thorax, while surgical warm ischemia time (WIT) was calculated from lung positioning into the recipient thorax to lung reperfusion. Lungs from DCD donors suffered a further warm ischemic time while on the donor site that was partitioned between low-flow, the time the patient spent at systolic arterial pressure below 50 mmHg, and no-flow, the time between the patient cardiac arrest and the initiation of lung cold flush during graft procurement.

Recipients' demographic data, diagnosis, time on waiting list, lung mechanics, gas exchange, and LAS at the moment of the last evaluation preceding LuTX were recorded [27]. All patients underwent right heart catheterization to evaluate pulmonary hypertension. Recipients' incidence of multidrug-resistant pulmonary bacterial colonization, hospital admission at the time of LuTX, and requirement of ECMO bridge to LuTX were recorded. Type of LuTX (single versus double), configuration and timing of eventual intraoperative ECMO support, warm ischemia time duration, and transfusions of blood products during surgery were collected.

During the first three postoperative days, type of ventilator support, respiratory mechanics, and gas exchange were recorded. We calculated compliance of respiratory

system and intrapulmonary shunt fraction according to standard formulae. To describe gas exchange function while accounting for the level of respiratory support, the PaO_2/FiO_2 level was normalized by the level of positive end-expiratory pressure (PEEP) applied. This modified Oxygenation Index (mOI) was calculated as follows $(FiO_2{*}PEEP)/PaO_2$, with higher values indicating worse respiratory function.

All parameters based on arterial oxygenation value (i.e., PaO_2/FiO_2, intrapulmonary shunt, mOI) were not calculated for patients on extracorporeal support. Primary graft dysfunction (PGD) was assessed at 72 h postlung reperfusion [28], and patients on ECMO were graded as PGD 3. Lung function was assessed at 3, 6, and 12 months after transplantation, while chronic lung allograft disease diagnosis was defined as a persistent decline in pulmonary function [25]. The following outcomes were recorded the following: requirement of post-transplant ECMO support, duration of ECMO support, ICU length of stay (LOS), ventilator-free days at 28 days, incidence of reintubation, tracheostomy, and post-transplant major and minor airway complication respectively requiring or not intervention (either pneumatic dilation or re-absorbable prosthesis positioning) [29]. Patients' survival was assessed with a minimum follow-up time of 1 year. Whether both transplantation and retransplantation occurred within the study period, both events were considered as separate cases and patients' follow-up was interpreted as interrupted at the time of retransplantation. Because of the retrospective nature of the study when analyzing patient's survival, we considered the following as covariates: recipient age, disease (cystic fibrosis vs. noncystic fibrosis), occurrence of PGD 2 or 3 at 72 h post-transplantation, hospitalization at the time of transplantation, donor cause of death (DBD versus DCD), and graft total preservation time.

Statistical analysis

All continuous variables are presented as mean ± standard deviation or as median [interquartile range], as appropriate. Categorical variables are expressed as absolute number (percentage). Comparisons of continuous data were performed with Student's *t*-test or the rank-sum test, as appropriate, while the chi-square test was used to compare categorical data. Survival analysis was performed by using log-rank Kaplan–Maier estimator and displayed as time to event data. The hazard ratio was computed performing a multivariable Cox regression model with restricted cubic splines for continuous covariates, as appropriate (Appendix S1). No missing data are observed concerning survival analysis, while for all other analysis, missing data were maintained below 20% of total data count. Effect size is expressed as mean difference (95% confidence interval) for continuous data and odds ratio (95% confidence interval) for categorical data. *P* value <0.05 was assumed as significant. Data were analyzed with SIGMAPLOT 11.0 software and R-CRAN software—version 3.5.3.

Results

From January 2011 to December 2018, 213 lung donors have been procured at our Institution (Fig. 1). One hundred sixty LuTX were performed from standard donor lungs. Fifty-two lungs from extended criteria DBD ($n = 37$) and DCD ($n = 15$) underwent EVLP, and among DBD donors, two grafts were treated with transportable *ex-vivo* perfusion through OCS device due only to expected preservation time longer than 6 h. Data from these two pair of donor and recipient are excluded from the present analysis. Thirty-one lungs (24 DBD and 7 DCD) were judged suitable for transplantation, corresponding to an overall acceptance rate of 62%. Causes of lung unsuitability for transplantation in the remaining 19 cases are summarized in Fig. 1. Figure 2 illustrates the number of transplantations per year partitioned between standard- and EVLP-treated grafts. Comparison between accepted and rejected lungs is presented in Table S1: Unsuitable grafts showed higher pulmonary vascular resistance [361 (285; 416) vs. 478 (362; 570) dyne*cm/s^5; $P = 0.030$; mean difference −120 (−233; −7)], lower PaO_2/FiO_2 [532 (487; 570) vs. 393 (283; 474) mmHg, $P < 0.001$; mean difference 142 (80; 203)], and lower compliance [132 (87; 172) vs. 70 (52; 83) ml/cmH_2O, $P < 0.001$; mean difference 62 (28; 97)].

Donors' characteristics and EVLP parameters

Donors' demographic parameters were similar in the two groups, except for a higher body mass index in the EVLP group and for the cause of death (Table 1). Oxygenation in the EVLP group was impaired resulting in a higher Oto Score, despite a similar duration of mechanical ventilation between the two groups. Grafts from DCD donors which resulted suitable for LuTX at the end of the EVLP procedure suffered further *in situ* WIT, respectively, for DCD II and III: 53 ± 58 and 13 ± 4 min of low-flow time and 223 ± 57 and 125 ± 37 min of no-flow time.

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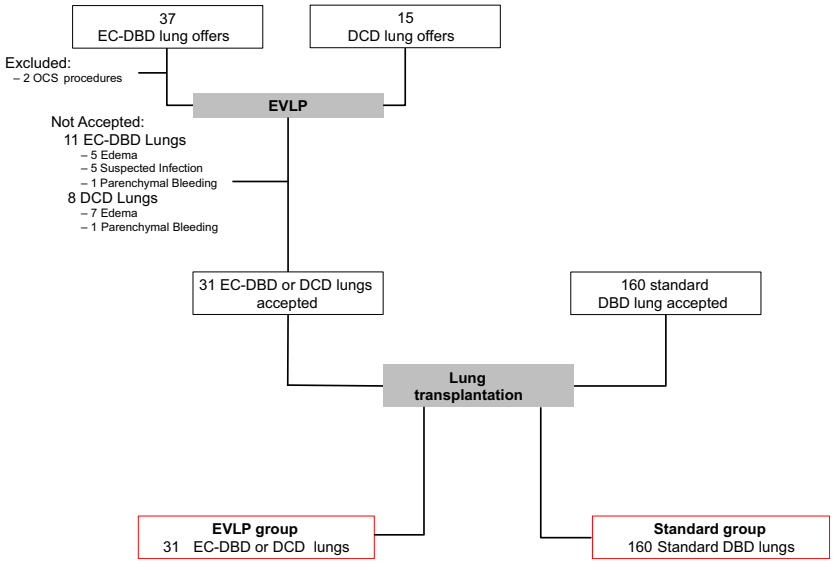

Figure 1 Donation process flowchart. The figure illustrates the decisional pathway of lung offering and acceptance for transplantation at the Milan Lung Transplant Center along the study period. DCD, donor after cardiocirculatory death; EC-DBD, extended criteria donor after brain death; EVLP, *Ex-vivo* lung perfusion; OCS, organ care system.

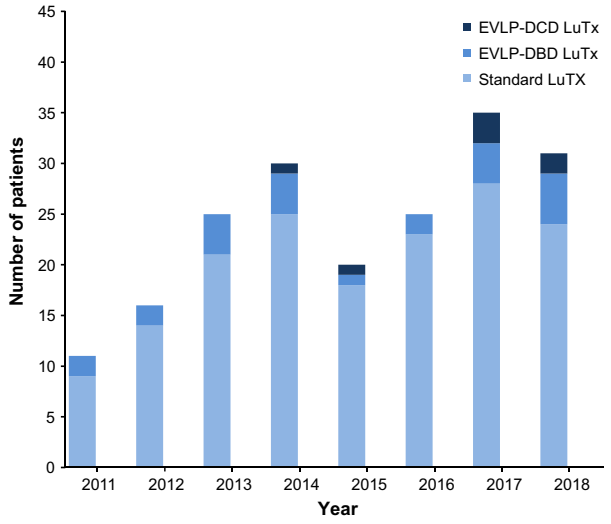

Figure 2 Lung transplant activity. The figure illustrates the progressive increase in the number of lung transplantation performed per year along the study period and the specific contribution of standard lung donors, extended criteria DBD-EVLP-treated lungs and DCD-EVLP-treated lungs. DBD, donor after brain death; DCD, donor after cardiocirculatory death; EVLP, *ex-vivo* lung perfusion.

Extended criteria DBD and DCD lungs judged suitable for transplantation underwent 250 [225; 296] min of EVLP and showed good function at the end of the procedure: PaO_2/FiO_2 = 532 [487; 570] mmHg, pulmonary vascular resistance = 361 [285; 416] dyne*s/cm^5 and lung compliance = 132 [87; 172] ml/cmH_2O. Lung weight increased along with the EVLP procedure [889 ± 60 g pre-EVLP vs. 1027 ± 64 g post-EVLP, $P < 0.001$; mean difference 140 (94; 186)]. Total preservation time was extended in EVLP-treated lungs due to both the EVLP time and a longer cold ischemia time.

Pre- and intraoperative recipients' characteristics

Table 2 shows the recipients' characteristics. We observed no statistically significant difference between patients' cohorts. Cystic fibrosis represented the most frequent indication for LuTX (i.e., 106, 55%). About one-third of the patients were hospitalized at the time of lung offer and 24 patients (12%) required urgent transplantation while on ECMO support as a bridge to LuTX.

Bilateral LuTX was the predominant surgical procedure (i.e., 152, 79%). Intraoperative ECMO support was more frequent in the EVLP cohort, as compared to the standard cohort [61% vs. 39%; $P = 0.026$, OR 2.4 (1.1; 5.4)]. The most common configuration was venoarterial ECMO with central cannulation (Table 4). Intraoperative warm ischemia time was not different between groups.

Postoperative function, follow-up, and survival

Early pulmonary function after LuTX is shown in Table 3. Immediately after transplantation, recipients of EVLP-treated lungs showed lower PaO_2/FiO_2 and required higher PEEP levels, thus showing a higher mOI [3.0 (2.1; 4.5) vs. 5.1 (3.9; 8.5), $P < 0.001$; mean difference −2.1 (−3.5; −0.7)]. Respiratory system compliance measured on the first postoperative day was similar between the standard and EVLP group recipients [36 (28; 45) vs. 32 (28; 45) ml/cmH_2O, $P = 0.491$, mean difference −1.5 (−7.5; 4.6)], while intrapulmonary shunt fraction was higher in the EVLP group [11 (6; 16)% vs. 17 (11; 28)%, $P = 0.015$, mean difference 10 (4; 15)]. Except for two patients who required to maintain venoarterial ECMO for both respiratory and hemodynamic support in the postoperative phase, peripheral

Table 1. Donors' characteristics.

Donors (n = 191)	Standard (n = 160)	EVLP (n = 31)	P value	Effect size
Age, years	47 [32; 56]	49 [42; 58]	0.315	4 (−2; 9)
Male sex, n (%)	88 (55)	23 (74)	0.074	2.3 (1.0; 5.6)
BMI, kg/m^2	24.1 [21.4; 26.2]	27.7 [24.9; 29.0]	<0.001	2.9 (1.4; 4.5)
Cause of death, n (%)				
DBD	160 (100)	24 (78)	<0.001	–
Cerebrovascular	90 (56)	17 (55)		0.9 (0.4; 2.0)
Trauma	45 (28)	3 (10)		0.3 (0.1; 0.9)
Postanoxic	14 (9)	4 (13)		1.5 (0.5; 5.0)
Other	11 (7)	0 (0)		–
DCD	0 (0)	7 (23)		–
Class II	–	2		–
Class III	–	4		–
Class IV	–	1		–
MV duration, days	2 [1; 3]	3 [1; 5]	0.095	1 (0; 3)
PaO_2/FiO_2, mmHg	456 [387; 518]	289 [230; 323]	<0.001	−167 (−127; −204)
Oto score	4 [2; 5]	8 [6; 10]	<0.001	4 (3; 5)
Total preservation time, min				
1st Lung	307 [240; 375]	867 [706; 932]	<0.001	567 (475; 558)
2nd Lung	520 [455; 582]	1052 [968; 1175]	<0.001	532 (472; 576)
Cold ischemia time, min				
1st Lung	307 [240; 375]	595 [498; 661]	<0.001	288 (222; 301)
2nd Lung	520 [456; 582]	788 [745; 912]	<0.001	268 (226; 326)

BMI, body mass index; DBD, donor after brain death; DCD, donor after cardiocirculatory death; MV, mechanical ventilation; PaO_2/FiO_2, arterial partial pressure of oxygen to fraction of inspired oxygen ratio.

P values <0.05 are assumed as statistically significant. Effect size is expressed as mean difference (95% CI) for continuous variables or OR (95% CI) for categorical variables.

veno-venous ECMO was the configuration of choice in cases of insufficient graft function at the end of the surgical procedure. More patients in the EVLP group required postoperative ECMO support [32% vs. 18% P = 0.053, OR 2.1 (0.9; 5.1)], but it was rapidly weaned in both groups [2 (1; 4) vs. 2 (1; 3) days, P = 0.674, mean difference −2 (−5; 2)]. Suboptimal gas exchange at 24 h after LuTX in EVLP recipients required longer duration of mechanical ventilation and higher levels of PEEP. However, lung function rapidly recovered leading to similar ICU-LOS. Rate of PGD grade 2–3 at 72 h from LuTX was similar between groups [i.e., 24% vs. 27%, P = 0.910, OR 0.9 (0.4; 2.1)]. Four patients died in ICU, two in each group: 3 because of septic shock and 1 for a cardiovascular event.

Mid-term outcomes are shown in Table 4. Airway complications were more frequent in the EVLP group, none of them affecting grafts from DCD donors. Among survivors, oxygenation was preserved in the majority of patients and forced vital capacity progressively improved along the first year of follow-up in both groups. Forced expiratory volume and oxygenation were slightly reduced at 6 months after LuTX in the EVLP group, but afterward, it improved similarly in both groups.

Log-rank survival analysis did not show statistically significant difference in survival between standard and EVLP-treated recipients: Survival at 28 days, 6 months, 1 year, and 2.5 years was, respectively, 99 [98–100]% vs. 97 [91–100]%, 92 [88–96]% vs. 87 [76–98]%, 85 [80–91]% vs. 74 [60–91]%, and 70 [63–78]% vs. 61 [44–83]%; P = 0.439 (see Fig. 3). Median follow-up time was 2.5 [1.3–4.6] years. Five patients, all in the standard group, received both transplantation and retransplantation within the study period, with one retransplantation performed with an EVLP-treated graft. Multivariable Cox proportional hazard model showed a higher probability of post-transplant survival among cystic fibrosis recipients, while no other association was detected among the variables analyzed (see Table 5). The On Line Supplement (Fig. S2) shows the whole population probability of survival adjusted for covariates.

The most frequent causes of death were sepsis (47% and 58% in standard and EVLP group, respectively) and chronic allograft rejection (26% and 25%). Time to

Table 2. Recipients' characteristics.

Recipients (n = 191)	Standard (n = 160)	EVLP (n = 31)	P value	Effect size
Age, years	44 [32; 58]	36 [25; 57]	0.281	−4 (−10; 2)
Male sex, n (%)	81 (51)	20 (64)	0.222	1.8 (0.8; 3.9)
BMI, kg/m^2	21.2 [18.9; 25.3]	21.0 [17.5; 23.0]	0.431	−0.6 (−2.2; 1.1)
Time on WL, days	136 [59; 277]	253 [44; 564]	0.157	114 (−3; 224)
Disease, n (%)				
Cystic fibrosis	77 (48)	18 (58)	0.617	1.5 (0.7; 3.2)
Pulmonary fibrosis	53 (33)	8 (26)		0.7 (0.3; 1.7)
COPD	13 (8)	2 (7)		0.8 (0.2; 3.6)
Bronchiectasis	4 (2)	0 (0)		–
Histiocytosis X	4 (2)	1 (3)		1.3 (0.1; 12.0)
LAM	1 (1)	1 (3)		5.3 (0.3; 87.1)
Re-LuTX	2 (1)	1 (3)		2.6 (0.2; 30.0)
Other	6 (4)	0 (0)		–
LAS	39.2 [34.6; 54.2]	44.8 [36.0; 60.5]	0.141	3.1 (−3.7; 9.9)
PaO_2/FiO_2, mmHg	235 ± 85	261 ± 78	0.143	26 (−9; 60)
$PaCO_2$, mmHg	44 [39; 50]	44 [38; 53]	0.529	−3 (−9; 3)
FVC, %	48 [38; 57]	42 [37; 59]	0.746	1 (−6; 8)
FEV1, %	32 [25; 51]	27 [23; 49]	0.193	−4 (−12; 4)
Pulmonary hypertension, n (%)				
Mild	62 (39)	11 (35)	0.606	0.9 (0.4; 1.9)
Moderate	19 (12)	3 (10)		0.8 (0.2; 2.9)
Severe	9 (6)	0 (0)		–
MDR bacterial colonization, n (%)	84 (52)	19 (61)	0.483	1.4 (0.6; 3.1)
Hospitalized at time of LuTX, n (%)	38 (24)	9 (30)	0.659	1.3 (0.6; 3.1)
Double LuTX, n (%)	126 (79)	26 (84)	0.686	1.4 (0.5; 3.9)
ECMO bridge to LuTx, n (%)	20 (12)	4 (13)	0.815	1.0 (0.3; 3.0)
Surgical approach, n (%)				
Bilateral thoracotomy	40 (25)	4 (13)	0.302	0.4 (0.1; 1.3)
Clamshell	88 (55)	21 (68)		1.7 (0.8; 3.9)
Monolateral thoracotomy	32 (20)	6 (19)		1.7 (0.6; 4.6)
Warm ischemia 1st lung*, min	81 [71; 95]	80 [60; 98]	0.629	0 (−7; 7)
Warm ischemia 2nd lung*, min	74 [62; 85]	72 [65; 90]	0.747	−3 (−10; 6)

BMI, body mass index; COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; FEV1, first second forced expiratory volume; FVC, forced vital capacity; LAS, lung allocation score; MDR, multidrug resistant; $PaCO_2$, arterial partial pressure of carbon dioxide; PaO_2/FiO_2, arterial partial pressure of oxygen to fraction of inspired oxygen ratio; VA, venoarterial; VV, veno-venous; WL, waiting list.

P values <0.05 are assumed as statistically significant. Effect size is expressed as mean difference (95% CI) for continuous variables or OR (95% CI) for categorical variables.

*Warm ischemia time considers only the intraoperative surgical time of graft implantation. Pulmonary hypertension is graded according to the mean pulmonary arterial pressure (PAPm) value: mild (PAPm ≥25 and ≤34 mmHg), moderate (PAPs ≥35 and ≤44 mmHg), or severe (PAPm >45 mmHg) [51].

CLAD occurrence did not differ between groups: Kaplan–Meier log-rank analysis P = 0.484 (Fig. S1).

Discussion

Main findings of the present study are as follows:

1. Initiation of an EVLP program allowed expansion of the lung donor pool to extended criteria DBD and to DCD, accounting for 16% of the total number of LuTX performed along the study period.

2. LuTX recipients of EVLP-treated lungs showed worse respiratory function immediately after transplantation and required more frequently postoperative ECMO support and longer duration of mechanical ventilation, with no difference in ICU length of stay.

3. Despite a higher rate of airway complications in the EVLP group, pulmonary function of patients within 1 year from LuTX was not different between recipient of either extended criteria DBD or DCD lungs treated

Table 3. Postoperative respiratory function.

	Admission		24 h		72 h	
	Standard group	EVLP group	Standard group	EVLP group	Standard group	EVLP group
PaO_2/FiO_2, mmHg	276 [206; 374] (n = 133)	204 [133; 245] (n = 21)	304 [228; 372] (n = 140)	276 [200; 304] (n = 26)	298 [231; 348] (n = 85)	282 [201; 357] (n = 21)
P value	<0.001		0.058		0.568	
Effect size	−88 (−140; −35)		−34 (−75; 6)		−6 (−53; 40)	
PEEP, cmH_2O	10 [8; 10] (n = 159)	10 [10; 12] (n = 31)	8 [0; 10] (n = 158)	10 [2; 12] (n = 31)	0 [0; 9] (n = 156)	6 [0; 10] (n = 31)
P value	0.006		0.139		0.027	
Effect size	−1 (−2; 0)		1 (−1; 3)		−2 (−4; 0)	
mOI	3.0 [2.1; 4.5] (n = 133)	5.1 [3.9; 8.5] (n = 21)	2.1 [0.0; 3.7] (n = 140)	3.7[0.7; 4.9] (n = 28)	1.8 [0.0; 4.1] (n = 83)	2.4 [1.6; 4.5] (n = 22)
P value	<0.001		0.180		0.150	
Effect size	−2.1 (−3.5; −0.7)		−0.8 (−2; 0.4)		−1.1 (−2.7; 0.4)	
Intubated, n (%)	160 (100)	31 (100)	62 (39)	22 (71)	48 (30)	14 (45)
P value	0.999		0.002		0.156	
Effect size	1 (1; 1)		3.9 (1.7; 8.9)		1.9 (0.9; 4.2)	
ECMO, n (%)	29 (18)	10 (32)	17 (11)	6 (19)	12 (7)	4 (13)
P value	0.053		0.287		0.522	
Effect size	2.1 (0.9; 5.1)		2.0 (0.7; 5.6)		1.8 (0.5; 6.1)	

ECMO, extracorporeal membrane oxygenation; mOI, modified oxygenation index; PaO_2/FiO_2, arterial partial pressure of oxygen to fraction of inspired oxygen ratio; PEEP, positive end-expiratory pressure.

Respiratory function assessed at ICU admission, 24, and 72 h post-transplantation. P values <0.05 are assumed as statistically significant. Effect size is expressed as mean difference (95% CI) for continuous variables or OR (95% CI) for categorical variables.

with EVLP and lungs from standard donors preserved with static cold storage.

4. Recipients of EVLP lungs showed similar mid-term probability of survival compared with recipients of standard organs.

Despite the recognized importance of optimizing lung function throughout brain death observation [30], up to 57% of the lungs are excluded from donation because of poor lung function at the time of procurement [31]. On the other hand, lung donation after circulatory death, despite being a potential source of suitable organs, might suffer from suboptimal lung function evaluation and the warm ischemia time ahead organ procurement. EVLP represents a unique platform that allows for the assessment of standard organ, the extension of the preservation time and—potentially—the restoration of organ homeostasis [32]. Based on these premises, and on the preliminary report of safety and efficacy of the procedure in increasing the number of transplantation from extended criteria DBD lungs [24], a structured an EVLP program was started at our Institution. During the study period, the total number of LuTX per year performed at our Center progressively increased, with EVLP-treated lungs corresponding to 16% of the total number of transplantations. In the Italian scenario, lung procurement from DCD represents a unique challenge due to the prolonged warm ischemia time on the donor site [33,34], since the legislation requires 20 min in the absence of cardiac activity to confirm death [35]. Nevertheless, experimental data show that, conversely to other solid organs, lung cells viability can be maintained even in the absence of blood flow by ensuring adequate alveolar oxygenation [36]. Lungs from DCD in our patients' cohort underwent an average warm ischemia time on the donor site longer than 120 min [37] and were all treated with EVLP before transplantation to evaluate lung function and eventually restore cells' energetic pool [38]. Remarkably, despite the prolonged warm ischemia time among DCD donors, we did not detect any airway complication among this subgroup. Notably, lung compliance and gas exchange and the pulmonary vascular resistance more than donor characteristics identified organs with suboptimal function. The oxygenation criterion is the most widely recognized parameter adopted to evaluate lung function both in the donor and during EVLP. Many

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Table 4. Recipients' outcome.

Outcome (n = 191)	Standard (n = 160)	EVLP (n = 31)	P value	Effect size
Intraoperative ECMO, n (%)	63 (39)	19 (61)	0.040	2.7 (1.2; 6.0)
VA-ECMO*	49 (78)	15 (79)		
VV-ECMO*	14 (22)	4 (21)		
RBC, units	3 [1; 7]	6 [1; 9]	0.137	2 (0; 5)
FFP, units	1 [0; 4]	3 [0; 6]	0.062	2 (0; 5)
PLT, units	0 [0; 0]	0 [0; 0]	0.032	2 (0; 3)
28-day ventilator-free days, days	27 [24; 28]	26 [19; 27]	0.028	−2 (−5; −1)
ECMO duration, days	2 [1; 4]	2 [1; 3]	0.674	−2 (−5; 2)
Tracheostomized, n (%)	23 (14)	5 (16)	0.980	1.1 (0.4; 3.3)
ICU-LOS, days	4 [2; 9]	6 [3; 12]	0.208	1 (−4; 6)
ICU readmission, n (%)	14 (9)	2 (6)	0.922	0.7 (0.1; 3.3)
Airway complications, n (%)*	9 (6)	5 (16)	0.040	2.6 (0.8; 8.4)
Dehiscence/stenosis, n	1/8*	0/5		
Minor, n (%)	3 (2)	1 (3)		
Bronchial dilatation, n (%)	3 (2)	4 (13)		
Bronchial stenting, n (%)	3 (2)	0 (0)		
3 months	n = 153	n = 28		
PaO_2/FiO_2, mmHg				
>300	138 (92)	25 (93)	0.639	0.9 (0.2; 3.4)
200–300	8 (5)	2 (7)		1.4 (0.3; 6.9)
<200	4 (3)	0 (0)		–
FEV1, %	72 [61; 86]	65 [49; 80]	0.074	−7 (−14; 1)
FVC, %	73 ± 17	69 ± 21	0.377	−3 (−11; 4)
6 months	n = 147	n = 27		
PaO_2/FiO_2, mmHg				
>300	137 (95)	24 (89)	0.004	0.6 (0.1; 2.3)
200–300	7 (5)	1 (4)		0.8 (0.1; 6.5)
<200	0 (0)	2 (7)		–
FEV1, %	77 [64; 93]	66 [58; 86]	0.047	−9 (−17; 0)
FVC, %	79 ± 18	74 ± 19	0.155	−5 (−13; 2)
12 months	n = 135	n = 23		
PaO_2/FiO_2, mmHg				
>300	126 (94)	22 (96)	0.763	1.4 (0.2; 11.4)
200–300	5 (4)	1 (4)		1.2 (0.1; 10.5)
<200	3 (2)	0 (0)		–
FEV1, %	84 ± 21	75 ± 19	0.074	−8 (−18; 0)
FVC, %	85 ± 18	82 ± 21	0.492	−3 (−11; 5)

ECMO, extracorporeal membrane oxygenation; FEV1: first second forced expiratory volume; FVC: forced vital capacity; ICU, intensive care unit; LOS, length of stay; PaO_2/FiO_2, arterial partial pressure of oxygen to fraction of inspired oxygen ratio.

Gas exchange at 3, 6, and 12 months from LuTX is expressed according to patients' oxygenation among survivors.

*Airway complications include bronchial anastomotic complications requiring either pneumatic dilation or re-absorbable prosthesis positioning. A single case of bronchial anastomosis dehiscence was secondary to surgical site infection. P values <0.05 are assumed as statistically significant. Effect size is expressed as mean difference (95% CI) for continuous variables or OR (95% CI) for categorical variables.

reports compare donor and EVLP PaO_2/FiO_2 without accounting for the lower hemoglobin content of the EVLP perfusate [4,7,9]: A level of mixed venous PO_2 of 450 mmHg might still represent a suboptimal threshold if the hemoglobin concentration is particularly low or absent [39]. For this reason, we believe that the oxygenation criteria should be associated with other parameters (e.g., lung compliance and imaging) and/or metabolic/biomolecular markers of organ function to better characterize donors' lung function. We emphasize the role of donors' lung evaluation to help the clinicians in donor-recipient matching, thus allowing expansion of lung donor pool without exposing recipients to the risk of receiving a damaged graft.

The median LAS score of our patients [39.9 (34.8–54.6)] indicates an intermediate disease severity, which

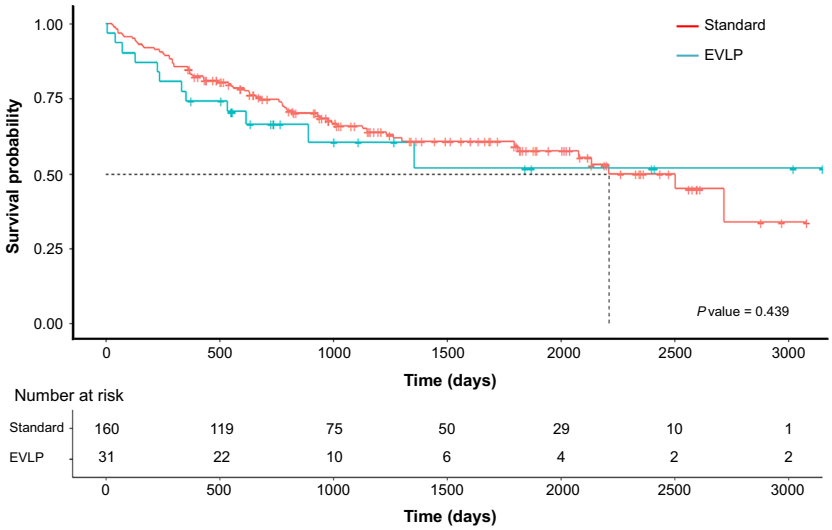

Figure 3 Graft survival analysis. Log-rank Kaplan–Maier analysis of actual survival between standard and EVLP-treated lung recipients. The number of patients at risk is displayed at predefined time intervals.

Table 5. Multivariable cox proportional hazard model analysis.

Variables	Hazard ratio [95% CI]	*P* value
EVLP	0.32 [0.05–2.03]	0.230
Age	–	0.102
Donation after cardiocirculatory death	1.08 [0.11–7.77]	0.949
Cystic fibrosis	0.44 [0.17–0.98]	0.045
PGD grade 2–3 at 72 h	1.41 [0.82–2.44]	0.207
Hospitalization at time of LuTX	0.89 [0.48–1.66]	0.723
Total preservation time	–	0.404

EVLP, *Ex-vivo* lung perfusion; LuTX, lung transplantation; PGD, primary graft dysfunction.

Variables included in the analysis are as follows: EVLP treatment (compared to standard treatment), donation after cardiocirculatory death (compared to donation after brain death), recipients' age, recipients' diagnosis of cystic fibrosis (compared to all other recipients' diagnosis), PGD grade 2–3 diagnosis at 72 h post-transplantation (compared to the absence of PGD grade 2–3 diagnosis), recipients' hospitalization at time of LuTX (compared to recipients' admitted from home at the time of LuTX), and graft total preservation time. For recipients' age and graft total preservation, we provide only the p value because estimated spline coefficients are not directly interpretable as HR [95% CI]. *P* values <0.05 are assumed as statistically significant.

might be explained by the high incidence of cystic fibrosis in the study population [40]. Our results are consistent with previous reports of delayed recovery of EVLP-treated lungs [8,9]. Recipients of EVLP-treated lungs had worse oxygenation immediately after transplantation, thus requiring higher PEEP or—in selected cases—continuation of ECMO after surgery. However, duration of extracorporeal support was similarly short in both patient groups and mechanical ventilation was just one day longer in the EVLP group, which did not result in delayed ICU discharge. In the overall study population, immediately after transplantation, the average PaO_2/FiO_2 was <300 mmHg but rapidly improved, being more probably explained by fluid overload than by an actual lung injury [41]. A higher incidence of bronchial anastomosis complications was observed in the EVLP group: We are unable to distinguish whether this is attributable to the extension of the total preservation due to the EVLP procedure or to the longer cold ischemia time occurred in the EVLP group because of logistic issues [42]. Airway complication rate is rarely reported when studying outcomes of EVLP-treated grafts [6,18]. It remains debated whether is there any advantage in performing the low-flow, acellular perfusate, and closed atrium EVLP technique in order to both reduce vascular shear stress and ensure a slightly positive left atrial pressure throughout the procedure. A possible advantage of the closed atrium EVLP technique has been hypothesized in order to maintain retrograde bronchial perfusion throughout the procedure [14,43,44].

Unlike the study of Fisher *et al.* [18] but similarly to the recent study of Divithotawela *et al.* [20], we did not observe a difference in survival between the two study groups. However, patients in the EVLP group had a 12% lower 1-year survival, a difference that, despite not reaching statistical significance, may be acknowledged as clinically relevant. Nevertheless, mortality rate of the EVLP group is in range with those previously presented [4,8-10]. The results of the EXPAND trial have been recently released [45]: The authors showed the efficacy (acceptance rate of 87%) and safety (91% one-year

patients' survival) of the portable normothermic *ex-vivo* lung perfusion system (OCS). The present study shows a different case-mix in both donor and recipient population compared with the EXPAND trial: Donor grafts enrolled in this study have lower PaO_2/FiO_2 (378 vs. 289 mmHg) and longer cold ischemic time, while recipients were younger and most of them diagnosed of cystic fibrosis. We observed a similar 28 days of survival rate 99% vs. 97% but a lower 12 months of survival 91% vs. 74% (with 85% survival in the standard group): It remains still not clear how much donor graft management affects 1-year survival. Two prospective multicenter nonrandomized clinical trials (NCT01365429 and NCT03343535) are currently underway to confirm the noninferiority of EVLP-treated extended criteria donor lungs compared with standard donor lungs preserved by static cold storage.

Primary graft dysfunction grading is commonly adopted to evaluate early postoperative lung performance [28]; however, the reported incidence of PGD is highly variable ranging from 0% to 30% [46]. The poor reproducibility of the index might be due to the confounding factors affecting CXR evaluation (e.g. fluid overload). Furthermore, grading PGD according to the sole PaO_2/FiO_2 value does not account for the level of ventilator support applied [39,47], despite mean airway pressure represents one of the main determinants of oxygenation in respiratory failure patients [48]. In the effort of better characterizing early graft function after LuTX, we report the oxygenation value normalized by the PEEP level applied, taking the latter as a surrogate for mean airway pressure. To our knowledge, this represents the first report of respiratory system mechanics and intrapulmonary shunt in the early phases after LuTX in a large patients' population. We observed impaired respiratory mechanics despite intermediate–low levels of shunt. This observation suggests that dead space, associated with lung collapse/edema, contributes significantly to impaired lung function after transplantation as previously demonstrated [49]. Further investigation of specific properties of the graft immediately after transplantation might provide evidence for tailoring mechanical ventilation of the newly transplanted organ.

Concerning the use of intraoperative ECMO support, the literature is highly heterogeneous. Some centers report the systematic use of "prophylactic" ECMO to avoid hemodynamic and ventilator stress on the newly transplanted lungs, while others adopt a more conservative strategy to avoid intraoperative anticoagulation and hemodilution, thus limiting positive fluid balance and transfusions. In our study, intraoperative ECMO support was more frequently instituted in the EVLP group. Comparing timing of intraoperative ECMO institution between the standard and EVLP groups, respectively, 20% vs. 23% of patients required ECMO before the first pneumonectomy while 10% vs. 29% ahead the second pneumonectomy. According to the available data, we are unable to disentangle whether intraoperative ECMO utilization represents an outcome, signifying a worse function of the transplanted lungs, or an explanation of the poorer lung function early after transplantation in the EVLP group. The most relevant confounding factor is that frequently the decision of ECMO initiation is not merely ascribable to impaired gas exchange or poor perfusion of the transplanted graft since also the evidence of right ventricular failure or surgical needs can play a significant role [50].

Limitations of the study

We acknowledge that the present study represents a single-center retrospective analysis covering a wide time frame and a relatively low number of LuTX procedures compared with other previous similar reports. However, we aimed to describe early and mid-term respiratory function after transplantation to identify potential factors affecting outcomes of LuTX from EVLP-treated lungs and finally to assess the potential advantages of the implementation of an EVLP program.

Conclusion

The initiation of the EVLP program at the Milan Lung Transplant Center increased the number of transplant procedures by 16% and allowed to expand the lung donor pool toward donors after cardiocirculatory death. Recipients of EVLP-treated lungs required more ventilatory and ECMO support in the early phase after transplantation and higher rate of airway complications, but this did not affect early and mid-term outcomes. The increase in LuTX procedures along with the noninferiority in patients' outcome justifies the adoption of an EVLP program even in an intermediate-volume LuTX center. The population described in the present study deserves specific considerations due to the peculiarities of Italian regulation regarding donation after cardiocirculatory death.

Authorship

JF, LR, FG, and AZ: conceived the study. JF and FG: collected data regarding pretransplantation function, intraoperative management, and ICU stay. LMC, VR, and PT: performed patients' follow-up and collected

survival data. EB: collected waiting list data. LR, AP, IR, PM, and DT: collected surgical data. JF, FG, VS, LR, and AZ: analyzed the data. GB: performed statistical consult. JF, FG, LR, and AZ: drafted the original version of the manuscript. MN, FB, FV, and GG: revised the manuscript critically; all authors approved the final version of the manuscript.

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Conflicts of interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial or nonfinancial interest in the subject matter discussed in this manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Freedom from CLAD. Log-Rank Kaplan-Maier analysis of time free from Chronic Lung Allograft Disease (CLAD) between Standard and EVLP treated lung grafts. The number of patients at risk is displayed at predefined time intervals.

Figure S2. Hazard function adjusted for covariates. The figure illustrates the hazard function with pointwise 95% confidence interval for the event death in the overall study patients' population.

Table S1. Comparison of donors' characteristics and EVLP parameters between EVLP-treated grafts judged as suitable (LuTX) and un-suitable (no LuTX) for transplantation.

Appendix S1. On Line Supplement Additional Methods.

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