ORIGINAL ARTICLE

A novel concept for evaluation of pulmonary function utilizing PaO2/FiO2 difference at the distinctive FiO2 in cellular *ex vivo* lung perfusion—an experimental study

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Meeting presentation: This manuscript was presented at the 38th annual International Society for Heart and Lung Transplantation meeting, Nice on April 13, 2018.

SUMMARY

For more accurate lung evaluation in *ex vivo* lung perfusion (EVLP), we have devised a new parameter, PaO_2/FiO_2 ratio difference (PFD); $PFD_{1–0.4}$ = P/F ratio at FiO_2 1.0 - P/F ratio at FiO_2 0.4. The aim of this study is to compare PFD and transplant suitability, and physiological parameters utilized in cellular EVLP. Thirty-nine human donor lungs were perfused. At 2 h of EVLP, $PFD_{1–0.4}$ was compared with transplant suitability and physiological parameters. In a second study, 10 pig lungs were perfused in same fashion. $PFD_{1–0.4}$ was calculated by blood from upper and lower lobe pulmonary veins and compared with lobe wet/dry ratio and pathological findings. In human model, receiver operating characteristic curve analysis showed $PFD_{1–0.4}$ had the highest area under curve, 0.90, sensitivity, 0.96, to detect nonsuitable lungs, and significant negative correlation with lung weight ratio ($R^2 = 0.26$, $P < 0.001$). In pig model, $PFD_{1–0.4}$ on lower and upper lobe pulmonary veins were significantly associated with corresponding lobe wet/dry ratios ($R^2 = 0.51$, $P = 0.019$; $R^2 = 0.37$, $P = 0.060$), respectively. $PFD_{1–0.4}$ in EVLP demonstrated a significant correlation with lung weight ratio and allowed more precise assessment of individual lobes in detecting lung edema. Moreover, it might support decision-making in evaluation with current EVLP criteria.

Transplant International 2019; 32: 797–807

Key words
donor lung assessment, *ex vivo* lung perfusion, lung lobes assessment, lung transplantation, PaO_2/FiO_2 ratio, PaO_2/FiO_2 ratio difference

Received: 28 November 2018; Revision requested: 30 December 2018; Accepted: 14 March 2019; Published online: 15 April 2019

doi:10.1111/tri.13426

Introduction

The shortage of donor lungs for lung transplantation remains a major problem, resulting in the death of many individuals on transplant waiting lists. Because the utilization rate of donor lungs is only approximately 20% in the United States [1], many attempts to utilize marginal donor lungs have been reported [2,3]. Currently, *ex vivo* lung perfusion (EVLP) has been recognized worldwide as a reliable and powerful tool when dealing with marginal lungs [4,5]. This tool provides us with donor lung preservation, [6,7] evaluation, [8–10] and treatment [11,12]. Although many studies have contributed to advancements in these three areas, we absolutely need to continue to improve their quality. Focusing on marginal lung assessment, there are still some challenges to overcome. For instance, pulmonary edema formation is a main finding of ischemia re-perfusion injury (IRI) of the current EVLP protocol [13]. However, current EVLP protocol is lacking in reliable objective parameters for pulmonary edema. Additionally marginal donor lungs are often not uniform in their ventilation, perfusion, and distribution of pulmonary edema, this heterogeneity makes the evaluation of the lungs more difficult. Therefore, more convenient and practical parameters for assessment of pulmonary function are strongly needed.

The partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) (P/F) ratio is one of the major parameters in the determination of donor lung utilization in cellular EVLP [14]. However, multiple factors including FiO_2, peak inspiratory pressure (PIP), positive end expiratory pressure (PEEP), ventilator mode, and cardiac output can influence the P/F ratio's predictive value for lung transplantation [15–18]. Our group previously demonstrated that P/F ratio varies at different FiO_2: 0.21, 0.4, and 1.0 in cellular EVLP [19]. Furthermore, we have shown that the pattern of P/F ratio at FiO_2 0.21 (P/F 0.21) < P/F 1.0 was significantly associated with higher P/F ratio, higher pulmonary compliance and lower shunt fraction, compared with the pattern of P/F 0.21 > P/F 1.0 in a porcine lung EVLP model [19]. To expand this concept, a new parameter for pulmonary function is proposed (Fig. 1). This new parameter, P/F ratio difference (PFD) is defined based on P/F 1.0 and P/F at other FiO_2. It is calculated as:

$$PFD_{1-0.4} = P/F\ 1.0 - P/F\ 0.4$$
$$PFD_{1-0.21} = P/F\ 1.0 - P/F\ 0.21$$

where P/F 1.0 is PaO_2/FiO_2 at FiO_2 1.0, P/F 0.4 is PaO_2/FiO_2 at FiO_2 0.4, and P/F 0.21 is PaO_2/FiO_2 at FiO_2 0.21.

We hypothesize that PFD may be an improved, predictive measurement of pulmonary function for donor lungs during EVLP. Furthermore, PFD may be used to evaluate pulmonary function for each lobe by blood gas analysis from assigned pulmonary veins (PV). The primary aim of this study is to compare PFD of human lungs to the following: physiological parameters, transplant suitability, and lung weight (LW). The secondary aim is to compare the calculated PFD of each PV in the pig lungs to the following: wet/dry (W/D) ratio, pathological findings, and cytokine analysis all from the corresponding lobe.

Material and methods

Study design

Human lung model

The human rejected donor lungs were procured in the standard fashion and stored in cold preservation solution for 4–6 h. Subsequently, the lungs were perfused for 2 h of cellular EVLP with Swedish protocol. The lungs were evaluated for transplant suitability with our established criteria (described below) at 2 h of EVLP. The calculated PFD was compared with transplant suitability, physiological parameters, and LW.

Pig donor after cardiac death (DCD) model

In order to observe the concept of PFD in each lobe, PFDs were calculated from upper or lower lobe PV and were compared with corresponding lobe W/D ratio, inflammatory cytokines, and pathological findings at 2 h of EVLP in the pig DCD model.

Human rejected donor lungs and selection criteria

Thirty-nine human donor lungs were rejected by the clinical transplantation team. The reasons are shown in Table 1. Our selection criteria for research lungs were as follows: (i) donor lungs were rejected by a clinical team; and/or (ii) P/F < 300 mmHg; and/or (iii) abnormal chest X-ray suggesting pulmonary edema or atelectasis. We excluded lungs from donors with a premorbid history of lung disease, pulmonary infection, bilateral contusion, bullous emphysema, and bilateral aspiration. The lungs were procured using the clinical protocol of regional organ procurement organizations [20]. The research consent for donation and the approval of the

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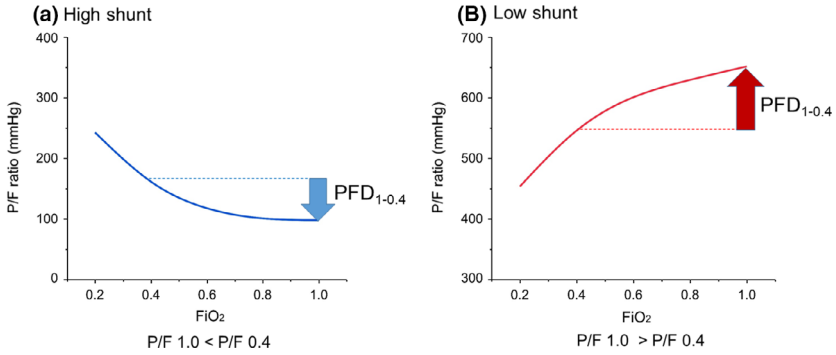

Figure 1 The conceptual image of two different correlations between partial pressure of oxygen/fraction of inspired oxygen (P/F) ratio and fraction of inspired oxygen (FiO_2). (a) High shunt pattern; P/F ratio decreases as FiO_2 increases. P/F ratio at FiO_2 1.0 is smaller than that at FiO_2 0.4. (b) Low shunt pattern; P/F ratio at FiO_2 1.0 is larger than that at FiO_2 0.4.

Institutional Research Board for our study were obtained.

Animal preparation

Ten female Yorkshire pigs were utilized for this study. Our previous report shows the details of preparation and lung procurement [10,19]. The pigs were anesthetized and euthanized by an intravenous injection of potassium chloride (2.0 mEq/kg). After 2 h of warm ischemia, the lungs were procured in the standard fashion. Then lungs were stored for 5 h at 4°C in Perfadex (XVIVO Perfusion Inc., Englewood, CO, USA) before perfusion. Based on our previous study, the combination of 2 h of warm storage and 5 h of cold storage resulted in significantly moderate IRI at 2 h of cellular EVLP [10,19]. All our procedures followed the Cleveland Clinic Institutional Animal Care and Use Committee guidelines.

Cellular EVLP system and Lung function assessment

The EVLP and lung assessment were performed as in previous reports [10,19]. Following is a brief overview: lungs were perfused in cellular EVLP using Vivoline LS1 (Vivoline Medical AB, Lund, Sweden) with mechanical ventilation (Servo-i: Maquet Critical Care, Solna, Sweden) of tidal volume (TV) 6 ml/kg ideal body weight (IBW) and PEEP 5 cmH_2O. This system required 70 ml/kg IBW/min for perfusate flow, open left atrium, and red blood cells in perfusate. Based on parameters at 2 h of EVLP, lungs were considered nonsuitable for transplant when the P/F ratio at FiO_2 1.0 was less than 300 mmHg and/or with significant deterioration of more than 15% from the baseline in airway parameters [i.e., PIP, mean pressure, dynamic compliance (Cdyn), and static compliance (Csta)] and/or vascular parameters did not reach 100% of 70 ml/kg IBW/minute. Furthermore, if airway fluid or visual findings of pulmonary edema or palpation findings were significant, lungs were considered nonsuitable.

PaO_2/FiO_2 difference (PFD) on EVLP

We performed 10 min of alveolar recruitment with a TV 10 ml/kg IBW, respiratory rate 10 breath/min, and PEEP 5 cmH_2O following manual recruitment maneuver with high PEEP (maximum PIP 25 cmH_2O). Then blood gas analysis (BGA) was performed on FiO_2 0.21, 0.4, and 1.0 at 2 h of EVLP in human models. PFDs were calculated from two consecutive analyses of BGA data, which were performed within 10 min of each other. During the calculation of the PFD, the ventilation was maintained at the same setting, including ventilation mode, tidal volume, PEEP, inspiratory–expiratory ratio and respiratory rate. Calculated PFDs were compared with physiological parameters, LW, and transplant suitability. In the pig model, blood gas samples were obtained synchronously from the lower lobe PV (LLPV) and upper lobe PV (ULPV) on FiO_2 0.4 and 1.0 to assess gas exchange.

LW and tissue samples

Before the initiation and the end of EVLP, whole LWs were measured. The LW ratio was defined as LW at 2 h/LW at 0 h of EVLP. Pig lung tissue samples were taken before and after EVLP from the middle of the upper and lower lobes for W/D ratio measurement, tissue cytokine analysis, and pathological assessment.

Tissue cytokine analysis

In the pig model, 20 samples [upper lobe (n = 10) and lower lobe (n = 10)] at 2 h of EVLP were homogenated and evaluated for three cytokines [interleukin (IL) -1beta, IL-8, and IL-10] as in previous report [21].

Table 1. Human donor demographics and transplant suitability in EVLP

Variables	All *N* = 39 Mean ± SD/Number (% of *N*)	Suitable *N* = 14 Mean ± SD/Number (% of *N*)	Nonsuitable *N* = 25 Mean ± SD/Number (% of *N*)
Age, years	45.2 ± 11.3	42.7 ± 11.8	46.6 ± 11.1
Gender, Male	19 (48.7%)	4 (28.6%)	15 (60.0%)
Height, cm	170.4 ± 10.0	169.3 ± 6.9	171.1 ± 11.5
Body mass index, kg/m^2	30.8 ± 7.5	33.1 ± 4.9	29.5 ± 8.4
Ventilation, days	4.9 ± 2.3	4.5 ± 2.6	5.1 ± 2.2
Donation after cardiac death	8 (20.5%)	2 (14.3%)	6 (24.0%)
Smoking, >20 pack-years	16 (41.0%)	6 (42.9%)	10 (40.0%)
Cold ischemia time, hours	5.1 ± 0.9	5.1 ± 1.1	5.2 ± 1.0
Cause of death			
Anoxia	22 (56.4%)	10 (71.4%)	12 (48.0%)
Cerebrovascular/Stroke	9 (23.1%)	2 (14.3%)	7 (28.0%)
Head trauma	8 (20.5%)	2 (14.3%)	6 (24.0%)
Last P/F ratio, mmHg	218.1 ± 102.1	207.4 ± 122.8	224.1 ± 90.8
Lung weight at 0 h, g	874 ± 232.4	771.6 ± 185.3	931.4 ± 239.5*
Main reason declined by clinical LTx			
Low P/F ratio	28 (71.8%)	9 (64.3%)	19 (76.0%)
Significant chest x-ray findings	7 (18.0%)	3 (21.4%)	4 (16.0%)
Smoking history	4 (10.2%)	2 (14.3%)	2 (8.0%)

SD, standard deviation; BMI, body mass index; P/F, partial pressure of oxygen/fraction of inspired oxygen ratio; LTx, lung transplantation.

*Lung weight at 0 h in nonsuitable lungs was significantly higher than that in suitable lungs ($P = 0.038$).

Cytokine analysis was performed in multiplex using the Luminex Platform (R&D Systems Inc., Minneapolis, MN, USA).

Pathological assessment

All pig tissue samples were stained with Hematoxylin & Eosin and given an acute lung injury (ALI) grade in a blinded manner by CF in the same fashion as in our previous report [21]. Following is a brief overview: ALI grades of 0–3 were used to represent the severity and the extent of ALI, with 0 standing for no visible evidence and 3 for maximum severity and complete involvement. Twenty samples from upper lobe ($n = 10$) and lower lobe ($n = 10$), were given an ALI grade of 0–3 in the standard fashion. Then, the difference of ALI grade was calculated as the difference of ALI grade = ALI grade at 2 h of EVLP—ALI grade at 0 h of EVLP.

Statistical analysis

All human variables were confirmed as normal distribution by Shapiro–Wilk's W test and were expressed as a mean ± standard deviation (SD). Correlations were analyzed using Pearson's correlation coefficient. By using the receiver operating characteristic (ROC) curve analysis, sensitivity was calculated to identify lungs that were nonsuitable for transplant. An optimal threshold was obtained to achieve the maximum Youden's Index [sensitivity − (1 − specificity)] in ROC curve analysis [22]. All variables in the pig model were given as median (interquartile range, IQR) because of the distribution. Correlations were analyzed using Spearman's correlation coefficient. Nonparametric Wilcoxon rank sums tests were used to analyze the differences in numeric data between groups. All statistical analyses were performed using JMP Version 13.1.0 (SAS Institute Inc, Cary, NC, USA). Probability values of $P < 0.05$ were considered as statistically significant.

Results

Donor lung demographics and transplant suitability in EVLP

Table 1 shows human donor lung demographics. Donor age was 45.2 ± 11.3 (Mean ± SD) years, consisting of

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19 men (48.7% of total). The last P/F ratio before donation was 218.1 ± 102.1 mmHg and the main reason for lungs to have been rejected from clinical lung transplantation was a P/F ratio lower than 300 mmHg. Fourteen lungs were judged as suitable in the evaluation at 2 h of EVLP, whereas 25 lungs were deemed nonsuitable. LW at 0 h in nonsuitable lungs was significantly higher than that in suitable lungs (931.4 ± 239.5 vs. 771.6 ± 185.3 g, $P = 0.038$, Table 1).

PFD and physiological parameters in suitable or nonsuitable lungs at 2 h of EVLP

In suitable lungs, $PFD_{1–0.4}$ and $PFD_{1–0.21}$ were significantly higher than those in nonsuitable lungs (53.2 ± 31.7 vs. −17.7 ± 49.6 mmHg, $P < 0.001$; −13.9 ± 45.0 vs. −119 ± 96.5 mmHg, $P = 0.005$, Table 2), respectively. There was no significant difference in P/F ratio at FiO_2 0.21 and 0.4 between suitable and nonsuitable group (382 ± 57.5 vs. 364 ± 132 mmHg, $P = 0.632$; 314.8 ± 57.2 vs. 262.4 ± 88.6 mmHg, $P = 0.055$, Table 2). In contrast, P/F 1.0 was significantly higher in the suitable group than in the nonsuitable group (368.1 ± 46.9 vs. 244.9 ± 72.7 mmHg, $P < 0.001$). Compared to nonsuitable lungs, suitable lungs were significantly associated with greater Cdyn ratio (Cdyn at 2 h EVLP/Cdyn at base line) (1.2 ± 0.2 vs. 1.0 ± 0.1, $P < 0.001$), better Csta ratio (Csta at 2 h EVLP/Csta at base line) (1.2 ± 0.3 vs. 0.9 ± 0.1, $P = 0.003$), lower pulmonary artery pressure (16.7 ± 1.9 vs. 18.8 ± 3.1 mmHg, $P = 0.031$), lower LW at 2 h EVLP (772.3 ± 174.1 vs. 1115.4 ± 291.0 g, $P < 0.001$), and lower LW ratio (LW at 2 h EVLP/LW at 0 h of EVLP) (1.01 ± 0.10 vs. 1.22 ± 0.27, $P = 0.007$).

ROC curve analysis on PFD

The threshold, area under the curve (AUC), specificity and sensitivity are demonstrated using ROC curve

Table 2. PFD and physiological parameters at 2 h of EVLP.

Variables	Suitable $N = 14$ Mean ± SD	Nonsuitable $N = 25$ Mean ± SD	*P* value
$PFD_{1–0.4}$, mmHg	53.2 ± 31.7	−17.7 ± 49.6	<0.001
$PFD_{1–0.21}$, mmHg	−13.9 ± 45.0	−119 ± 96.5	0.005
General EVLP parameters			
P/F ratio 0.21, mmHg	382 ± 57.5	364 ± 132	0.632
P/F ratio 0.4, mmHg	314.8 ± 57.2	262.4 ± 88.6	0.055
P/F ratio 1.0, mmHg*	368.1 ± 46.9	244.9 ± 72.7	<0.001
PIP, cmH_2O	10.9 ± 1.4	12.7 ± 1.9	0.004
Cdyn, ml/cmH_2O	66.0 ± 15.3	52.9 ± 16.2	0.018
Cdyn ratio*	1.2 ± 0.2	1.0 ± 0.1	<0.001
Plateau pressure, cmH_2O	8.5 ± 1.0	8.8 ± 1.2	0.390
Csta, ml/cmH_2O	114.6 ± 33.4	113.3 ± 49.7	0.933
Csta ratio*	1.2 ± 0.3	0.9 ± 0.1	0.003
Mean pressure, cmH_2O	5.8 ± 0.3	6.2 ± 0.7	0.092
Mean pressure ratio*	0.97 ± 0.05	1.04 ± 0.13	0.102
PAP, mmHg	16.7 ± 1.9	18.8 ± 3.1	0.031
PVR, dyne ▫ s/cm^5	313 ± 49.3	366.1 ± 114.9	0.109
PVR ratio*	1.00 ± 0.08	1.02 ± 0.09	0.448
Additional EVLP parameters			
Lung weight at 2 h, g	772.3 ± 174.1	1115.4 ± 291.0	<0.001
LW ratio	1.01 ± 0.10	1.22 ± 0.27	0.007

SD, standard difference; $PFD_{1–0.4}$, partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio difference [PaO_2/FiO_2 (P/F) ratio at FiO_2 1.0 - P/F ratio at FiO_2 0.4 (P/F 0.4)]; $PFD_{1–0.21}$, P/F 1.0 – P/F 0.21; P/F ratio 0.21, P/F ratio at FiO_2 0.21; P/F ratio 0.4, P/F ratio at FiO_2 0.4; P/F ratio 1.0, P/F ratio at FiO_2 1.0; PIP, peak inspiratory pressure; Cdyn, dynamic compliance; Cdyn ratio, Cdyn at 2 h of *ex vivo* lung perfusion (EVLP)/Cdyn at base line; Csta, static compliance; Csta ratio, Csta at 2 h of EVLP/Csta at base line; Mean pressure, mean airway pressure; Mean pressure ratio, Mean pressure at 2 h of EVLP/Mean pressure at base line; PAP, pulmonary artery pressure; PVR, pulmonary vascular resistance; PVR ratio, PVR at 2 h of EVLP/PVR at base line; LW, lung weight; LW ratio, LW at 2 h of EVLP/LW at 0 h of EVLP.

*Parameters used for judgment of transplant suitability.

analysis in Table 3. $PFD_{1–0.4}$ (threshold 37.5) showed higher AUC of 0.90 and sensitivity of 0.96 compared with sensitivity of 0.84 and AUC of 0.86 when using $PFD_{1–0.21}$ (threshold −46.1). P/F 1.0 also showed high AUC of 0.91, sensitivity of 0.88, specificity of 0.92 with optimal threshold of 330. On the other hand, P/F 0.4 and P/F 0.21 did not show more than 0.70 of AUC. Although Cdyn ratio (Cdyn at 2 h of EVLP/Cdyn at base line) and LW at 2 h showed high AUC of 0.81 and 0.86, respectively, neither of them reached the value of sensitivity on $PFD_{1–0.4}$ and the specificity on P/F ratio 1.0. Other parameters, such as plateau pressure, Cdyn, Csta, and pulmonary artery pressure did not show more than 0.75 of AUC.

Correlation between PFD and physiological parameters

The correlation of simple regression analysis between PFD, LW ratio, and physiological parameters are shown in Table 4. $PFD_{1–0.4}$ showed significant negative correlation with LW ratio ($R^2 = 0.26$, $P < 0.001$, Table 4). $PFD_{1–0.21}$ and P/F ratio 1.0 also showed negative correlation with LW ratio, but both of R^2 were small (0.16 and 0.12, respectively). No significant correlation was found between pulmonary vascular resistance (PVR) ratio and the other parameters. Based on these results, a multiple linear was calculated to predict LW ratio based on six variables ($PFD_{1–0.4}$, P/F ratio 1.0, mean pressure ratio, Cdyn ratio, Csta ratio, and PVR ratio). A significant regression equation was found ($F = 3.47$, $P = 0.009$) with R^2 of 0.39. This analysis revealed that both $PFD_{1–0.4}$ and mean pressure ratio were significant predictors of LW ratio (Table 5, model 1). Furthermore, the same analysis based on $PFD_{1–0.4}$ and mean pressure ratio, a significant regression equation was also found ($F = 9.80$, $P < 0.001$) with R^2 of 0.35 (Table 5, model 2).

Correlation between PFD and LW in upper lobe and lower lobe using porcine DCD model

Twenty blood gas samples were taken from ULPV ($n = 10$) and LLPV ($n = 10$). $PFD_{1–0.4}$ on LLPV was

Table 3. Receiver operating characteristic (ROC) curve analysis in EVLP parameters at 2 h.

Variables	AUC	Threshold*	Specificity	Sensitivity
$PFD_{1–0.4}$, mmHg	0.90	37.5	0.71	0.96
$PFD_{1–0.21}$, mmHg	0.86	−46.1	0.78	0.84
General EVLP parameters				
P/F ratio 0.21, mmHg	0.61	295.2	1.00	0.48
P/F ratio 0.4, mmHg	0.67	260.0	0.85	0.56
P/F ratio 1.0, mmHg	0.91	330.0	0.92	0.88
PIP, cmH_2O	0.79	12.0	0.78	0.76
Cdyn, ml/cmH_2O	0.71	51.2	0.85	0.56
Plateau pressure, cmH_2O	0.59	9.0	0.64	0.60
Csta, ml/cmH_2O	0.55	88.0	0.78	0.40
Mean pressure, cmH_2O	0.62	7.0	1.0	0.16
PAP, mmHg	0.70	19.0	0.85	0.60
PVR, dyne s/cm^5	0.61	409	1.0	0.32
Cdyn ratio	0.81	1.14	0.71	0.88
Csta ratio	0.75	1.0	0.42	0.92
PVR ratio	0.53	0.9	0.14	1.0
Additional EVLP parameters				
Lung weight at 2 h, g	0.86	915.0	0.85	0.84
LW ratio	0.77	1.16	0.92	0.64

AUC, area under curve; $PFD_{1–0.4}$, partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio difference [PaO_2/FiO_2 (P/F) ratio at FiO_2 1.0 - P/F ratio at FiO_2 0.4(P/F ratio 0.4)]; $PFD_{1–0.21}$, P/F ratio 1.0 – P/F ratio 0.21; EVLP, *ex vivo* lung perfusion; P/F ratio 0.21, P/F ratio at FiO_2 0.21; P/F ratio 0.4, P/F ratio at FiO_2 0.4; P/F ratio 1.0, P/F ratio at FiO_2 1.0; PIP, peak inspiratory pressure; Cdyn, dynamic compliance; Csta, static compliance; Mean pressure, mean airway pressure; PAP, pulmonary artery pressure; PVR, pulmonary vascular resistance; Cdyn ratio, Cdyn at 2 h of EVLP/Cdyn at base line; Csta ratio, Csta at 2 h of EVLP/Csta at base line; PVR ratio, PVR at 2 h of EVLP/PVR at base line; LW, lung weight; LW ratio, LW at 2 h of EVLP/LW at 0 h of EVLP.

*Optimal thresholds were obtained to achieve the maximum Youden's Index [sensitivity – (1 − specificity)] in ROC curve analysis.

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Table 4. Simple regression analysis.

	LW ratio			Cdyn ratio			PVR ratio		
Variables	Estimate	R^2	*P* value	Estimate	R^2	*P* value	Estimate	R^2	*P* value
$PFD_{1–0.4}$	−116.9	0.26	<0.001	84.6	0.10	0.054	−10.0	0.0002	0.920
$PFD_{1–0.21}$	−161.4	0.16	0.009	81.0	0.03	0.294	−30.8	0.0008	0.859
LW ratio	–	–	–	−0.1	0.02	0.371	−0.03	0.0002	0.928
P/F ratio 1.0	−127.9	0.12	0.026	61.1	0.02	0.388	34.9	0.001	0.826
Cdyn ratio	−0.17	0.02	0.371	–	–	–	0.01	0.0006	0.879
Csta ratio	−0.15	0.02	0.321	0.65	0.65	<0.001	0.06	0.03	0.263
Mean pressure ratio	0.63	0.09	0.053	−0.11	0.004	0.683	0.14	0.03	0.226
PVR ratio	−0.03	0.0002	0.928	0.05	0.0006	0.879	–	–	–

LW, lung weight; LW ratio, LW at 2 h of *ex vivo* lung perfusion (EVLP)/LW at 0 h of EVLP; Cdyn, dynamic compliance; Cdyn ratio, Cdyn at 2 h of EVLP/Cdyn at base line; PVR, pulmonary vascular resistance; PVR ratio, PVR at 2 h of EVLP/PVR at base line; $PFD_{1-0.4}$, partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio difference [PaO_2/FiO_2 (P/F) ratio at FiO_2 1.0 - P/F ratio at FiO_2 0.4]; $PFD_{1–0.21}$, P/F ratio at FiO_2 1.0 (P/F 1.0) – P/F 0.21; LW, lung weight; LW ratio, LW at 2 h of EVLP/LW at 0 h of EVLP; P/F ratio 1.0, P/F ratio at FiO_2 1.0; Cdyn, dynamic compliance; Cdyn ratio, Cdyn at 2 h of *ex vivo* lung perfusion (EVLP)/Cdyn at base line; Csta, static compliance; Csta ratio, Csta at 2 h of EVLP/Csta at base line; Mean pressure ratio, mean airway pressure at 2 h of EVLP/mean airway pressure at base line; PVR, pulmonary vascular resistance; PVR ratio, PVR at 2 h of EVLP/PVR at base line.

Table 5. Multiple regression analysis for lung weight ratio.

	Model 1				Model 2			
	Estimate	SE	Beta	*P* value	Estimate	SE	Beta	*P* value
$PFD_{1–0.4}$, mmHg	−0.001	0.0006	−0.45	0.007	−0.0022	0.0005	−0.51	<0.001
Mean pressure ratio	0.657	0.2858	0.32	0.028	0.605	0.272	0.29	<0.001
P/F ratio 1.0, mmHg	−0.0004	0.0004	−0.17	0.273				
Cdyn ratio	0.171	0.295	0.14	0.565				
Csta ratio	−0.124	0.241	−0.12	0.609				
PVR ratio	−0.162	0.391	−0.06	0.680				
R^2	0.39				0.35			
F for change in R^2	3.47 ($P = 0.009$)				9.80 ($P < 0.001$)			

SE, standard error; $PFD_{1–0.4}$, partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio difference [PaO_2/FiO_2 (P/F) ratio at FiO_2 1.0 - P/F ratio at FiO_2 0.4(P/F ratio 0.4)]; Mean pressure ratio, mean airway pressure at 2 h of *ex vivo* lung perfusion (EVLP)/mean airway pressure at base line; P/F ratio 1.0, P/F ratio at FiO_2 1.0; Cdyn ratio, dynamic compliance (Cdyn) at 2 h of EVLP/Cdyn at base line; Csta ratio, static compliance (Csta) at 2 h of EVLP/Csta at base line; PVR, pulmonary vascular resistance; PVR ratio, PVR at 2 h of EVLP/PVR at base line.

significantly associated with lower lobe W/D ratio ($R^2 = 0.51$, $P = 0.019$, Fig. 2). $PFD_{1–0.4}$ on ULPV also showed a negative correlation with upper lobe W/D ratio (not statistically significant, $R^2 = 0.37$, $P = 0.060$, Fig. 2).

Correlation between PFD and Pathological findings

Out of 20 samples, nine cases were "ALI grade difference ≥1" group and 11 cases were "ALI grade difference = 0" group. $PFD_{1–0.4}$ in "ALI grade difference = 0" group was significantly higher than that in "ALI grade difference ≥1" group [33.5 (−2 to 129.5) vs. −20.5 (−65.3 to 28.8) mmHg, $P = 0.044$, Fig. 3].

The correlation between PFD and the level of inflammatory cytokines

Of the following three cytokines, IL-1beta, IL-8, and IL-10, only IL-1beta showed significant negative correlation with $PFD_{1–0.4}$ ($R^2 = 0.21$, $P = 0.041$).

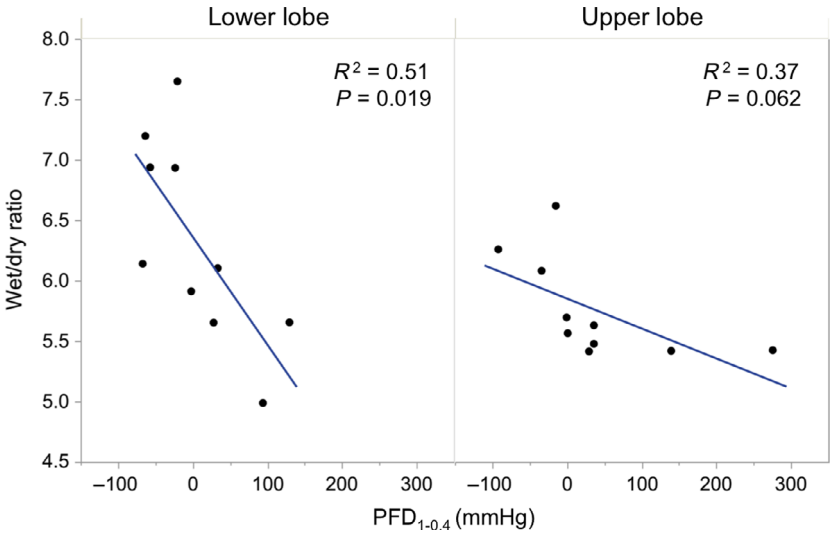

Figure 2 The correlation between $PFD_{1–0.4}$ and wet/dry (W/D) ratio of lower and upper lobe in porcine *ex vivo* lung perfusion model.

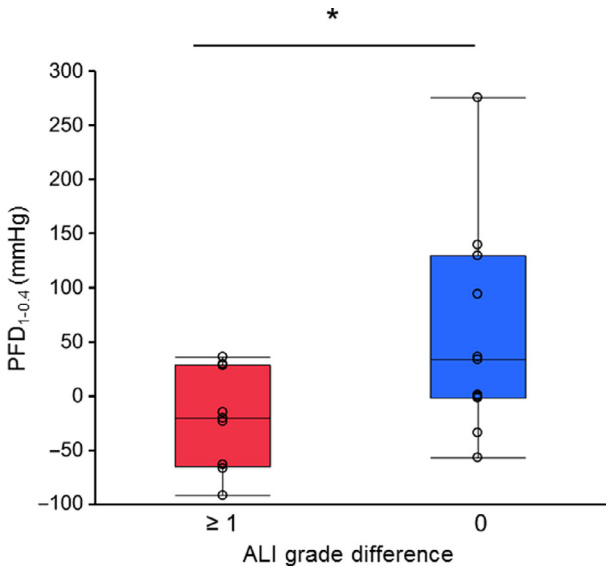

Figure 3 A comparison of $PFD_{1–0.4}$ between the difference of acute lung injury (ALI) grade ≥1 versus difference of ALI grade = 0 represented by box and whiskers plots. The difference of ALI grade was defined as the difference of ALI grade = ALI grade at 2 h of *ex vivo* lung perfusion (EVLP) – ALI grade at 0 h of EVLP. The middle horizontal line represents the median, and the upper and lower whiskers represent the maximum and minimum values. Each box represents the middle 50% of the data (25–75% range). *$P < 0.05$.

Discussion

The most prominent finding in this study was that $PFD_{1–0.4}$ had a significant correlation with LW ratio. We compared $PFD_{1–0.4}$ with existing EVLP parameters such as ventilator mechanics, hemodynamics, and LW. As a result of simple regression analysis, $PFD_{1–0.4}$ had the most significant correlation with LW at 2 h of EVLP and LW ratio. Furthermore, a multiple regression analysis revealed that $PFD_{1–0.4}$ was a significant predictor of LW ratio. So far many attempts have been made to measure donor lung water accurately. Using transpulmonary thermodilution, Katzenelson *et al.* [23] reported that extra vascular lung water (EVLW) was very closely correlated to the gravimetric measurement of lung water in pulmonary edema. Trebbia *et al.* [24] demonstrated in a pig model that transpulmonary thermodilution during EVLP could accurately measure EVLW. Recently, they also demonstrated that a larger amount of EVLW in EVLP resulted in a worse primary graft dysfunction grade after lung transplantation using the same method [25]. However, this method requires a closed circuit (not feasible in Swedish protocol EVLP with an open left atrium) and produces inaccurate EVLW measurements in severely damaged lungs [26]. Therefore, in Swedish protocol EVLP, $PFD_{1–0.4}$ might be a useful tool to evaluate pulmonary edema rather than the thermodilution method and it might be an improved parameter in suggesting pulmonary edema than any existing EVLP parameters.

A second important finding was that the $PFD_{1–0.4}$ produced the highest AUC and sensitivity to identify lungs that are nonsuitable for transplant in ROC curve analysis, which was performed using the current standard criteria in cellular EVLP protocol [27,28]. P/F 1.0 and $PFD_{1–0.21}$ also demonstrated a high AUC, but sensitivity in either case did not reach that of $PFD_{1–0.4}$. Originally, our group had reported that the pattern of P/F 0.21 < P/F 1.0 was significantly associated with higher pulmonary compliance and lower shunt fraction compared with the pattern of P/F 0.21 > P/F 1.0 in porcine lungs [19]. However, ROC curve analysis in the current

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study using human donor lungs showed that AUC at FiO_2 0.4 was better than that at FiO_2 0.21 and that the optimal threshold was not 0. It was interesting that $PFD_{1–0.4}$ showed higher sensitivity to identify "nonsuitable" lungs because sensitivity for "nonsuitable" should be critical in making a decision in a clinical situation. In clinical practice, it is imperative that nonsuitable lungs be eliminated from the transplant inventory. From the viewpoint of patient safety, this means that sensitivity (sensitivity to nonsuitable lungs for transplantation) should be given priority over specificity.

The third important finding was that $PFD_{1–0.4}$ had showed potential as a valid parameter for assessment of each lobe. The lungs from a marginal donor are often heterogeneous because atelectasis and aspiration are more likely in the lower lobe than in the upper lobe. Sometimes differences can also be seen between the right and left. Although single lung transplantation has a higher mortality rate than double lung transplantation [29], single lung transplantation may be preferable in patients with higher risk during the prolonged anesthesia time [30]. Lung lobar transplantation has also been shown to be an effective way to reduce the mortality rate of patients on the waiting list for transplantation [2,31,32]. Therefore, single and/or lobar lung assessment in EVLP is absolutely needed for these procedures. Although many studies have reported that EVLP is the preferred evaluation method for whole donor lungs [9,10], the precise evaluation for suitability of a single lung or lobe is still unclear. Cost *et al.* [33] reported that selective PV gases provide corroborative objective support to the findings in bronchoscopy, palpation, and visual assessment in the donor hospital. This concept might be useful in EVLP setting. In the current study, PFDs were calculated from PV blood at each upper or lower lobe as well as P/F ratio. As a result, $PFD_{1–0.4}$ was significantly correlated with lung lobe W/D ratio rather than P/F 1.0. These results demonstrated that lobe PFD might be useful for evaluating lung lobes weight. Interestingly, the lobe PFD correlated not only with lobe W/D ratio but also with the pathological findings of ALI grade and level of inflammatory cytokines in each lobe. We believe that these results also suggest that lobe PFD can be an indicator of lobe IRI. Although directly measuring LW was simple and reliable, there is still no precise method to measure the lung lobes weight during EVLP.

Discussing the features of $PFD_{1–0.4}$ would be worthwhile in understanding this unique parameter. It is known that the correlation between FiO_2 and PO_2 changes depending on the shunt fraction of individual lungs (Fig. 1) [17,18]. Furthermore, two physiological effects contribute to the correlation between FiO_2 and PO_2. One is hypoxic pulmonary vasoconstriction at low FiO_2 [34] and the other is absorption atelectasis at FiO_2 1.0 [35]. Based on these correlations, the concept of PFD is to assume the shunt fraction, estimating the condition of the lungs using two available P/F ratios at different FiO_2. Generally, there are two main factors for increasing the shunt fraction, which are anatomical shunt and a physiological shunt of nonventilated alveoli. The main causes for physiological shunt are atelectasis and diseases with alveolar filling as in pulmonary edema. Even with EVLP settings different from *in vivo* settings, these two could be related to shunt fraction. In the current study, atelectasis had been eliminated from donor lungs using alveolar recruitment with high PEEP before cold preservation in donor hospital and blood gas sampling in EVLP. Therefore, pulmonary edema might be a main factor for affecting shunt fraction which determines PFD. PFD may be more accurately correlated to weight gain with the perquisite that atelectasis is resolved. On the other hand, the effect of atelectasis on PFD has not been clear and it should be clarified in the future.

Pulmonary edema is important in evaluation for transplant suitability, but there are no established criteria to evaluate it objectively in the current EVLP protocol. The investigators always have to judge pulmonary edema subjectively by visual inspection or palpation during EVLP. Our results demonstrated that $PFD_{1–0.4}$ was significantly associated with LW ratio. $PFD_{1–0.4}$ could indicate pulmonary edema based on objective values and may provide evidence for the surgeon's subjective assessment. Furthermore, $PFD_{1–0.4}$ might be useful in evaluating pulmonary edema in isolated lung lobes. Therefore, we believe that these are advantages of PFD than the other existing EVLP parameters and that PFD will have the capacity to improve graft selection compared to classical methods.

There are several limitations to this study. First, we recognize that this ROC curve analysis was performed on the parameters in EVLP using rejected, human donor lungs. Current findings are only valid in "rejected" donor lungs and further study is needed for standard donor lungs. Second, because the current study has no clinical outcomes on transplanted EVLP lungs with a favorable $PFD_{1–0.4}$, we should verify the consistency of PFD on clinical outcomes. Furthermore, the optimal threshold should be considered under extensive clinical outcomes after lung transplantation. However, it was surprising that 14 pairs of lung were

judged as "Suitable" in the current study. This result might be attributed to the fact that clinical teams declined these donor lungs because of low P/F ratios caused by atelectasis, which could be improved easily by alveolar recruitment. As a result of feedback from the research team to the clinical transplant team, the number of such cases has rapidly decreased in our institute recently. Third, because of the anatomical differences between human lungs and pig lungs, this PFD concept developed in isolate lung lobes of the pig model may not be feasible in the human model.

In conclusion, PFD in EVLP demonstrated significant correlation with LW ratio and allowed more precise assessment of individual lobes in detecting lung edema. Moreover, the role of PFD measurements of PFD in the evaluation of donor lungs during EVLP might support decision-making in evaluation using current EVLP criteria.

Authorship

HN: designed research/study, performed research/study, analyzed data, and wrote the paper. TO: designed research/study, performed research/study, and wrote the paper. KSA and YI: performed research/study. CFF: performed research/study and contributed important reagents. JSH: contributed important reagents and analyzed data. KRM: designed research/study and contributed important reagents.

Funding

The authors have declared no funding.

Conflict of interest

The authors have declared no conflicts of interest.

Acknowledgements

This research was supported by the donation of Rosy and Ray Park. We express our deepest gratitude to the organ donors and their families. We thank all members of Lifebanc, Life Connection of Ohio, and transplant teams in the Cleveland Clinic. We are grateful to XVIVO perfusion Inc. and Maquet for their support. We are also grateful to Jennifer Bucey and David Wheeler for editorial support .

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