Normothermic Ex Vivo Lung Perfusion in Clinical Lung Transplantation

Marcelo Cypel, M.D., Jonathan C. Yeung, M.D., Mingyao Liu, M.D., Masaki Anraku, M.D., Fengshi Chen, M.D., Ph.D., Wojtek Karolak, M.D., Masaaki Sato, M.D., Ph.D., Jane Laratta, R.N., Sassan Azad, C.R.A., Mindy Madonik, C.C.P., Chung-Wai Chow, M.D., Cecilia Chaparro, M.D., Michael Hutcheon, M.D., Lianne G. Singer, M.D., Arthur S. Slutsky, M.D., Kazuhiro Yasufuku, M.D., Ph.D., Marc de Perrot, M.D., Andrew F. Pierre, M.D., Thomas K. Waddell, M.D., Ph.D., and Shaf Keshavjee, M.D.

BACKGROUND

More than 80% of donor lungs are potentially injured and therefore not considered suitable for transplantation. With the use of normothermic ex vivo lung perfusion (EVLP), the retrieved donor lung can be perfused in an ex vivo circuit, providing an opportunity to reassess its function before transplantation. In this study, we examined the feasibility of transplanting high-risk donor lungs that have undergone EVLP.

METHODS

In this prospective, nonrandomized clinical trial, we subjected lungs considered to be high risk for transplantation to 4 hours of EVLP. High-risk donor lungs were defined by specific criteria, including pulmonary edema and a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (\(\text{PO}_{2}/\text{FiO}_{2}\)) less than 300 mm Hg. Lungs with acceptable function were subsequently transplanted. Lungs that were transplanted without EVLP during the same period were used as controls. The primary end point was primary graft dysfunction 72 hours after transplantation. Secondary end points were 30-day mortality, bronchial complications, duration of mechanical ventilation, and length of stay in the intensive care unit and hospital.

RESULTS

During the study period, 136 lungs were transplanted. Lungs from 23 donors met the inclusion criteria for EVLP; in 20 of these lungs, physiological function remained stable during EVLP and the median \(\text{PO}_{2}/\text{FiO}_{2}\) ratio increased from 335 mm Hg in the donor lung to 414 and 443 mm Hg at 1 hour and 4 hours of perfusion, respectively (\(P<0.001\)). These 20 lungs were transplanted; the other 116 lungs constituted the control group. The incidence of primary graft dysfunction 72 hours after transplantation was 15% in the EVLP group and 30% in the control group (\(P=0.11\)). No significant differences were observed for any secondary end points, and no severe adverse events were directly attributable to EVLP.

CONCLUSIONS

Transplantation of high-risk donor lungs that were physiologically stable during 4 hours of ex vivo perfusion led to results similar to those obtained with conventionally selected lungs. (Funded by Vitrolife; ClinicalTrials.gov number, NCT01190059.)

From the Toronto Lung Transplant Program (M.C., J.C.Y., M.L., M.A., F.C., W.K., M.S., J.L., S.A., M.M., C.W.C., C.C., M.H., L.G.S., K.Y., M.P., A.F.P., T.K.W., S.K.) and the Interdepartmental Division of Critical Care Medicine (A.S.S.), University of Toronto; the McEwen Centre for Regenerative Medicine, Toronto General Research Institute (M.C., M.L., T.K.W., S.K.); and the Keenan Research Centre, Li Ka Shing Knowledge Institute, St. Michael’s Hospital (A.S.S.) — all in Toronto. Address re-print requests to Dr. Keshavjee at Toronto General Hospital, 200 Elizabeth St., 9N946, Toronto, ON M5G 2C4, Canada, or at shaf.keshavjee@uhn.on.ca.

Lung transplantation is lifesaving for patients with end-stage lung diseases. However, the number of patients waiting for a lung transplant greatly exceeds the number of available donors. On average, only 15% of lungs from multiorgan donors are used for transplantation; the rest are considered unsuitable owing to the lung injury that occurs after brain death and to complications associated with treatment in the intensive care unit (ICU) (e.g., barotrauma and pulmonary edema). Although nonstandard donor lungs (i.e., lungs with suboptimal gas-exchange function or infiltrates visible on chest radiographs) have been used successfully, increased primary graft dysfunction — an acute lung injury that appears within 72 hours after transplantation — has been reported in some studies. Such injury affects early outcomes and is associated with an increased risk of chronic graft dysfunction. Thus, clinicians tend to be highly conservative when selecting donors, and because of the relatively small number of organs that are deemed to be acceptable, mortality is high among patients awaiting transplantation.

Increased use of available lungs is a promising means of augmenting the number of lung transplants. Although management strategies in the multiorgan donor are important in preventing lung deterioration, organs are often retrieved before the lungs can recover from brain death or related injuries. The use of static hypothermia is widely accepted for preserving lung viability after removal, but the inhibition of cellular metabolism as a result of hypothermia makes it difficult to repair lungs or assess them (i.e., retest the organ) during the preservation period. Ex vivo lung perfusion (EVLP) with the use of acellular normothermic perfusate after organ retrieval is a potential method for assessing lung viability. With EVLP, lungs are perfused and ventilated ex vivo at body temperature to mimic physiologic conditions. Preclinical studies have shown that normal and injured donor lungs that were maintained for up to 12 hours in the EVLP system had excellent, sustained lung function after transplantation. In this study, we examined the feasibility of assessing the physiological integrity of high-risk lungs before implantation by means of EVLP.

**Methods**

In this single-institution, prospective, nonrandomized trial, we compared outcomes in recipients of high-risk donor lungs that had been subjected to normothermic EVLP and contemporaneous recipients of conventionally assessed lung transplants. Donor lungs that met the entry criteria (i.e., were at high risk for nonuse) were retrieved, delivered to our center by means of standard cold-storage transport in a low-potassium dextran solution (Perfadex, Vitrolife), and perfused in the EVLP system for 4 hours. Lungs were considered suitable for transplantation if during EVLP the \( \text{PO}_2/\text{FiO}_2 \) ratio — that is, the partial pressure of oxygen ex vivo (\( \text{PO}_2 \)) to the fraction of inspired oxygen (\( \text{FiO}_2 \)) — was 350 mm Hg or more and if deterioration from baseline levels of all three physiological measurements (pulmonary vascular resistance, dynamic compliance, and peak inspiratory pressure) was less than 15% while the lungs were ventilated with the use of a tidal volume of 7 ml per kilogram of donor body weight and a rate of 7 breaths per minute during the perfusion period.

Recipients provided written informed consent to participate in this study in accord with the protocol, which was approved by the University Health Network research ethics board. Recipients were chosen sequentially, independently of their assignment to either the EVLP group or the control group, and were selected on the basis of blood type, size of the organ (total lung capacity), and waiting-list status, in keeping with our usual practice. Group assignments were based solely on whether the donor lung met the entry criteria; if it did, it was implanted in the intended recipient after EVLP. There was one exception, since one recipient had not provided consent for participation (90% of patients on the waiting list provided consent during the pretransplantation visits). In that case, the next recipient was chosen. A separate consent form was used for the three initial patients in the safety and feasibility study. Recipients were informed before transplantation that they would be receiving EVLP lungs. After the lungs were transplanted, standard care was provided in both groups, including fluid manage-
ment, antibiotic prophylaxis, immunosuppression, and surveillance bronchoscopy.\textsuperscript{21}

The study was conducted in accordance with the protocol. The sponsor (Vitrolife) was not involved in the conduct of the study, analysis or storage of the data, or preparation of the manuscript.

**INCLUSION AND EXCLUSION CRITERIA**

**Donors**
High-risk donor lungs were defined as those meeting any one of the following criteria: best ratio of the partial pressure of arterial oxygen (PaO\textsubscript{2}) to F\textsubscript{i}O\textsubscript{2} of less than 300 mm Hg; pulmonary edema, defined as bilateral interstitial infiltrates without evidence of infection, detected on the last chest radiograph by the lung-transplantation physician assessing the donor; poor lung deflation or inflation during direct intraoperative visual examination at the donor site; blood transfusions exceeding 10 units; and donation after cardiac death, as defined by Maastricht category III (donor without a heartbeat and with cardiocirculatory death imminent after withdrawal of treatment) or category IV (cardiocirculatory death in a brain-dead donor).\textsuperscript{22} Donor lungs with established pneumonia, severe mechanical lung injury (i.e., contusions in more than one lobe), or gross gastric aspiration were excluded. Donor age was neither an exclusion nor an inclusion criterion.

A previously described donor score based on age, status with respect to smoking, chest radiographic findings, bronchopulmonary secretions, and arterial blood gas measurements was used to compare the severity of donor risk factors between groups.\textsuperscript{23} The score ranges from 0 to 18, with higher scores indicating more risk factors; a maximum score of 7 was considered to be the cutoff level for transplant acceptability.\textsuperscript{23}

**Recipients**
All patients on our waiting list for single or bilateral transplantation or re-transplantation were eligible. Candidates for combined heart–lung transplantation were excluded.

**STUDY LOGISTICS**
Donor lungs were offered to our lung-transplantation program through our provincial organ procurement organization (Trillium Gift of Life Network). Assessment of potential donor lungs was based on the usual constellation of clinical factors, including history, PaO\textsubscript{2}:F\textsubscript{i}O\textsubscript{2}, bronchoscopic findings, radiologic assessment, and direct examination of the lung during procurement. As is our practice, we pretreated all potential lung donors with glucocorticoids (methylprednisolone, 15 mg per kilogram of body weight given intravenously every 24 hours); according to the ICU policy of the donor hospital, antibiotics were given to donors if a specific organism had been identified on bronchoalveolar lavage. Donor lungs were included in the EVLP group only after a complete assessment by our team was carried out at the donor hospital before harvesting. Once accepted for EVLP, donor lungs were transported to our center, where a team of trained nurses, perfusionists, and transplantation surgeons had prepared the EVLP system in a sterile operating room. All lung-transplantation surgeons were aware of the design and objectives of the study.

A safety and logistic feasibility study was first performed in three patients who underwent bilateral lung transplantation, with the use of standard criteria for donor lungs. One lung was transplanted according to conventional practice, and the other was transplanted after 1 hour of EVLP (see Fig. 1A in the Supplementary Appendix, available with the full text of this article at NEJM.org).

Post-transplantation chest radiographs and bronchoscopic inspection of the anastomosis in the lungs transplanted according to conventional practice and the lungs transplanted after EVLP were similar (Fig. 1B and 1C in the Supplementary Appendix).

**EX VIVO LUNG PERFUSION**

**Technique**
The acellular EVLP technique has been described in detail elsewhere (Fig. 2 in the Supplementary Appendix, and Video 1, available at NEJM.org).\textsuperscript{18,19,24} For details concerning the EVLP technique and the composition of the perfusate, see the description and Table 1 in the Supplementary Appendix. After 4 hours of EVLP, the lungs were cooled to 10°C over a period of 10 minutes. Thereafter, perfusion and ventilation were stopped (with F\textsubscript{i}O\textsubscript{2} changed to 0.5 for the purpose of lung storage),
Ex Vivo Functional Assessment
For the functional assessment ex vivo, tidal volume was set at 10 ml per kilogram of donor body weight and 10 breaths per minute, with FiO\textsubscript{2} at 1.0. Lung function was evaluated hourly during EVLP according to the following calculations: PO\textsubscript{2} = [left atrial PO\textsubscript{2} – pulmonary-artery PO\textsubscript{2} (in mm Hg)], and pulmonary vascular resistance = [(pulmonary-artery pressure – left atrial pressure) × 80] ÷ pulmonary-artery flow (in dynes · sec⁻¹ · cm⁻²), and peak inspiratory pressure (in centimeters of water). Radiography of the ex vivo lung and flexible bronchoscopy were performed at 1 hour and 3 hours of EVLP.

STUDY END POINTS
The primary end point was primary graft dysfunction of grade 2 (PaO\textsubscript{2}:FiO\textsubscript{2} of 200 to 300 mm Hg) or grade 3 (PaO\textsubscript{2}:FiO\textsubscript{2} <200 mm Hg), according to the International Society for Heart and Lung Transplantation classification (ISHLT),\textsuperscript{25} at 72 hours after transplantation. Grades 0 and 1 represent good graft function (PaO\textsubscript{2}:FiO\textsubscript{2} >300 mm Hg) without abnormalities on chest radiographs (grade 0) or with radiographic abnormalities (grade 1). Secondary end points were PaO\textsubscript{2}:FiO\textsubscript{2} at the time of arrival in the ICU and at 24 hours, and 48 hours after lung transplantation; the need for extracorporeal membrane oxygenation; bronchial complications requiring intervention; duration of mechanical ventilation; length of stays in the ICU and hospital; and mortality at 30 days.

STATISTICAL ANALYSIS
All statistics were calculated with GraphPad Prism 5, with results expressed as medians and ranges. A nonparametric Mann–Whitney test was performed to compare numerical data; Fisher’s exact test was used for categorical data. Percentage-point differences (with 95% confidence intervals) are provided for study end points expressed as proportions. A post hoc analysis was performed to compare the effects of EVLP stratified according to the two subgroups of donors — those without a heartbeat and those who were brain-dead. For differences in lung function at several time points over the 4-hour period of EVLP, repeated-measures analysis of variance was used. P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

DONOR LUNGS
From September 2008 through January 2010, lungs from 306 multiorgan donors were offered to our program, and 136 lung transplantations were performed. Lungs from 23 donors were identified as high-risk on the basis of entry criteria and were evaluated for 4 hours while being perfused in the EVLP system (Table 2 in the Supplementary Appendix). Twenty of these lungs had physiologically stable pulmonary function and were accepted for transplantation (Fig. 1); 9 were from donors without a heartbeat (Maastricht category III, 45%) and 11 were from brain-dead donors (Maastricht category IV, 55%).\textsuperscript{22}

Donor age and status with respect to smoking did not differ significantly between the EVLP group and the control group, but there were significant between-group differences in donor lung characteristics at baseline (Table 1). Donor lungs in the EVLP group had significantly worse gas exchange and more radiographic and bronchoscopic abnormalities (e.g., mucopurulent or bloody airway secretions and evidence of charcoal aspiration). Within the EVLP group, lungs from donors without a heartbeat had higher PaO\textsubscript{2}:FiO\textsubscript{2} values and lower donor scores than did lungs from brain-dead donors.

The median time from harvest to implantation for the EVLP group was 653 minutes (range, 267 to 1021) versus 370 minutes (range, 163 to 662) for the control group (P<0.001). The three preservation periods (i.e., cold ischemic time 1, EVLP time, and cold ischemic time 2) for the EVLP group are shown in Figure 3 in the Supplementary Appendix.

LUNG FUNCTION DURING EVLP
There was significant improvement in gas exchange during EVLP in the 20 lungs used for transplantation (Fig. 2). The median PaO\textsubscript{2}:FiO\textsubscript{2} while the lungs were still in the donors was 335 mm Hg; after 1 hour and 4 hours of EVLP, the PO\textsubscript{2}:FiO\textsubscript{2} increased to 414 and 443 mm Hg, respectively (P<0.001). In addition, pulmonary vascular resistance, dynamic compliance, and peak inspiratory pressure were stable throughout the 4 hours of EVLP in all 20 lungs used for lung transplantation. Lung function worsened in 3 of the 23 donor
normothermic ex vivo perfusion in lung transplants

lungs, with the EVLP $P_O_2:F_iO_2$ decreasing to less than 350 mm Hg in some cases, and these lungs were not transplanted (Fig. 4 in the Supplementary Appendix). The 20 lungs selected for transplantation showed stable or improved radiographic findings during EVLP (e.g., reduced pulmonary edema or decreased consolidation and atelectasis between 1 hour and 3 hours of EVLP) (Fig. 5 in the Supplementary Appendix).

**Clinical Outcome in the EVLP Group**

Primary graft dysfunction was defined as impaired gas exchange meeting ISHLT criteria for grade 2 or 3 dysfunction ($P_O_2:F_iO_2 <300$ mm Hg at 2, 3, or 4 hours of EVLP) after lung transplantation, in the absence of other causes of impaired gas exchange. The incidence of primary graft dysfunction 72 hours after lung transplantation tended to be lower in the recipients of EVLP lungs than in the controls (15% vs. 30.1%; 95% confidence interval [CI], –2.6% to 32.8%; $P=0.11$) (Table 2). None of recipients in the EVLP group had severe primary graft dysfunction at 72 hours ($P_O_2:F_iO_2 <200$ mm Hg) (Fig. 6 in the Supplementary Appendix), as compared with 9.4% of controls ($P=0.36$). A post hoc analysis of the effects of EVLP stratified according to donor subgroup (i.e., donors without a heartbeat and brain-dead donors) (Table 2) showed no significant difference in primary graft dysfunction, although the statistical power of the study to detect such a difference was low.

With regard to secondary end points, there were no significant differences between the EVLP and control groups in the occurrence of primary graft dysfunction at the other three time points (at ICU arrival and 24 and 48 hours after transplantation) (Table 2). None of the patients with EVLP lung transplants had gas-exchange abnormalities sufficient to warrant extracorporeal membrane oxygenation. The incidence of bronchial complications requiring intervention (e.g., dilation) was similar in the EVLP and control groups (5% and 4%, respectively; $P=1.0$), and no significant differences between the groups were observed in the median duration of post-transplantation mechanical ventilation (2 days for both groups).
Table 1. Donor, Recipient, and Transplantation Characteristics of Ex Vivo Lung Perfusion (EVLP) Lungs and Control Lungs.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lungs from Donors without a Heartbeat (N = 9)</th>
<th>EVLP Lungs (N = 20)</th>
<th>Control Lungs (N = 116)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td>Median</td>
<td>40</td>
<td>38</td>
<td>39</td>
<td>45</td>
</tr>
<tr>
<td>Range</td>
<td>16–68</td>
<td>17–69</td>
<td>16–69</td>
<td>6–79</td>
</tr>
<tr>
<td>Best PaO₂:FiO₂ (mm Hg) while in donor</td>
<td>420</td>
<td>275</td>
<td>335</td>
<td>459</td>
</tr>
<tr>
<td>Abnormal chest radiograph (%)</td>
<td>33</td>
<td>91</td>
<td>70</td>
<td>45</td>
</tr>
<tr>
<td>Abnormal bronchoscopic results (%)</td>
<td>89</td>
<td>91</td>
<td>90</td>
<td>52</td>
</tr>
<tr>
<td>Smoking history &gt;10 packs/day (%)</td>
<td>33</td>
<td>9</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>Positive culture on bronchoalveolar lavage (%)</td>
<td>78</td>
<td>82</td>
<td>80</td>
<td>60</td>
</tr>
<tr>
<td>Donor score‡</td>
<td>5</td>
<td>8</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Recipient</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td>Median</td>
<td>52</td>
<td>57</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>Diagnosis of pulmonary fibrosis (%)</td>
<td>33</td>
<td>36</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>UNOS lung-allocation score§</td>
<td>34</td>
<td>37</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Transplantation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral transplantation (%)</td>
<td>89</td>
<td>63</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>Retransplantation (%)</td>
<td>0</td>
<td>9</td>
<td>5</td>
<td>4.3</td>
</tr>
<tr>
<td>Total preservation time (min)</td>
<td>267–760</td>
<td>467–1021</td>
<td>267–1021</td>
<td>163–662</td>
</tr>
</tbody>
</table>

* PaO₂:FiO₂ denotes ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen, and UNOS United Network for Organ Sharing.
† P values are for comparisons of all EVLP lungs with control lungs and were calculated with the use of Fisher’s exact test for discrete variables and the Mann–Whitney test for continuous variables.
‡ The donor score ranges from 0 to 18 and is based on age, status with respect to a history of smoking, PaO₂:FiO₂, chest radiographs, and bronchoscopic findings; higher scores indicate the presence of more risk factors.23
§ The UNOS lung-allocation score ranges from 0 to 100, with higher numbers indicating worse clinical status and greater potential transplantation benefit.

P=0.15), length of stay in the ICU (4 days for both, P=0.68), and hospital length of stay (23 and 27 days, respectively; P=0.39). Two of 20 patients (10%) died within 30 days after transplantation in the EVLP group, as compared with 6 of 116 in the control group (5.2%, P=0.33). Two of the patients who received EVLP lungs died; one patient died on day 7 after lung transplantation, owing to gram-negative sepsis unrelated to the donor, and the other died on day 16 from massive retroperitonsal.
toneal bleeding due to anticoagulation therapy for atrial fibrillation. Both causes of death were confirmed by autopsy. No serious adverse events were directly related to EVLP (Table 3 in the Supplementary Appendix). The survival rate at 1 year was 80% in the EVLP group and 83.6% in control group (P = 0.54); 15 of the 20 recipients of EVLP lungs (75%) and 94 of the 116 controls (81%) survived, with median follow-up times of 561 days (range, 7 to 821) and 542 days (range, 9 to 828), respectively.

**DISCUSSION**

Lung transplantation is limited by the shortage of available organs and by complications that occur after transplantation, such as primary graft dysfunction. The condition of the transplanted lung during the perioperative period strongly influences both short-term and long-term outcomes. In our study, we found that it was feasible to improve the outcome of transplantation if lungs from donors without a heartbeat or brain-dead donors were subjected to 4 hours of normothermic ex vivo perfusion during the organ-preservation phase.

To confirm that donor lung function was adequate for transplantation, we used a combination of physiological variables that addressed gas exchange (PO$_2$/FiO$_2$ > 350 mm Hg), pulmonary mechanics (stable peak inspiratory pressure and dynamic compliance), and pulmonary vasculature (stable pulmonary vascular resistance). One could argue that the advantage of EVLP is that it can be used to identify lungs that are not suitable for transplantation. Indeed, two of the three lungs that were rejected after EVLP appeared to function reasonably well on the basis of assess-
ments carried out while the lungs were still in vivo (i.e., the PaO\textsubscript{2}:FiO\textsubscript{2} was adequate and the chest radiograph was normal), but because they were obtained from donors without a heartbeat, they were included in the study (see cases 17 and 18 in Table 2 in the Supplementary Appendix). However, these lungs were not transplanted because pulmonary vascular resistance, peak inspiratory pressure, or dynamic compliance deteriorated during EVLP. Unfortunately, the study design does not allow us to conclude that this decision was correct, since there was no group of matched controls in whom such lungs were transplanted.

We chose primary graft dysfunction at 72 hours as the primary end point on the basis of evidence suggesting that it is the best determi-

### Table 2. Outcomes in the EVLP and Control Groups.

<table>
<thead>
<tr>
<th>End Point</th>
<th>Donors without a Heartbeat (N = 9)</th>
<th>Brain-Dead Donors (N = 11)</th>
<th>Total (N = 20)</th>
<th>Control Lungs (N = 116)</th>
<th>Absolute Difference†</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary end point§</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PGD grade 2 or 3 at 72 hr (%)</td>
<td>11</td>
<td>18</td>
<td>15</td>
<td>30</td>
<td>15 (−3 to 33)</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Secondary end points¶</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGD grade 2 or 3 at ICU arrival (%)</td>
<td>33</td>
<td>18</td>
<td>25</td>
<td>30</td>
<td>5 (−15 to 26)</td>
<td>0.30</td>
</tr>
<tr>
<td>PGD grade 2 or 3 at 24 hr (%)</td>
<td>11</td>
<td>18</td>
<td>15</td>
<td>36</td>
<td>21 (3 to 39)</td>
<td>0.07</td>
</tr>
<tr>
<td>PGD grade 2 or 3 at 48 hr (%)</td>
<td>33</td>
<td>27</td>
<td>30</td>
<td>35</td>
<td>5 (−17 to 27)</td>
<td>0.46</td>
</tr>
<tr>
<td>ECMO (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>PaO\textsubscript{2}:FiO\textsubscript{2} on arrival in ICU (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.51</td>
<td></td>
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<tr>
<td>Median</td>
<td>420</td>
<td>423</td>
<td>422</td>
<td>372</td>
<td></td>
<td></td>
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<tr>
<td>Range</td>
<td>85–518</td>
<td>86–538</td>
<td>85–538</td>
<td>49–591</td>
<td></td>
<td></td>
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<tr>
<td>Mechanical ventilation after transplantation (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.15</td>
<td></td>
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<tr>
<td>Median</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Range</td>
<td>1–27</td>
<td>1–101</td>
<td>1–101</td>
<td>1–43</td>
<td></td>
<td></td>
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<tr>
<td>ICU stay after transplantation (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.68</td>
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<tr>
<td>Median</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Range</td>
<td>1–34</td>
<td>1–101</td>
<td>1–101</td>
<td>1–103</td>
<td></td>
<td></td>
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<tr>
<td>Hospital stay after transplantation (days)</td>
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<td></td>
<td>0.39</td>
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<tr>
<td>Median</td>
<td>19</td>
<td>34</td>
<td>23</td>
<td>27</td>
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<tr>
<td>Range</td>
<td>7–54</td>
<td>11–101</td>
<td>7–101</td>
<td>9–156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Airway complications (%)¶</td>
<td>11</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>−1 (−10 to 10)</td>
<td>1.0</td>
</tr>
<tr>
<td>Mortality at 30 days (%)</td>
<td>22</td>
<td>0</td>
<td>10</td>
<td>5</td>
<td>−5 (−19 to 9)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

ECMO denotes extracorporeal membrane oxygenation, ELVP ex vivo lung perfusion, ICU intensive care unit, and PGD primary graft dysfunction.

† The differences between all EVLP lungs and control lungs are shown in percentage points (with 95% confidence intervals [CI]) for each study end point.

‡ P values were calculated with the use of Fisher’s exact test for discrete variables and the Mann–Whitney test for continuous variables and are for the comparison between all EVLP lungs and control lungs.

§ Primary graft dysfunction was defined as a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO\textsubscript{2}:FiO\textsubscript{2}) of less than 300 mm Hg, according to the International Society for Heart and Lung Transplantation classification. Grade 0 indicates PaO\textsubscript{2}:FiO\textsubscript{2} ≥300 mm Hg with clear chest radiographs, grade 1 PaO\textsubscript{2}:FiO\textsubscript{2} ≥300 mm Hg with infiltration on chest radiographs, grade 2 PaO\textsubscript{2}:FiO\textsubscript{2} <200 mm Hg, and grade 3 PaO\textsubscript{2}:FiO\textsubscript{2} <200 mm Hg.

¶ Airway complications were defined as those requiring interventions, such as bronchial dilation.
EVLP is limited to six lung transplants that un-
amely early outcomes; 30-day mortality was 36% among patients with grade 3 primary graft dys-
function at 72 hours versus 5% among patients without grade 3 dysfunction.6,26 In the patients in our study who received lungs assessed during EVLP, the incidence of primary graft dysfunction was low, and severe dysfunction (grade 3) at 72 hours was absent, despite the fact that the function of the EVLP lungs was significantly more impaired at baseline (on the basis of the donor score) than that of the control lungs. In light of the confidence intervals for the differences between the two groups (95% CI, −3% to 33%), we are 97.5% confident that the incidence of primary graft dysfunction in the EVLP group was not more than 3% higher than that in the control group. Other measures of the outcome in recipients, such as the number of days of mechanical ventilation and the duration of the ICU and hospital stays, were also acceptable and were similar in the two groups. Mortality at 30 days was 10% in the EVLP group — twice that in the control group; however, this difference represented only one excess death, and the causes of death, as assessed on autopsy, were not directly related to graft dysfunction.

To our knowledge, the use of EVLP in humans has not previously been assessed prospectively in a systematic fashion. Clinical experience with EVLP is limited to six lung transplants that underwent short-term (1-hour), blood-based EVLP.27 Our study involved a normothermic, acellular, blood-free perfusate; protective perfusion and ventilation strategies; and a prolonged perfusion time. This approach was associated with gradual improvement in the function of most of the lungs that were subjected to EVLP.

The few series of cases of lung transplantation involving lungs from donors without a heartbeat (Maastricht category III) have indicated good early outcomes;28-32 however, one recent study showed increased rates of primary graft dysfunction, as well as increased in-hospital mortality.33 Therefore, most lung-transplantation centers do not use lungs from donors without a heartbeat. Indeed, we were hesitant to transplant such lungs, even if they seemed otherwise adequate, until we were able to assess lung function using EVLP.

The limitations of this study are inherent in studies with a small number of cases and lack of randomization. Thus, our study does not provide definitive evidence that the lungs subjected to EVLP would have performed well if they had been directly transplanted, without the use of EVLP. Although a randomized clinical trial of EVLP would be ideal, overcoming the ethical difficulty of directly transplanting so-called questionable organs will present a challenge.

In conclusion, our study shows that the use of extended normothermic EVLP allows an objective assessment of high-risk donor lungs. When these lungs are transplanted, acceptable rates of primary graft dysfunction are achieved, and the early outcomes are similar to those with conventionally selected and transplanted lungs.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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REFERENCES

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supp. Methods – Perfusate Composition and Ex vivo Lung Perfusion Technique

Perfusate

The circuit was primed with 2 L of Steen Solution™ (XVIVO, Vitrolife), a buffered dextran containing extracellular-type solution with an optimized colloid osmotic pressure developed specifically for EVLP (Supplementary table 1). In addition, 500 mg of Solumedrol (Sandoz, USA), 500 mg of Primaxin (Merck, USA) and 3000 IU of Heparin (Organon, Canada) were added to the perfusate. Every hour after the initiation of EVLP, 500 ml of circulated perfusate was removed and replenished with 500 ml of fresh solution.

Technique

After the lungs were transferred to the XVIVO™ chamber (Vitrolife), the left atrial (LA) cannula was connected to the circuit. Flow was initiated slowly in a retrograde fashion to de-air through the pulmonary artery (PA) cannula. The PA cannula was then connected to the circuit and anterograde flow was started at 150 ml/min with the perfusate at room temperature. The temperature of the perfusate was then gradually increased to 37°C. When 32°C was reached (usually over 30 min), ventilation was started and the perfusate flow rate was gradually increased. The flow of gas used to deoxygenate and provide carbon dioxide to the inflow perfusate via a gas exchange membrane was then initiated at 1L/min. We used 40% of the estimated donor cardiac output (CO) as the target maximum maintenance perfusate flow rate to perfuse both lungs. A positive LA pressure was maintained between 3 and 5 mmHg by adjusting the height of the hard-shell reservoir. A protective mode of mechanical ventilation was applied using a tidal volume of 7 ml/kg (based on donor ideal body weight), at 7 breaths per min, positive end-expiratory pressure (PEEP) of 5 cmH₂O and an inspired oxygen fraction (FiO₂) of 21%. The lungs were recruited with inspiratory holds to a peak airway pressure (PawP) of 20 cmH₂O every hour. The pH, pCO₂, electrolytes and glucose were maintained at physiologic levels in the perfusate.

At the end of 4h of EVLP, the lung block was cooled down in the circuit to 10°C over 10 minutes. Thereafter perfusion and ventilation were stopped (FiO₂ was increased to 50% for lung storage), and the trachea was clamped to maintain the lungs in an inflated state. The lungs were then statically preserved at 4°C in Perfadex® until transplantation.
Supplementary Material - Legends

Supplementary Figure 1. Safety and logistic feasibility study was first performed in 3 patients who underwent bilateral LTx using standard criteria donor lungs, where one lung was transplanted as per current practice and the other lung transplanted following 1h of EVLP (A). No differences were observed between the perfused (left) and non-perfused (right) lung in CXR performed at ICU arrival (B). Six weeks after transplantation flexible bronchoscopy demonstrated normal bronchial healing of bronchial anastomosis (C). CSP: cold static preservation; EVLP: ex vivo lung perfusion.

Supplementary Figure 2. (A) Schematic of the EVLP System. The lungs are placed within the XVIVO™ chamber (Vitrolife AB, Sweden). The perfusate leaves the lungs via the LA cannula and enters the reservoir. From there, the perfusate is pumped using a centrifugal pump into the oxygenator and heat exchanger where it is deoxygenated by a gas mixture (86% N₂, 8% CO₂ and 6% O₂) and warmed to normothermia. The perfusate then passes through a leukocyte filter before reentering the lungs via the PA cannula for oxygenation by the lung. (B) Human donor lung in the EVLP system prior to transplantation.

Supplementary Figure 3. Subdivisions of total preservation time in EVLP donor lungs.

Supplementary Figure 4. Lung function during EVLP in 3 lungs that did not meet criteria for transplantation. Case 17, deterioration in all 4 functional parameters and EVLP delta P/F < 350 mmHg. Case 18, increase in PawP and EVLP delta P/F < 350 mmHg. Case 19 - poor deflation likely related to some degree of emphysema in donor lungs.

Supplementary Figure 5. Illustration of a lung ex vivo x-ray. After 3 h of EVLP, there is marked improvement in lung aeration and decreased radiologic evidence of pulmonary edema (upper panel) and improvement in a right lower lobe focal non-pneumonic consolidation/atelectasis after EVLP (lower panel).

Supplementary Figure 6. Distribution of primary graft dysfunction grades within 72h in the EVLP group. Note that at 72 h after transplantation 100% of the patients were free of PGD III (P/F < 200 mmHg).

Supplementary Table 1. Components of Steen Solution™ (Vitrolife).

Supplementary Table 2. Specifications of donor lungs in EVLP arm

Supplementary Table 3. Adverse events in EVLP group
Supplementary Figure 2
Preservation time in EVLP group

Supplementary Figure 3
Supplementary Figure 4
Supplementary Figure 5
Supplementary Table 1. Components and properties of Steen solution. This is a buffered extracellular solution containing dextran with an optimal colloid osmotic pressure developed specifically for extra-corporeal perfusion of lungs.