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ORIGINAL ARTICLE

Incidence and severity of primary graft dysfunction after lung transplantation using rejected grafts reconditioned with *ex vivo* lung perfusion[†]

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Abstract

OBJECTIVES: *Ex vivo* lung perfusion (EVLP) is a novel technique used to evaluate and recondition marginal or rejected grafts. Primary graft dysfunction (PGD) is a major early complication after lung transplantation (LTx). The use of marginal or initially rejected grafts may increase its incidence and severity. The aim of this study is to evaluate the incidence of PGD after LTx using rejected grafts reconditioned with EVLP.

METHODS: PGD has been evaluated immediately after LTx (t0) and after 72 h (t72) in patients receiving standard (Group A) or reconditioned (Group B) grafts. EVLP was performed using a controlled acellular perfusion according to the Toronto technique.

RESULTS: From July 2011 to February 2013, 36 LTxs have been performed: 28 patients (21 M/7 F, mean age 51.7 ± 14.7 years) in Group A and 8 (6 M/2 F, mean age 46.6 ± 9.8 years) in Group B (successful recondition rate of 73%, 8 of 11 cases). Incidence rate of PGD 3 at t0 and at t72 (Group A versus Group B) was 50 vs 37% (P = NS) and 25 vs 0% (P = NS), respectively. Post-transplant extracorporeal membrane oxygenation was required in 5 and 2 patients in Groups A and B, respectively (P = NS).

CONCLUSIONS: The use of initially rejected grafts treated with EVLP does not increase the incidence and severity of PGD after LTx. Although comparison of PGD 3 incidence in the two groups did not reach a statistical difference, all EVLP patients suffering from severe PGD early after transplant recovered normal lung function at 72 h, suggesting a protective role of EVLP against PGD occurrence and severity.

Keywords: Lung transplantation • Primary graft dysfunction • *Ex vivo* lung perfusion

INTRODUCTION

The number of lung transplants is influenced by the low rate of graft suitability [1]. With an incidence rate up to 25%, primary graft dysfunction (PGD) is a major early complication of lung transplantation, resulting in impaired oxygenation and poor lung compliance [2]. According to the severity of lung dysfunction, PGD is classified in four classes [1, 2]. Although risk factors have been identified, no specific treatments have been shown to be effective. Its management is based on supportive strategies (i.e. prolonged mechanical ventilation, extracorporeal membrane oxygenator). Reported PGD risk factors are both donor related (older age, prolonged mechanical ventilation, head trauma, history of smoking, female gender, aspiration) and recipient or transplant related (body mass index >25, female gender, pulmonary hypertension, pulmonary fibrosis, prolonged ischaemic time, use of cardiopulmonary bypass, blood transfusion, type of preservation solution, single lung transplant) [3].

It has been shown that normothermic *ex vivo* lung perfusion (EVLP) is a safe and feasible strategy to increase the pool of transplantable lungs [3]. Its clinical application is still limited but increasing worldwide.

The aim of this study is to evaluate and compare the incidence and severity of PGD after lung transplantation using reconditioned or standard grafts.

MATERIALS AND METHODS

To increase the number of transplants, a programme of reconditioning of marginal or initially rejected lungs has been started in our centre. Both standard (Group A) and reconditioned (Group B) lung-transplanted patients from July 2011 to February 2013 have been evaluated to better understand the clinical impact of the EVLP program.

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Ex vivo lung perfusion

Indications to EVLP were bad gas exchange [(ratio of arterial oxygen concentration to the fraction of inspired oxygen) ratio <300] at initial donor referral or at final graft assessment before retrieval and/or evidence of pulmonary oedema at chest X-ray or computed-tomography (CT) scan and/or presence of wet lung at surgical inspection in the absence of significant infection and/or contusion.

From brain-dead donors, the lung block was retrieved following the standard technique. After a period of cold storage (4°C) using Perfadex®, the lungs were transferred in a lung chamber. According to the Toronto protocol, the pulmonary artery (PA) and left atrial (LA) cuff were sewn to specially designed funnel-shaped cannulas with built-in pressure probes (Vitrolife®) and then connected to the perfusion circuit, primed with Steen Solution™, broad-spectrum antibiotics (imipenem/cilastatin 500 mg/500 mg), heparin (10 000 IU) and methylprednisolone (500 mg). Materials used were the following: a Euroset™ circuit with Admiral oxygenator, an anti-leucocyte filter (Pall LeukoGuard-6® Arterial Filter) and a Medtronic Bio-Medicus® pump. The lung block was perfused and gradually rewarmed at low flow to reach 40% of ideal cardiac output in the first hour. Protective ventilation (tidal volume 7 ml/kg/min, positive end-expiratory pressure 5 cmH_2O, respiratory rate 7 acts/min, FiO_2 0.21) was started when lung perfusate temperature reached 32°C. EVLP was maintained for 4–6 h and lung function was evaluated every hour. During evaluation, ventilation parameters were changed as follows: tidal volume 10 ml/kg/min, positive end-expiratory pressure 5 cmH_2O, respiratory rate 10 acts/min and FiO_2 1. Pulmonary function was evaluated on the following parameters: PA pressure, LA pressure, pulmonary vascular resistance, airways pressure (peak, mean and plateau), lung compliance (static and dynamic) and oxygenation capacity (delta pO_2: perfusate LA pO_2 – perfusate PA pO_2). Bronchoscopy and lung X-ray were performed after 1 h and after 3 h of perfusion. The lungs were used for transplantation if the criteria reported in Table 1 were met.

Conventional lung transplantation

Optimal lung grafts were flushed with Perfadex® through the PA and pulmonary veins and, after a period of cold storage (4°C), were directly implanted.

Primary graft dysfunction classification

PGD was defined following ISHLT guidelines and classified in four groups: PGD 0 (P/F ratio >300 without radiographic infiltrates), PGD 1 (P/F ratio >300 with radiographic infiltrates), PGD 2 (P/F ratio from 200 to 300 with radiographic infiltrates) and PGD 3 (P/F ratio <200 with radiographic infiltrates) [4]. PGD was evaluated at two time points: immediately after lung transplantation (t0) and 72 h post-transplant (t72).

Table 1: Ideal parameters for transplantation at the end of EVLP

Delta pO_2	>350 mmHg
Left atrial pressure	From 3 to 5 mmHg
Pulmonary artery pressure	Stable or <15 mmHg
Airway pressure	Stable or decreased
Pulmonary vascular resistance	Stable or decreased
Compliance	Stable or decreased
Bronchoscopy	Negative
Lung X-ray	Negative

EVLP: *Ex vivo* lung perfusion.

Statistical analysis

Ischaemic time, mechanical ventilation length, ICU stay, 30-day mortality, PGD incidence and severity at t0 and at t72 have been recorded and compared in both groups. Descriptive statistics are presented as means, medians, standard deviations and ranges for the continuous variables, and as counts and percentages for categorical variables. Comparisons between groups were performed using a χ^2 test or ANOVA test as appropriate. Significant statistical difference has been considered for $P < 0.05$.

RESULTS

From July 2011 to February 2013, 36 lung transplants (27 males, 9 females, mean age 49.4 ± 15.6 years) have been performed at San Giovanni Battista Hospital, Turin. Twenty-eight patients (Group A: 21 males, 7 females, mean age 51.7 ± 14.7 years) received a conventional lung transplant. From the beginning of the reconditioning programme, 11 lung blocks, initially considered unsuitable for direct transplant, underwent EVLP. Among those, in 8 cases, ideal parameters for transplantation were reached, allowing bilateral lung transplants in those patients (Group B: 6 males, 2 females, mean age 46.6 ± 9.8 years). In 3 cases, lungs were rejected after EVLP: 2 for infection and 1 for poor gas exchange related to emphysema associated to a heavy history of smoking. Infections were suspected on the basis of lung X-ray and bronchoscopic findings and later confirmed by bronchoalveolar lavage and lung tissue cultures. In 1 case, EVLP was helpful to identify a clear right lower lobe infection, although the chest X-ray and the CT scan performed in the donor the same day of donation were negative [5]. EVLP was run for a mean period of 282.8 ± 57.1 min. The mean P/F ratio increased from 200 ± 85 (range 98–300) before EVLP to 426 ± 52 ($P < 0.05$), 450 ± 67 ($P < 0.05$), 449 ± 77 ($P < 0.05$) and 438 ± 8 ($P < 0.05$) at 1, 2, 3 and 4 h during EVLP, respectively. Mean delta pO_2 was over 350 mmHg at each evaluation time point. Pulmonary vascular resistances remained stable during the whole period of perfusion (312 ± 136, 312 ± 120, 392 ± 112 and 352 ± 176 dyne/s/cm^{-5} at 1, 2, 3 and 4 h, respectively). Bronchoscopies at 1 and 3 h during perfusion were normal in 6 cases and showed trivial secretions in the remaining 2 cases. Lung X-rays showed a complete resolution of oedema detected in the donors' chest X-rays or at surgical inspection.

Donor characteristics are summarized in Table 2. No significant differences were found between the two groups, except for the P/F ratio at referral, as expected (mean PaO_2/FiO_2 at 100% oxygen Group A: 498 ± 62.5; Group B: 338 ± 126, $P = 0.00004$). Comparing clinical results of lung transplantation in the two groups, no differences can be noticed in terms of duration of mechanical ventilation (median, Group A: 46.5; Group B: 52 h, P = NS), ICU stay (median, Group A: 8, Group B: 9 days, P = NS) and 30-day mortality (Group A: 17.8%; Group B: 12.5%, $P = 0.64$).

PGD incidence and severity at t0 and at t72 did not show any significant difference (Table 3). The overall incidence rate of PGD 3 was 47 and 19% at t0 and t72, respectively. In Group A, 14 of 28

Table 2: PGD risk factors—donor and recipient characteristics

	Group A (n = 28)	Group B (n = 8)	P
Donor			
Mean age (years)	43.3 ± 16.8	44.7 ± 16.2	0.84
Female gender (%)	46	85	0.08
Smoking history (%)	29	29	0.97
Smoking >10 packs/year (%)	29	25	0.88
Mean mechanical ventilation (days)	3.3 ± 2.9	2.1 ± 1.6	0.30
Trauma as the cause of death (%)	25	0	0.18
P/F ratio at referral	498 ± 62.5	338 ± 126	<0.01
Recipient			
Body mass index	24.1 ± 5.8	24.8 ± 5.8	0.78
Female gender (%)	75	71	0.06
Pulmonary hypertension (%)	0	0	NS
Pulmonary fibrosis (%)	61	57	0.86
Transplant			
Single lung transplant (%)	43	0	0.03
Mean ischaemic time (min)	341 ± 103	916 ± 232	0.003
Use of cardiopulmonary bypass (%)	21	57	0.09
Blood transfusion (%)	61	100	0.05

PGD: primary graft dysfunction.

Table 3: PGD incidence and severity between groups (% ± confidence interval)

	Overall (n = 36)	Group A (n = 28)	Group B (n = 8)	P	Overall (n = 36)	Group A (n = 28)	Group B (n = 8)	P
PGD 0–1	36 ± 4.1% (13)	29 ± 10.5% (8)	63 ± 43.3% (5)	0.11	62 ± 20.1% (22)	54 ± 19.8% (15)	88 ± 60.6% (7)	0.10
PGD 2	17 ± 5.4% (6)	21 ± 7.6% (6)	0% (0)	0.19	19 ± 6.0% (7)	21 ± 7.6% (6)	12 ± 7.9% (1)	0.65
PGD 3	47 ± 15.2% (17)	50 ± 18.3% (14)	37 ± 25.3% (3)	0.57	19 ± 6.0% (7)	25 ± 9.1% (7)	0% (0)	0.14

In the absence of significant differences, our data show that all EVLP patients suffering from severe PGD recovered normal respiratory function after 72 h from lung transplantation.
PGD: primary graft dysfunction.

patients (50%) suffered from PGD 3 at t0, and in 7 of those (50%) PGD 3 was still present at t72. Once more, PGD 3 at t72 had a significant impact on hospital mortality because 4 of 7 patients died during the hospital stay without recovering satisfactory pulmonary function. Conversely, no cases of PGD 3 at t72 were recorded in Group B due to a full resolution of the 3 cases of PGD 3 at t0. Extracorporeal membrane oxygenator (ECMO) implantation early after transplant was similar in the two groups: 5 (18%) patients in Group A and 2 (25%) patients in Group B, P = NS. In Group A, 3 of 5 patients did not recover optimal lung function, and these patients eventually died on ECMO because of multiorgan failure. In the remaining 2 patients, ECMO was weaned on Day 1 and Day 4, respectively. In Group B, both patients requiring ECMO recovered an excellent pulmonary function after 48 h of support. The following postoperative course of these patients was straightforward with a total mechanical ventilation time of 68 and 90 h, respectively.

DISCUSSION

PGD is a major early complication of lung transplantation. It is characterized by poor gas exchange as a result of acute lung injury similarly to what happens in acute respiratory distress syndrome. Its incidence is variable, depending also on the definition criteria used for the diagnosis. Its clinical impact is significant in terms of both early and long-term morbidity and mortality [6]. Its pathogenesis is multifactorial. Whatever the initial cause, pulmonary endothelium injury leads to capillary–alveolar membrane leakage resulting in gas exchange impairment and inflammatory agents' activation [7]. Unfortunately, no specific treatments are available at the moment and only supportive treatment with mechanical ventilation and extracorporeal oxygenation is applicable. Many risk factors related with PGD have been identified [3]. Among these, 'graft quality' and ischaemic time are two aspects intimately associated with the EVLP procedure. Therefore, the use of initially rejected grafts reconditioned with EVLP may have a theoretical impact on PGD incidence and severity after transplant.

EVLP is a novel technique that may positively recondition initially rejected lungs [8–10]. In our experience, it allowed lung transplantation in 8 of 11 cases (73%). The high rate of positive reconditioning may suggest an accurate selection of grafts to be perfused. In our experience, all of the grafts that were treated with EVLP came from relatively good donors (younger, shorter history of smoking or non-smokers, shorter duration of mechanical ventilation, and no infection). The exception was poor oxygenation, as it was due to their existing pulmonary oedema. Without a reconditioning programme, those lungs would not have been accepted for transplantation in our centre. EVLP increased the volume of

Table 4: Experiences reported in the literature

	LTx with EVLP grafts	Positive reconditioning (%)	PGD 3 at t72 (%)	ECMO implantation
Cypel *et al.* [12]	50	86	8.5	1 patient–2%
Zich *et al.* [13]	6	46	NA	2 patients–33%
Wallinder *et al.* [14]	11	100	18	1 patient–9%
Aigner *et al.* [15]	9	69	22[a]	1 patient–11%
Ingemansson *et al.* [16][b]	6	66	NA	NA
Boffini *et al.* (These data are those reported in the present article)	8	73	0	2 patients–25%

[a]PGD 3 at 24 h.
[b]All patients discharged and alive at 3 months post-transplant.
LTx: lung transplantation; EVLP: *ex vivo* lung perfusion; PGD: primary graft dysfunction; ECMO: extracorporeal membrane oxygenator.

our lung-transplant activity by nearly one-third in the first 18 months of practice [11]. Our results are comparable with other experiences reported so far [12–16].

Although reconditioned lung transplants were performed with initially rejected grafts and these lungs had a longer ischaemic time (although perfused), clinical results are similar to those coming from standard lung transplantation. Very interestingly, incidence and severity of PGD were similar in both groups at t0 and t72. In Group A, 50% of patients still experienced PGD 3 at t72, and only in 3 cases a full recovery of pulmonary function has been observed. Moreover, PGD had a significant impact on hospital mortality; in fact 4 of 7 patients suffering from PGD 3 at t72 died. The high rate of PGD-3 in our population could be attributed to our use of the lowest value of arterial blood gas analysis for the classification of PGD-3. In our series of transplanted patients, incidence of ECMO implantation was high. This may be explained with a low threshold of P/F for ECMO indication in our centre. We prefer to be aggressive and give ECMO support earlier to those patients with unsatisfactory gas exchange. ECMO is usually implanted percutaneously through a femoro-femoral access when the P/F ratio is lower than 120. However, reconditioned lungs required ECMO only in 2 cases, and some considerations are mandatory. First of all, ECMO support was required in the very first 2 cases of our series and that may be related to an initial learning curve. Secondly, the clinical features of those 2 cases of PGD were peculiar: an abnormal inflammatory response has been noticed, with an elevated WBC count and very high pro-calcitonin levels that completely normalized in POD 2. The weaning of the ECMO device was very fast (<48 h in both cases) with a complete recovery of lung function, and a subsequent uneventful post-operative period with early extubation in both patients. On the contrary, in Group A, only 1 of 5 patients was weaned from ECMO within the first 72 h post-transplant and, among this subgroup, 3 patients died on ECMO. Table 4 shows all the reconditioning experiences reported in the literature so far. The largest series comes from the Toronto Lung Transplant group encompassing 50 patients transplanted with reconditioned lungs from September 2008 to December 2011. Cypel *et al.* [12] demonstrated that, in EVLP recipients, incidence of PGD at t72 tended to be lower than in the control group (2.5 vs 8.5%, $P = 0.14$) and ECMO was required in only 1 patient (2%). The main limitation of their experience is that, in a significant number (22 donors, 44%), EVLP was performed on grafts coming from controlled donation after cardiac death (DCD) donors with optimal lung function before death. Another important paper comes from the UK. The Harefield Hospital group [12] performed 13 EVLP procedures between January 2009 and December 2010 and 6 grafts were eventually transplanted (utilization rate 46%). Two patients developed severe PGD after transplant and required ECMO (1 patient with veno-venous ECMO for 10 days and 1 patient with veno-arterial ECMO for 2 days and veno-venous ECMO for a further 2 days). Wallinder *et al.* [14] from Gothenburg ran 11 perfusions from January 2011 to June 2012, allowing 9 double-lung and 2 single-lung transplants. In that population, 2 patients had severe PGD 72 h after transplant, and 1 patient required ECMO support. All patients recovered normal lung function and were discharged alive from hospital. Aigner *et al.* [15] from Vienna reported on 13 EVLP procedures allowing 9 lung transplants (utilization rate 69%). Significant PGD at t24 was found in 2 patients and 1 of those required ECMO according to the strategy of the centre (pulmonary hypertension). Another significant experience comes from the Lund group [15], using the Vivoline technique. Nine initially rejected lungs have been treated with EVLP, allowing lung transplant in 6 of those. Incidence and severity of PGD were not reported but all patients were alive after 3 months from transplant.

The main limitation of our study is represented by the small number of patients but, although limited, our initial results are encouraging. We do believe that this experience could be of interest also because it comes from a small-volume centre, suggesting a potential widespread use of the reconditioning techniques among every lung transplant programme. Another important limitation is related with the uncontrolled statistical analysis that does not eliminate the possible presence of significant confounders.

Only early and medium-term results are available worldwide on the small cohort of reconditioned lung transplanted patients. Long-term results of this procedure are therefore mandatory before definitely considering EVLP as a standard procedure in lung transplantation. However, the excellent clinical results in terms of both early mortality and graft function reported so far may suggest a significant role of EVLP even in standard grafts that should be confirmed by a prospective randomized clinical trial.

Conflict of interest: none declared.

REFERENCES

[1] Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Dobbels F *et al.* The registry of the International Society for Heart and

Lung Transplantation: 29th adult lung and heartlung transplant report-2012. J Heart Lung Transplant 2012;31:1073–86.
[2] Boffini M, Ranieri VM, Rinaldi M. Lung transplantation: is it still an experimental procedure? Curr Opin Crit Care 2010;16:53–61.
[3] Lee JC, Christie JD. Primary graft dysfunction. Clin Chest Med 2011;32:279–93.
[4] Christie JD, Carby M, Bag R, Corris P, Hertz M, Weill D *et al*. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part II: definition. A consensus statement of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2005;24:1454–9.
[5] Boffini M, Bonato R, Rinaldi M. The potential role of *ex vivo* lung perfusion for the diagnosis of infection before lung transplantation. Transpl Int 2014;27:e5–7.
[6] Daud SA, Yusen RD, Meyers BF, Chakinala MM, Walter MJ, Aloush AA *et al*. Impact of immediate primary lung allograft dysfunction on bronchiolitis obliterans syndrome. Am J Respir Crit Care Med 2007;175:507–13.
[7] de Perrot M, Liu M, Waddell TK, Keshavjee S. Ischemia-reperfusion induced lung injury. Am J Respir Crit Care Med 2003;167:490–511.
[8] Wierup P, Haraldsson A, Nilsson F, Pierre L, Scherstèn H, Silverborn M *et al. Ex vivo* evaluation of non acceptable donor lungs. Ann Thorac Surg 2006;81:460–6.
[9] Steen S, Ingemansson R, Eriksson L, Pierre L, Algotsson L, Wierup P *et al*. First human transplantation of a nonaccepatable donor lung after reconditioning ex vivo. Ann Thorac Surg 2007;83:2191–4.
[10] Cypel M, Yeung JC, Hirayama S, Rubacha M, Fischer S, Anraku M *et al*. Technique for prolonged normothermic ex vivo lung perfusion. J Heart Lung Transplant 2008;27:1319–25.
[11] Boffini M, Ricci D, Barbero C, Bonato R, Ribezzo M, Mancuso E *et al*. Ex-vivo lung perfusion (EVLP) increases the pool of lung grafts: analysis of its potential and real impact on a lung transplant program. Transpl Proc 2013;45:2624–6.
[12] Cypel M, Yeung JC, Machuca T, Chen M, Singer LG, Yasufuku K *et al*. Experience with the first 50 ex vivo lung perfusions in clinical transplantation. J Thorac Cardiovasc Surg 2012;144:1200–6.
[13] Zych B, Popov AF, Stavri G, Bashford A, Bahrami T, Amrani M *et al*. Early outcomes of bilateral sequential single lung transplantation after ex-vivo lung evaluation and reconditioning. J Heart Lung Transplant 2012;31:274–81.
[14] Wallinder A, Ricksten SE, Silverborn M, Hansson C, Riise GC, Liden H *et al*. Early results in transplantation of initially rejected donor lungs after ex vivo lung perfusion: a case-control study. Eur J Cardiothorac Surg 2014;45:40–4; discussion 44–5.
[15] Aigner C, Slama A, Hotzenecker K, Scheed A, Urbanek B, Schmid W *et al*. Clinical ex vivo lung perfusion—pushing the limits. Am J Transplant 2012;12:1839–47.
[16] Ingemansson R, Eyjolfsson A, Mared L, Pierre L, Algotsson L, Ekmehag B *et al*. Clinical transplantation of initially rejected donor lungs after reconditioning ex vivo. Ann Thorac Surg 2009;87:255–60.

APPENDIX. CONFERENCE DISCUSSION

Dr A. Haverich *(Hannover, Germany)*: This is a very thoroughly investigated small series, and I think we learnt a lot from the data you presented. I have two questions. We in Hannover recently published a series of patients where we used borderline donor organs for lung transplantation in elective patients and found that in the low-risk patient population on the recipient side, the results were comparable with the overall results if we used borderline lungs in this population.

So my first question is in relation to your patient selection. Did you use this in elective patients or in emergency case patients, or didn't you care at all about the indication?

Dr Boffini: I do apologize because I didn't say that. This is a consecutive series. The indication to use the graft, the type of graft, was related with the presence of an available recipient. We did not select any patient. And although the statistical analysis may be quite weak, I can say that this can be considered a picture of real daily practice, and the basic take-home message is that you can use this graft quite safely with acceptable results.

Dr Haverich: The second question is in regard to the logistics. I learnt from your slides that you probably require 10 hours or so of extracorporeal perfusion. What about the anaesthesia, the operating room, are they waiting all the time? At what time do you take the recipient to the operating room for anaesthesia? What are the logistics around it? Or do you say, after 10 hours now the lungs are good, we do the implant. The question relates to the fact that in Hannover we are using continuous perfusion during transportation, and at the time of the end of transportation of the organs we can say this organ is good and we could do the transplant using the Organ Care System.

Dr Boffini: Yes, the organization is quite important. We have an operating room dedicated for this procedure; basically it is an operating room for emergencies. And we don't move the patient for the transplant until we are sure that the graft will be used. And the criteria to decide whether the graft will be used is based on two time points at least. So we think that the single parameter or the single value is not the important thing, but much more the trend. So we have to wait up to 2 to 3 hours before deciding. And when we have decided, we go ahead with perfusion just to reduce the ischaemic time before transplantation, but our local committee allowed us to go on with perfusion for up to 6 hours. After 6 hours we stop the perfusion, we cool the graft, and proceed as for the standard lung transplant.

Dr D. Wood *(Seattle, WA, USA)*: You've shown in your small series that you are effective with marginal or even potentially rejected donors in rehabilitating them and have successful results. And yet your outcomes would suggest that the results are even better with those that have been on ex vivo perfusion. Are you going to change your standards for which donors get ex vivo perfusion and potentially use it in more and more donors, making it a part of the standard of your practice for lung transplantation?

Dr Boffini: This is a good point. We have the feeling that because the incidence of PGD is lower and less severe with this type of graft, it would be very interesting to set up a prospective randomized clinical trial to also compare standard grafts treated with ex vivo lung perfusion versus the standard procedure. I must say that without the ex vivo lung perfusion, these organs would not have been accepted in our centre. So they were all rejected grafts for us.

Dr P. Ariyaratnam *(Hull, UK)*: What I wanted to ask is: do you use EVLP for a few hours? We know from some studies that hypoxia-reoxygenation can cause a lot of injury to the lungs. But you use a high level of reoxygenation, I think 100% of oxygenation, to get your sort of criteria for an optimized lung. I just wondered if there was any sort of detrimental effect on the lungs of using prolonged periods of perfusion with a high oxygenation?

Dr Boffini: During the perfusion, the lungs are ventilated on room air for all the period and the ventilator is switched on 100% only for 5 minutes just for the evaluation point phase. After that we go back to room air for the so-called maintaining period.

Dr P. Ariyaratnam: Okay.