Ex vivo lung perfusion in clinical lung transplantation—State of the art

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Summary

Ex vivo lung perfusion (EVLP) has emerged as a new technique for assessing and potentially reconditioning human donor lungs previously unacceptable for clinical transplantation with the potential to dramatically push the limits of organ acceptability. With the recent introduction of portable EVLP, a new era in lung preservation may be upon us with the opportunity to also limit organ ischaemic times and potentially improve the outcome of donor lungs already deemed acceptable for transplantation. It took over half a century for the technique to evolve from basic theory to semi-automated circuits fit for clinical use that are now rapidly being adopted in transplant centres across the globe. With this field in constant evolution and many unanswered questions remaining, our review serves as an update on the state of the art of EVLP in clinical lung transplantation.

Keywords: Ex vivo lung perfusion • Lung transplantation • State-of-the-art review

INTRODUCTION

Lung transplantation is now a well-established treatment for end-stage lung disease with a median survival of over 10 years in specific patient groups [1]. There is, however, a critical shortage of suitable donor lungs available for many patients and one in five of those on the lung transplant waiting list will die before a suitable organ is identified. Less than 20% of lungs from multiorgan donors are at present deemed suitable for transplantation in the USA and the UK, and despite a year on year increase in absolute transplant numbers, an annual waiting list mortality of 10–20% persists [2–5].

A major challenge facing the lung transplant community is how to increase the number of usable donor lungs without compromising the success of the procedure. What have previously been regarded as ‘ideal’ donor lung criteria by the International Society for Heart and Lung Transplantation (ISHLT) are becoming less representative of what is now deemed acceptable in most centres (Table 1) [6]. This is in part due to improved management of the potential donor and also due to a redefinition of accept-ance criteria extending outside published standards [7–9]. This has increased absolute numbers of donor lungs available for transplant, but may have led to a more complex clinical management postoperatively [10].

Ex vivo lung perfusion (EVLP) has emerged as a promising new technique for evaluating and reconditioning donor lungs that would previously have been regarded as unusable. By increasing the number of lung transplant procedures in individual centres by 15–30%, EVLP is showing potential to substantially increase the availability of suitable donor lungs [11, 12]. Sanchez et al. [13] provided a detailed review of the basic EVLP technique in 2012. However, clinical experience has been rapidly evolving and with the introduction of portable EVLP, we might now face a new era of lung preservation where EVLP may also improve outcomes from donor lungs already deemed acceptable.

This review compiles the available findings on the use of EVLP in clinical lung transplantation, reporting on all publications, published abstracts to international meetings and ongoing multicentre trials, to provide an update on the state of the art of EVLP and the basis for a discussion of the key questions that remain unanswered in clinical use of EVLP.

MATERIALS AND METHODS

The literature search was carried out with the assistance of our Library and Information Services Manager with specialty training in the field. For full manuscripts, we searched Medline and EMBASE databases between year 1999 and January 2014 using the following search terms and keywords individually or combined: ex, vivo, lung, perfusion, transplantation, OCS and ‘organ care system’. For abstracts to international meetings, we used the same search terms and time period and searched the individual databases of the American Journal of Transplantation (ASTS, AST), Interactive Cardiovascular and Thoracic Surgery (EACTS, ECGTS), Journal of Heart and Lung Transplantation (ISHLT), Transplant International (ESOT) and Transplant Proceedings (TTS). For ongoing trials, the search terms were used in Medline’s clinical trials database, the Current Controlled Trials Database [http://www.controlled-trials.com], the ClinicalTrials.gov database [http://clinicaltrials.gov], the EU Clinical Trials Register [http://www.clinicaltrialsregister.eu] and the UK Clinical Research Network Portfolio Database [http://public.ukcrn.org.uk]. All search results were reviewed in detail and any report on one or more human
lung transplantation after EVLP assessment and any relevant randomized controlled or multicentre trial was included.

EX VIVO LUNG PERFUSION IN CLINICAL LUNG TRANSPLANTATION

Background

With the development of the first cardiopulmonary bypass machines in the 1950s grew an interest of its potential in organ transplantation, and various groups came to investigate the effect that perfusion, storage and preservation had on tissue viability and function [14–16]. In the case of hearts and lungs, the preserved organs were found particularly delicate yet had to be capable of performing at almost full normal function immediately following transplantation. Early attempts at perfusing the lung were almost invariably frustrated by oedema formation and deteriorating function (e.g. Jirsch et al. [17] at the University of Alberta, Canada, with canine lobes). This era is well described by Sanchez et al. [13].

Contribution of Steen

Originally designed to enable an improved assessment of lungs from donors after circulatory death (DCD), Steen and colleagues in Lund developed the first well-functioning EVLP circuit for clinical use. A fundamental step towards success was the development of Steen Solution™, a buffered perfusate solution with high albumin concentration to create an ideal colloid osmotic pressure allowing physiologic perfusion pressures and flow to be maintained without causing pulmonary oedema [18].

The Lund group EVLP circuit provided the blueprint for all circuits currently used in clinical centres worldwide. A pump generates a perfusate flow through a leucocyte filter and an oxygenator connected to a heater–cooler unit and gas exchange membrane before entering the lung through a cannula in the pulmonary artery. Pulmonary venous return is collected in a sterile reservoir either through an open left atrium (LA) or through a closed LA cannula and then recirculated. A ventilator is connected to the trachea allowing ‘protective’ ventilation to be carefully started only after the lungs have been rewarmed to 32°C and steadily increased while approaching normothermia (Fig. 1, Table 2) [18–20]. Figure 2 shows a timeline of the EVLP development in clinical lung transplantation.

Since the initial descriptions from Sweden, three differing philosophies have emerged from the centres in Lund, Toronto and Hannover.

The first two perfuse the cold-flushed and cold-stored lungs after arrival at the recipient centre, but differ in rate of flow, the presence of blood in the perfusate and the left atrial pressure. The last seeks to minimize lung ischaemia and cooling by perfusing in transit after a brief initial flush, with perfusion at normothermia almost from the start.

Lund—pioneers of clinical ex vivo lung perfusion

The preclinical work was translated by Steen et al. [22, 23] in 2000 when they performed the first human lung transplant using a DCD lung assessed by EVLP and the first successful lung transplant of an initially unacceptable donor lung reconditioned ex vivo in 2005.

The Lund EVLP protocol is designed to mimic the physiologic conditions a transplanted lung will face after reperfusion in the recipient. It serves primarily as a thorough assessment of borderline lungs not meeting standard acceptance criteria to reclaim those demonstrating suitability for clinical transplantation on the ex vivo circuit.

Table 1: ISHLT ‘ideal’ lung donor criteria

<table>
<thead>
<tr>
<th>Lung donor acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 55 years</td>
</tr>
<tr>
<td>ABO compatibility</td>
</tr>
<tr>
<td>Clear chest radiograph</td>
</tr>
<tr>
<td>PaO2 &gt; 300 mmHg (40 kPa) on FiO2 1.0, PEEP 5 cmH2O</td>
</tr>
<tr>
<td>Tobacco history &lt; 20 pack-years</td>
</tr>
<tr>
<td>Absence of chest trauma</td>
</tr>
<tr>
<td>No evidence of aspiration/sepsis</td>
</tr>
<tr>
<td>No prior cardiopulmonary surgery</td>
</tr>
<tr>
<td>Sputum gram stain—absence of organisms</td>
</tr>
<tr>
<td>Absence of purulent secretions at bronchoscopy</td>
</tr>
</tbody>
</table>

PaO2: partial pressure of oxygen; FiO2: fraction of inspired oxygen; PEEP: peak end-expiratory pressure; ISHLT: International Society for Heart and Lung Transplantation. (Adapted from Orens et al. [6].)
In the Lund protocol, an adequate period of perfusion is essential after reaching target temperature, ventilation and full flow to ensure stable respiratory and circulatory parameters and to give opportunity for lung reconditioning. EVLP reconditioning involves recruitment of atelectasis, reduction of the inflammatory burden by means of a leucocyte filter, circulating antimicrobials and glucocorticoids and sufficient time for oedema fluid to, if possible, be drawn out of the parenchyma by the hyperoncotic perfusate. In the clinical reports from Lund, perfusion times ranged from 1 to 2 h. If the initial evaluation was satisfactory, the lungs were immediately cooled and transplanted. If criteria were not met and potential for improvement remained, the lungs were perfused further to allow reconditioning; otherwise, if they failed to improve, they were discarded [22–24].

The prospective non-randomized multicentre trial DEVELOP-UK is currently running in all five UK transplant centres aiming to evaluate the clinical outcome, cost-effectiveness and quality of life for patients transplanted with reconditioned EVLP lungs compared with standard donor lungs using the Lund protocol and the Vivoline® LS1 system (Vivoline Medical AB, Lund, Sweden) (Table 3) [25].

### Table 2: EVLP protocols in clinical lung transplantation

<table>
<thead>
<tr>
<th></th>
<th>Lund</th>
<th>Toronto</th>
<th>OCS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Perfusion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target flow</td>
<td>100% of cardiac output (70 ml/kg/min)</td>
<td>40% of cardiac output ≤ 15</td>
<td>2–2.5 l/min</td>
</tr>
<tr>
<td>Pulmonary arterial pressure (mmHg)</td>
<td>≤ 10</td>
<td>≤ 15</td>
<td>≤ 20</td>
</tr>
<tr>
<td>Left atrial pressure (mmHg)</td>
<td>0 (open LA)</td>
<td>3–5</td>
<td>0 (open LA)</td>
</tr>
<tr>
<td>Pump</td>
<td>Roller</td>
<td>Centrifugal</td>
<td>Piston (pulsatile)</td>
</tr>
<tr>
<td>Per fusate</td>
<td>2 l Steen Solution™ with red-cell concentrates (haematocrit 10–15%)</td>
<td>2 l Steen Solution™</td>
<td>1.5 l OCS lung solution™ with red-cell concentrates (haematocrit 15–25%)</td>
</tr>
<tr>
<td><strong>Ventilation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode</td>
<td>Volume controlled</td>
<td>Volume controlled</td>
<td>Volume controlled</td>
</tr>
<tr>
<td>Tidal volume (ml/kg)</td>
<td>6–8</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Frequency (bpm)</td>
<td>10–15</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Peak end-expiratory pressure (cmH2O)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Fraction of inspired oxygen (%)</td>
<td>50</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>Start of ventilation</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Start of perfusion</td>
<td>37</td>
<td>37</td>
</tr>
</tbody>
</table>

*The ‘OCS Lung solution’ is similar in composition to low potassium dextran (LPD) with added glucose, and does not contain any albumin (Warnecke, Study Director of the OCS™ Lung INSPIRE Trial, personal communication). OCS: Organ Care System™; LA: left atrium; bpm: breaths per minute; EVLP: ex vivo lung perfusion.*

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The prospective non-randomized multicentre trial DEVELOP-UK is currently running in all five UK transplant centres aiming to evaluate the clinical outcome, cost-effectiveness and quality of life for patients transplanted with reconditioned EVLP lungs compared with standard donor lungs using the Lund protocol and the Vivoline® LS1 system (Vivoline Medical AB, Lund, Sweden) (Table 3) [25].

**Figure 2:** Timeline of EVLP in clinical lung transplantation. Labelled year is referring to the centre’s first reported EVLP transplant and the launch of on-going multicentre EVLP trials. EVLP: ex vivo lung perfusion.

**Toronto—developing and validating ex vivo lung perfusion**

Keshavjee and colleagues in Toronto have, with their extensive contributions, changed the landscape of EVLP into a technique to significantly expand the limited donor pool currently used in transplant centres all around the world [11, 19, 29, 30].

The focus of Keshavjee and Cypel’s studies has not been to just evaluate if a graft is usable or not, but to prolong the perfusion times to be able to potentially treat and better recondition injured lungs before transplantation (Table 2). They have most notably revised the Lund protocol to potentially increase the option of longer term perfusion with

- An acellular perfusate to avoid potential detrimental haemolysis.
- A low flow strategy with only 40% of estimated cardiac output to reduce pulmonary vascular shear stress and oedema formation.
- A closed circuit with both the PA and LA cannulated creating a positive LA pressure.

In six porcine double lungs and five human single lungs, they reported successful perfusion for 12 h with stable macroscopic, 

### Table 3: Ongoing multicentre trials investigating EVLP in clinical lung transplantation

<table>
<thead>
<tr>
<th>Brief title</th>
<th>Design</th>
<th>Location</th>
<th>EVLP protocol</th>
<th>Start date</th>
<th>Estimated enrolment</th>
<th>Estimated completion date</th>
<th>Primary end-point</th>
<th>Estimated date</th>
<th>EVLP system</th>
<th>EVLP protocol</th>
</tr>
</thead>
</table>
| NOVEL Lung Trial [25] | Prospective non-randomized, multicentre study | Germany, Belgium, France, Italy, Spain, UK, USA, Canada, Australia | OCS™ Lung (TransMedics, Inc., Andover, MA, USA) | Apr 2012 | 408 transplants (102 OCS preserved and 306 OCS reconditioned) | Dec 2015 | Composite of patient and graft 30-day survival and PGD Grade 3 at T72 h | Oct 2015 | Toronto XPS Lung Trial is now being conducted in the USA with the Toronto EVLP protocol and the XPS™ system to approve its clinical use (XVIVO Perfusion AB, Gothenburg, Sweden) [26].

### Hannover/Madrid—portable ex vivo lung perfusion

In a pilot study published in Lancet 2012, Warnecke et al. investigated the effect of normothermic preservation and transportation of standard criteria human donor lungs on a portable EVLP system. Twelve pairs of standard donor lungs were, instead of being brought to their centres by means of cold preservation on ice, preserved by normothermic perfusion and ventilation on the transportable Organ Care System™ (OCS) Lung (TransMedics, Inc., Andover, MA, USA). This was the first report of a portable EVLP system used in clinical transplantation, with short-term outcomes non-inferior to controls [20].

The OCS protocol used in the pilot study was a hybrid of the Lund and Toronto EVLP protocols. A cellular perfusate based on Steen Solution™ supplemented with erythrocytes and an open LA was combined with a perfusate flow limited to 2.5 l/min resembling the protective approach developed by the Toronto group. The Steen Solution™ has later been replaced by a low potassium dextran solution with added glucose, made by the system manufacturer (Table 2).

The OCS protocol is currently being evaluated on a larger scale in a prospective randomized multicentre pivotal trial, OCS™ Lung INSPIRE Trial, comparing transplant outcomes of standard criteria lungs preserved and transported by either normothermic EVLP or standard cold preservation [27]. Moreover, the International EXPAND Lung Pivotal Trial was just launched as a clinical pilot to also evaluate the more traditional use of assessing and possibly reconditioning lungs deemed unusable for standard transplantation on the OCS Lung portable system (Table 3) [28].

All current reports on clinical transplantation after EVLP are summarized in (Table 4).

### THE UNANSWERED QUESTIONS

#### Protocol strategy

EVLP offers a possible solution to some of the problem of scarcity of donor organs, but as an assessment/reconditioning tool, it is...
potentially expensive and time consuming. The choice between the three different EVLP techniques used in clinical lung transplantation today is therefore strongly linked to the development of commercial EVLP systems and endorsements of clinical trials. From initially consisting of kits put together in-house from cardiopulmonary bypass machines and ventilators in Sweden, Canada and the UK, the market for complete organ assessment systems is now thriving, and having a signature multicentre study seems vital for any company wanting to compete for market share (Fig. 3, Table 3). The outcomes of these trials could therefore have a central role in which protocols and systems will endure.

### Cellular or acellular perfusate?

One of the fundamental questions in EVLP is whether or not to use blood in the circulating perfusate. The Lund and OCS protocols advocate a cellular perfusate, whereas the Toronto technique uses an acellular solution.

The EVLP logistics are simplified, costs lowered and ethical conflicts arising from the use of limited blood products in studies of organs that may not be used clinically are prevented by avoiding erythrocyte concentrates [49]. The oxygen supply to the lung cells during EVLP also appears to be sufficiently provided by the ventilator alone without the need of oxygen carriers for parenchymal preservation [19, 50]. The most common reason for choosing an acellular perfusate is the concern of potential haemolysis due to mechanical trauma to the red blood cells during prolonged perfusion and the possible detrimental effects this might have on the donor lung [19, 49, 51, 52]. However, evidence of this being of concern in EVLP is limited. In a frequently cited study from Erasmus et al., lungs from four DCD porcine donors showed rising pulmonary vascular resistance (PVR) and airway pressures after 6 h of perfusion on their Lund-resembling EVLP circuit. This observation was later related to the use of a cellular perfusate and hence compared with the successful Toronto experiments of prolonged perfusion described above [19, 52, 53]. The reports are both limited by small sample sizes and only partly based on human preclinical experiments. A possible confounding factor to the raised PVR seen in Erasmus’ experiments was the absence of heparin or any other anticoagulant in the cellular perfusate, an essential component of the protocols used today as a precaution against micro-thrombi responsible for this sort of lung deterioration [38, 40].

### Table 4: Reports on EVLP in clinical lung transplantation

<table>
<thead>
<tr>
<th>Author</th>
<th>Transplant centre</th>
<th>Year of transplant</th>
<th>EVLP protocol</th>
<th>No. of EVLP assessments</th>
<th>Conversion rate to transplant</th>
<th>Median PaO2/FiO2 (kPa)</th>
<th>Rate of ECMO post-Tx (non-elective)</th>
<th>Rate of PGD Grade 3 at 72 h</th>
<th>30-day survival (%)</th>
<th>1-year survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingemansson et al. [34]</td>
<td>Lund</td>
<td>2006–7</td>
<td>Lund</td>
<td>8</td>
<td>75% (6)</td>
<td>21.1</td>
<td>17% (1)</td>
<td>NK</td>
<td>100</td>
<td>67</td>
</tr>
<tr>
<td>Fildes et al. [35]</td>
<td>Manchester</td>
<td>2008–10</td>
<td>Lund</td>
<td>NK</td>
<td>NK (8)</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>87.5</td>
<td>NK</td>
</tr>
<tr>
<td>Cypel et al. [11]</td>
<td>Toronto</td>
<td>2008–11</td>
<td>Toronto</td>
<td>58</td>
<td>86% (50)</td>
<td>44.5</td>
<td>2% (1)</td>
<td>2% (1)</td>
<td>96</td>
<td>87</td>
</tr>
<tr>
<td>Dark et al. [36]</td>
<td>Newcastle</td>
<td>2009–11</td>
<td>Toronto</td>
<td>18</td>
<td>39% (7)</td>
<td>34.6</td>
<td>0% (0)</td>
<td>14% (1)</td>
<td>100</td>
<td>86</td>
</tr>
<tr>
<td>Zych et al. [37]</td>
<td>Harefield</td>
<td>2009–10</td>
<td>Toronto</td>
<td>13</td>
<td>46% (6)</td>
<td>47.0a</td>
<td>33% (2)</td>
<td>NK</td>
<td>100</td>
<td>NK</td>
</tr>
<tr>
<td>Aigner et al. [38]</td>
<td>Vienna</td>
<td>2010–11</td>
<td>Toronto</td>
<td>13</td>
<td>69% (9)</td>
<td>28.8</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100</td>
<td>NK</td>
</tr>
<tr>
<td>Moradiellos et al. [39]</td>
<td>Madrid</td>
<td></td>
<td>NK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valenza et al. [40]</td>
<td>Milan</td>
<td>2011</td>
<td>Toronto</td>
<td>NK</td>
<td>NK (2)</td>
<td>24.5</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100</td>
<td>NK</td>
</tr>
<tr>
<td>Warnecke et al. [41]</td>
<td>Hannover</td>
<td>2011</td>
<td>OCS</td>
<td>12</td>
<td>100% (12)</td>
<td>61.5</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>Wallinder et al. [21]</td>
<td>Gothenburg</td>
<td>2011–12</td>
<td>Lund</td>
<td>11</td>
<td>100% (11)</td>
<td>27.9</td>
<td>9% (1)</td>
<td>9% (1)</td>
<td>100</td>
<td>NK</td>
</tr>
<tr>
<td>Hopkins et al. [42]</td>
<td>Brisbane</td>
<td>2011–12</td>
<td>Lund</td>
<td>5</td>
<td>80% (4)</td>
<td>25.2b</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100</td>
<td>NK</td>
</tr>
<tr>
<td>Boffini et al. [43]</td>
<td>Turin</td>
<td>2011–13</td>
<td>Toronto</td>
<td>NK</td>
<td>NK (8)</td>
<td>NK</td>
<td>25% (2)</td>
<td>0% (0)</td>
<td>NK</td>
<td>NK</td>
</tr>
<tr>
<td>Sage et al. [44]</td>
<td>Paris</td>
<td>2011–12</td>
<td>Toronto</td>
<td>21</td>
<td>95% (20)</td>
<td>34.1</td>
<td>NK</td>
<td>10% (2)</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Cypel et al. [45]</td>
<td>Toronto/Vienna/Madrid</td>
<td>2008–12</td>
<td>Toronto</td>
<td>125</td>
<td>82% (103)</td>
<td>35.7c</td>
<td>NK</td>
<td>5% (5)</td>
<td>96</td>
<td>88</td>
</tr>
<tr>
<td>Steen et al. [22]</td>
<td>Lund</td>
<td>2000</td>
<td>Lund</td>
<td>1</td>
<td>100% (1)</td>
<td>12.9</td>
<td>NK</td>
<td>0% (0)</td>
<td>100</td>
<td>NK</td>
</tr>
<tr>
<td>Steen et al. [23]</td>
<td>Lund</td>
<td>2005</td>
<td>Lund</td>
<td>1</td>
<td>100% (1)</td>
<td>34.4</td>
<td>0% (0)</td>
<td>NK</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Wigfield et al. [46]</td>
<td>Chicago/Toronto</td>
<td>2011</td>
<td>Toronto</td>
<td>1</td>
<td>100% (1)</td>
<td>24.0</td>
<td>0% (0)</td>
<td>NK</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>García Sáez et al. [47]</td>
<td>Harefield</td>
<td>2012</td>
<td>Toronto</td>
<td>1</td>
<td>100% (1)</td>
<td>&gt;40.0</td>
<td>NK</td>
<td>NK</td>
<td>100</td>
<td>NK</td>
</tr>
<tr>
<td>Patil et al. [48]</td>
<td>Harefield</td>
<td>2012</td>
<td>Toronto</td>
<td>1</td>
<td>100% (1)</td>
<td>10.0</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100</td>
<td>NK</td>
</tr>
<tr>
<td>Wallinder et al. [49]</td>
<td>Gothenburg</td>
<td>2012</td>
<td>Lund</td>
<td>2</td>
<td>100% (1)</td>
<td>40.0</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100</td>
<td>NK</td>
</tr>
<tr>
<td>Methangkool et al. [50]</td>
<td>UCLA</td>
<td>2012</td>
<td>OCS</td>
<td>1</td>
<td>100% (1)</td>
<td>35.0</td>
<td>0% (0)</td>
<td>NK</td>
<td>100</td>
<td>NK</td>
</tr>
</tbody>
</table>

*a* 90-day survival.  
*b* Mean PaO2/FiO2.  
*c* Toronto protocol with a cellular perfusate.  
*d* Three-centre experience presented at ISHLT 2013, overlapping individual centre reports.  
EVLP: ex vivo lung perfusion; ECMO: extracorporeal membrane oxygenation; PGD: primary graft dysfunction; NK: not known; PaO2/FiO2: ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen.
during lung evaluation [21, 55]. It is also argued that the presence of red blood cells provides a more physiologically relevant assessment of flow through the pulmonary microvasculature. In an elegant animal study, Yeung and colleagues in Toronto showed that blood gas analyses during EVLP with an acellular perfusate are unreliable and could disguise oxygenation deficits during assessment, especially in the case of a ventilation–perfusion mismatch. The shunting effect became obvious first when erythrocytes were added to the perfusate, and then vanished again when the perfusate was replaced with fresh acellular Steen Solution™ [51]. This was again a very small study, but the results and reasoning merit emphasis and further study as they fundamentally question the reliability of PaO₂ measurements during EVLP when using an acellular perfusate. Blood gas analyses drawn from the oxygenated perfusate returning to the LA are presently indispensable in evaluating lung function during perfusion and part of every published list of criteria on transplant suitability after EVLP in clinical lung transplantation.

**Assessment or reconditioning?**

A major limitation of *ex vivo* organ perfusion has since the beginning been injury and oedema formation inflicted by the circuit itself. Perfusion time is becoming a more and more central marker defining a system’s quality. The longer you can safely perfuse an organ, the longer you have for reconditioning, possible treatments, transportation and other logistics surrounding a transplant.

From available reports, Toronto runs the most stringent reconditioning process promoting perfusion to be maintained for at least 3–4 h with hourly assessments before determining transplant suitability [11]. In the Vienna and Harefield centres, they used the Toronto protocol, but questioned whether lungs demonstrating a rapid recovery derived any benefit from longer perfusion periods, and were accepting donor lungs after 2 h of EVLP [37, 38]. The Gothenburg and Lund groups went further and claimed that no additional time should be spent on the circuit if initial blood gas values, circulatory and ventilator parameters and the macroscopic evaluations were deemed satisfactory, and accepted a single evaluation time point for decision-making on transplant suitability [21, 34]. This approach has to our knowledge been unique to these two centres and has now also been adopted by the DEVELOP-UK study protocol [25]. More commonly, at least two sequential evaluations are considered necessary to back up a transplant decision [29, 36, 38, 41, 56].

**Full or reduced perfusate flow?**

The reduced perfusate flow used in the Toronto and OCS protocols is potentially less damaging to the donor lung and could allow longer perfusion times, supported by reports of successful experimental perfusion of 12 h with both methods [19, 57]. The alternative view is that a low flow strategy risks hypoperfusion with failure to adequately circulate areas of the capillary network. The assessment is at the same time less representative of the post-transplant load on the lung circulation, and could therefore require a longer and more thorough evaluation.

A complete evaluation with the Lund protocol, on the other hand, seems to give the assessor confidence in making a quick prediction of the organ’s transplant suitability, reflected in high conversion rates though shorter perfusion times in these centres (Table 4). The early decision-making with this approach could also indicate a remaining reluctance to assess the organ longer than necessary, potentially restricting the reconditioning potential. The Lund protocol has shown excellent transplant outcomes so far, but still has to prove its potential in long-term perfusion.

The EXPAND lung trial will add additional insight into the option of starting reconditioning of an injured lung immediately in the donor hospital and possibly avoid additional damage caused by cold ischaemia [28].

With the protocols running parallel in different centres with very little overlap and no existing comparative studies, claiming the superiority of one or the other is still merely speculation. A head-to-head randomized, controlled trial of the protocols and machines could now certainly be approved on ethical grounds and could be highly valuable to identify their individual benefits and limitations.

**Antimicrobial treatment**

The hazard of respiratory infections in the early post-transplant period is notorious and donor-to-host transmission of bacterial...
and fungal infections is a known cause of morbidity in the immunosuppressed lung transplant recipient [58–61].

With a localized closed circuit, EVLP is ideal for high-dose antimicrobial treatment with no risk of side effects to other organs. Extended criteria donor lungs subjected to the assessment form, by nature, a sub-population likely to have a higher microbial load than standard lungs. All clinical EVLP protocols therefore use prophylactic broad-spectrum antibiotics in their perfusate solution. Surprisingly, none of the clinical reports mention an analogous use of prophylactic fungicides. In Newcastle, we have recently reported that EVLP with high-dose antimicrobials in the perfusate is associated with an effective reduction of both the bacterial and fungal burden of the donor lung. We empirically used meropenem and amphotericin B, and recommend the addition of a broad-spectrum fungicide to the perfusate [62]. Yeast is a well-known cause of infections in the immunosuppressed post-transplant patient and was frequently cultured in the bronchoalveolar lavage fluid from these borderline donor lungs [6, 7, 62]. The results provide reassurance that normothermic EVLP does not increase the microbial load in the human donor lung, but clearly reduces it, and is therefore likely to increase the chance of a good outcome from a lung that would not otherwise have been used for transplantation.

**Ex vivo lung perfusion centres or ex vivo lung perfusion in every centre?**

Around 350 transplants of lungs perfused ex vivo have now been performed in nearly 30 centres. These centres are still mainly concentrated in Western Europe and North America, but the Brisbane group has been transplanting EVLP lungs since 2011, the São Paulo team is on the forefront of EVLP research, and in October 2013, Okayama became the first centre to transplant an EVLP-reconditioned lung in Asian (personal communication with X Vivo Perfusion AB and TransMedics, Inc.). Meanwhile, there is an ongoing debate on how the EVLP service should best be delivered in the future. The Toronto group has shown the feasibility of the ‘EVLP Centre’ approach in a case report with the team in Chicago. To manoeuvre around the restriction on EVLP in the USA outside the NOVEL Lung trial, a pair of unacceptable donor lungs was transported on ice to Toronto for reconditioning, and then flown back to Chicago for successful implantation [32]. Thus, concentrating the volume to fewer centres will presumably increase the experience of the EVLP team. The most obvious drawbacks of establishing such specialized EVLP centres are transportation and environmental costs, more complex logistics and the inevitable prolonged cold ischaemia inflicted on an already injured organ. By either approach, there is witness of less quantifiable benefits with the mere availability of the EVLP technique. In Milan, they stress how the implementation of EVLP in their low-volume centre has facilitated the safe use of extended criteria donor lungs in recent years [40]. In Newcastle, we have seen a clear increase in the numbers of donors assessed by our retrieval teams since EVLP was put into practice in 2009. Currently, the retrieving surgeon frequently manages to optimize intended EVLP donors, which would previously not have been approached, to reach standard transplant criteria. Initially rejected lungs are therefore now being brought immediately to implantation as often as taken back to our centre for reconditioning, an important ‘side effect’ of EVLP provision. This has also contributed to the launch of a National Scout Programme in 2012 to further optimize our donor management [63]. Another possible direction of future donor retrieval is the development of specialized procurement teams, independent from the surgical implant team. Such a team may provide an integrated organ perfusion service and offer adequately assessed and reconditioned donor lungs to transplant centres.

**Role of lungs from donors after circulatory death**

The resurgent interest in DCD donation from the early 1990s onwards ran in parallel with the work of Egan, Steen and others interested in using EVLP to evaluate these lungs [53, 64, 65]. The Toronto group initially used EVLP to assess a high proportion of DCD lungs before transplantation and all in which the time to donor arrest was longer than 30 min, even if they otherwise met standard criteria [29, 66]. Reports now indicate the safe use of these organs and equivalent early and intermediate outcomes compared with donation after brain death transplants. A compulsory EVLP assessment of DCD grafts therefore appears redundant [66–68]. However, EVLP may continue to play a pivotal role in supporting centres to safely start up DCD programmes, not least in Scandinavia, Germany and other regions where EVLP has preceded the implementation of DCD transplantation. We are also beginning to see the potential of EVLP in reassessing uncontrolled (Maastricht Category II) DCD donors as described by Moradiellos et al. in Madrid, which has the potential to completely alter donor graft availability [39].

**Static or portable ex vivo lung perfusion?**

The optimal method for lung preservation remains unclear. In the process of lung transportation, cold storage at 4–8°C has routinely been used to decrease cellular metabolic activity and preserve lung function [69]. This can, however, compound lung injury due to ischaemia, reperfusion and ATP depletion and increase the risk of primary graft dysfunction [79]. A large registry review in 1999 of 5052 lung transplants reported higher 30-day mortality with cold ischaemia times exceeding 8 h. Since the introduction of improved extracorporeal lung-preservation solutions, ischaemic times of up to 10–12 h have been safely reported [71–73]. Cypel et al. showed that normothermic EVLP can interrupt hypothermic ischaemic lung injury after 12 h of cold storage and ameliorate immediate transplant outcomes in a porcine model [74]. Nakajima et al. reported similar beneficial effects of EVLP in beagle DCD lungs [75]. What effect shorter periods of cold ischaemia have on graft function and patient survival in lung transplantation is still to be elucidated. The INSPIRE Lung trial will bring us a step closer [27]. There is still much uncertainty as to whether or not the benefits of this approach will outweigh its considerable costs and logistical hurdles. If it does, however, the portable EVLP technique has the potential of leading the way to a new era in lung assessment and organ preservation.

**Financial considerations of ex vivo lung perfusion**

The absolute cost of generating an additional donor organ for transplantation using EVLP is difficult to calculate, and has not yet been addressed in any clinical report on EVLP. In addition to the costs of equipment, consumables, drugs and perfusate solutions, are the associated staff costs. The available commercial systems have differing capital and running costs, and these need to be balanced against the outcomes for recipients of EVLP assessed and reconditioned lungs after transplantation, such as intensity of intensive therapy unit support and duration of hospitalization. Another
important consideration is the conversion rate from unsuitable donor lungs to usable organs, as a lower conversion rate will increase the overall cost of generating each additional successful lung transplantation with that particular system. More work on the formal health economics of EVLP needs to be performed to help institutions, insurers and commissioners of health services to make informed decisions about service provision.

CONCLUSION

EVLP has rapidly become an important method offering hope of dramatic increases in the number of donor lungs suitable for transplantation. The available methods are being investigated in ongoing multicentre trials, and the full safety, cost-effectiveness and potential impact on graft availability could soon be revealed. Donor selection criteria have undoubtedly varied between centres in the early use of clinical EVLP, perhaps related to contemporaneous transplant cultures and visions. A realistic conversion rate depends crucially on entry criteria, but might be 50–70% in experienced centres aiming to extend the donor pool with poorly functioning organs despite optimal donor management. True comparisons between different philosophies and different centres will depend on some broad definitions of the characteristics of lungs placed on EVLP; these do not yet exist.

As organ perfusion has become one of the ‘hot topics’ in all fields of transplantation in recent years, EVLP continues to lead its progression. Its application in clinical lung transplantation is spreading rapidly across the globe at the same time as exciting new ways to treat and recondition the perfused lung are spreading rapidly across the globe at the same time as exciting new ways to treat and recondition the perfused lung are spreading rapidly across the globe at the same time as exciting new ways to treat and recondition the perfused lung.

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Conflict of interest: Andrew J. Fisher is the Chief Investigator for the multicentre study of ex vivo lung perfusion in the United Kingdom (DEVELOP-UK) [25]. John H. Dark is the local Principal Investigator for the DEVELOP-UK trial at Freeman Hospital, Newcastle upon Tyne.

REFERENCES


Feldes RE, 274:115.


