Brain death and organ donation
Hoeksma, Dane

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CHAPTER 3

Quality of donor lung grafts: a comparative study between explosive and gradual brain death induction models in rats

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In preparation
**ABSTRACT**

**Introduction**

Despite the fact that brain death induces pro-inflammatory changes, correlating with the reduction of graft quality and outcome after transplantation, brain dead donors are the major source for transplantation. This study is designed to test whether explosive or gradual increase in intracranial pressure, to induce brain death, have a differential effect on the graft quality, and to identify deleterious mechanisms during brain death.

**Materials and methods**

Fisher (F344) rats were randomly assigned into three donor groups: 1) no intervention and immediate sacriﬁcation, 2) explosive - and 3) gradual brain death induction model, the latter were subdivided in sacriﬁcation time points 30 minutes, 1 hour, 2 hrs. and 4 hrs. after brain death induction. During the brain death period the animals were hemodynamically stabilized (MAP > 80 mmHg) and lung protective ventilated (VT = 6.5 ml/kg of body weight and a PEEP of 3 cmH2O. Hemodynamic changes and pulmonary inspiratory pressure were monitored, the lungs [n = 8/ group; excluding lost animals] were analyzed with a histological scoring system and for pro-inﬂammatory changes in gene expression with polymerase chain reaction.

**Results**

Immediately after explosive traumatic brain death induction 6 rats were lost, developing severe lung edema and subsequent failure of ventilation compared to none in the gradual model. Remarkable was the difference in mean arterial pressure before onset of brain death, followed by a considerably higher need of inotropic support in the explosive brain death model. In both groups patho-histological changes were found, but in the explosive model parenchyma injury was already pronounced immediately after conﬁrmation of brain death as a result of a more pronounced edema formation in the explosive model. The over time increasing pro-inﬂammatory changes in gene expression were not substantially different between the models, with the exception of the Vcam1 gene expression that was more pronounced in the gradual model.

**Conclusion**

The results of this study suggest that donor lungs suffer more injury after explosive onset of brain death, possibly making them unsuitable for transplantation, compared to gradual onset. However, ﬁndings in gene expression lead us to conclude that if lungs are considered suitable for transplantation the outcome after transplantation should be independent of the etiology of brain death.

**INTRODUCTION**

Lung transplantations are commonly performed using lungs derived from brain dead organ donors, who died of an extensive central nervous system injury secondary to an event of trauma, hemorrhage or infarction. At onset of brain death series of detrimental processes is initiated as a result of increased intracranial pressure. Initially, a massive increase of catecholamines in the blood occurs, also known as autonomic storm, this is accompanied by a signiﬁcant increase in systemic vascular resistance (SVR). The sudden increase in systemic vascular resistance results in the pooling of a large proportion of the total blood volume in the cardio pulmonary vasculature. Shortly after the autonomic storm the SVR decreases again and the aortic blood ﬂow either normalizes or in most of the subjects even results in hypotension.

Along with the hemodynamic changes brain death initiates an immune response in the peripheral organs. Brain death related changes have been identiﬁed as underlying reason for inferior outcome after lung transplantation. The lungs seems to be more susceptible to brain death induced injury than other organ systems because from reported brain dead donors 90% of the kidneys and 70% of the livers were procured for transplantation in contrast to only 20% of the lungs in 2007. Lungs are selected dependent on their quality. Reduced graft quality is the result of both, the early hemodynamic changes and the pro-inﬂammatory immune response which are also linked to the formation of pulmonary edema and increased pulmonary capillary leakage. Nevertheless, more pronounced hemodynamic and hormonal changes have been described for the explosive brain death model. As a consequence of the more pronounced alterations in the explosive model ischemic injury in the donor hearts is more severe, explaining the inferior outcome after transplantation. The hemodynamic changes in the explosive model have been suggested to impact the graft quality before as well as af ter transplantation. In difference to other organ systems clinical data are not conclusive whether cause of brain death affects lung transplantation outcome. We investigated in an animal experiment if the etiology of brain death affects the quality of the donor lung graft and whether this is due to differences in hemodynamics, immune response or both.

**MATERIALS AND METHODS**

**Animals**

All rats were kept under clean conventional conditions, with a 12/12h light-dark cycle at 22°C and were fed standard rat chow ad libitum. All animals received humane care in compliance with the Principles of Laboratory Animal Care (NIH Publication No.86-23, revised 1985) and the Dutch Law on Experimental Animal Care. The experiments were approved by the local animal care committee.

**Experimental groups**

Fisher (F344) rats weighing 270-300g at the time of experiment were obtained from Harlan, Netherlands. In total seventy-two (excluding 6 animals lost after BD induction) rats were randomly assigned into three groups: 1) no intervention (control), 2) explosive (1 minute) or 3) gradual (30 minutes) brain death induction model. Animals in group 2 and 3 were sacrificed at four different time points: 30 minutes, 1 hour, 2 hours and 4 hours after onset of brain death.

**Experimental groups**

Fisher (F344) rats weighing 270-300g at the time of experiment were obtained from Harlan, Netherlands. In total seventy-two (excluding 6 animals lost after BD induction) rats were randomly assigned into three groups: 1) no intervention (control), 2) explosive (1 minute) or 3) gradual (30 minutes) brain death induction model. Animals in group 2 and 3 were sacrificed at four different time points: 30 minutes, 1 hour, 2 hours and 4 hours after onset of brain death.
Brain death model

Anaesthesia was induced with oxygen (1 l/min) /isoflurane 5% and reduced to oxygen/isoflurane 2% for continuation. Subcutaneously 2 ml of saline were administered before the start of surgery. Animals were tracheotomized in supine position, intubated with a 14G polyethylene tube and mechanically volume controlled ventilated with a Harvard apparatus (model 683). The ventilation was set at a tidal volume of 6.5 ml/kg of body weight (BW), PEEP of 3 cmH2O and fraction of inspired oxygen (FO2) of 1 until the end of brain death induction when it was reduced to 0.5. The respiratory frequency was initially set at 120/min and was throughout the experiment adjusted as needed to keep the ETCO2 between 20-22 mmHg.

The left femoral artery was cannulated and used for continuous monitoring of mean arterial pressure (MAP), while the left femoral vein was cannulated and used to stabilize the MAP above 80 mmHg by fluid bolus (with a maximum of 1 ml per hour) of colloidal solution (HAES- sterin 10%, Fresenius Kabi, Bad Homburg, Germany) and infusions of norepinephrin (0.01 mg/ml) as needed with a perfusor pump (Terufusion Syringe Pump, model STC-521). Brain death was induced with 0.6 mg/kg of bodyweight rocuronium was given. The cardiac index was determined with 4% of formalin fixed with 4% of formalin in the presence of dNTPs (Invitrogen, Breda, Netherlands) after an initial using M-MLV (Moloney murine leukemia virus) Reverse Transcriptase (Invitrogen, Breda, Netherlands) according to the manufacturer’s instructions. Guanidine thiocyanate was added before continuation. The integrity of total RNA was measured before and after they were placed for 24 hrs at 100°C on aluminum foil. The weights of the primarily in Eppendorf tubes snap frozen right middle lung lobes were measured before drying at 100°C – alu foil) = Wet-Dry ratio and is given as the mean ± standard deviation. W/D ratio was calculated ((weight before drying at 100°C – alu foil) / (weight after drying at 100°C – alu foil)) = Wet-Dry ratio and is given as the mean ± standard deviation. RNA isolation and reverse transcriptase Polymerase-Chain Reaction

Total RNA was isolated from snap frozen right middle lung lobes were measured and before after they were placed for 24 hrs at 100°C on aluminium foil. The W/D ratio was calculated ((weight before drying at 100°C – alu foil) / (weight after drying at 100°C – alu foil)) = Wet-Dry ratio and is given as the mean ± standard deviation.

Gene expression analyses were performed at mRNA level by TaqMan low density array (TDLA). Designed primer sets (Table 1) were loaded with 5 µl cDNA (2ng/µl) and SYBR green (Applied Biosystems) into a well of a Taqman low density Array (TLDA) card. Amplification and detection were performed with the ABI Prism 7900-HT Sequence Detection System (Applied Biosystems) measuring the emission of SYBR green. Each sample was measured in triplicate and a pool of sample cDNA served as calibrator. PCR reaction consisted of 40 cycles at 95°C for 15 sec. and 60°C for 60 sec. after initiation for 2 min. at 50°C and 10 min. at 95°C. A dissociation curve analysis was performed for each reaction to ensure the amplification of specific products.

Table 1. Designed PCR primers

<table>
<thead>
<tr>
<th>Primer</th>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tnf</td>
<td>Tumor necrosis factor</td>
<td>AAGGCTTGTGGACACTCATGCTGAA</td>
<td>TGACCCCGAGGGGCGATTACCA</td>
<td>67</td>
</tr>
<tr>
<td>IL6</td>
<td>Interleukin 6</td>
<td>CCAACTCTCAAGTGCACCTCGAATG</td>
<td>TCCAAAGTGGTTACAAAGGGGAT</td>
<td>89</td>
</tr>
<tr>
<td>Cc1</td>
<td>Chemokine (C-C motif) ligand 1</td>
<td>TGGTCTCGAGAGTATGTCTCCAAAGA</td>
<td>AGAGCCATCGTGCAATCTCA</td>
<td>78</td>
</tr>
<tr>
<td>Cc1l</td>
<td>Chemokine (C-C motif) ligand 2</td>
<td>TGGTCTCGAGAGTATGTCTCCAAAGA</td>
<td>AGAGCCATCGTGCAATCTCA</td>
<td>78</td>
</tr>
<tr>
<td>ICAM1</td>
<td>Intercellular adhesion molecule 1</td>
<td>CCGAGACCTGTGAGATGGAAGTT</td>
<td>AGAGCCATCGTGCAATCTCA</td>
<td>251</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Vascular adhesion molecule 1</td>
<td>TGGTGGAGGTGGCCCGAAA</td>
<td>ACGGCCATTAACAGACCTTTGCA</td>
<td>74</td>
</tr>
<tr>
<td>Gapdh</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>GTATGTACCTTACCCCAGGAAG</td>
<td>GATGGTTTCCCTGGTGATA</td>
<td>97</td>
</tr>
<tr>
<td>B-actin</td>
<td>Actin, beta</td>
<td>GGAAGATTGCGTGACGAAATTA</td>
<td>GGCACAGGTCACGCTC</td>
<td>74</td>
</tr>
<tr>
<td>Hox1</td>
<td>Heme- oxygenase 1</td>
<td>CTCGCCATGAACACTGGAGAGT</td>
<td>GCAGAAAGCCGCTCTGAGC</td>
<td>74</td>
</tr>
<tr>
<td>Ppia</td>
<td>Peptidylprolyl isomerase A</td>
<td>TCCCGAGTCTGGACAACCTCAATT</td>
<td>CGAGCTACAGAAGAAGTTGTTGA</td>
<td>76</td>
</tr>
<tr>
<td>Eif2b1</td>
<td>Eukaryotic translation initiation factor 2B</td>
<td>ACCTGTATGCAACAGGCTTCAATT</td>
<td>TGGGACAGGCGTTCAAGTG</td>
<td>77</td>
</tr>
</tbody>
</table>

Histological scoring

Lungs were fixed in 4% formalin, embedded in paraffin and cut in four-µm-thick slices, subsequently stained with haematoxylin-eosin. A five-point semiquantitative severity-based scoring system was used as previously described21. The pathological findings were graded as negative = 0, slight = 1, moderate = 2, high = 3, and severe = 4. The amount of intra- and extra-alveolar haemorrhage, intra-alveolar edema, inflammatory infiltration of the interalveolar septa and airspace and overinflation were rated. Deviating from the original scoring scheme erythrocyte accumulation below the pleura was scored as 1 –
CHAPTER 3  

BRAIN DEATH INDUCTION SPEEDS AND PULMONARY EFFECTS

Table 2. Physiological parameters selected in the brain dead donor groups.

<table>
<thead>
<tr>
<th></th>
<th>BL</th>
<th>BD 0.5h</th>
<th>BD 1h</th>
<th>BD 2h</th>
<th>BD 4h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EM</td>
<td>GM</td>
<td>EM</td>
<td>GM</td>
<td>EM</td>
</tr>
<tr>
<td>n</td>
<td>= 30</td>
<td>n = 29</td>
<td>n = 19</td>
<td>n = 22</td>
<td>n = 15</td>
</tr>
<tr>
<td>Paw</td>
<td>348 ± 8</td>
<td>326 ± 9</td>
<td>378 ± 7</td>
<td>375 ± 8</td>
<td>375 ± 9</td>
</tr>
<tr>
<td>HR</td>
<td>384 ± 10</td>
<td>415 ± 14</td>
<td>375 ± 12</td>
<td>345 ± 7</td>
<td>353 ± 9</td>
</tr>
<tr>
<td>RTD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The given values are presented as mean ± standard error of the mean.

RESULTS

Failure of hemodynamic stabilization

Fisher rats (F344) were randomly assigned to control or the brain death models with subgroups of different stabilization periods. In the explosive brain death arm 6 animals were lost immediately after the induction of brain death since ventilation was not feasible (excluded from analysis and replaced), while no animal was lost in the gradual brain death model. At dissection of the thoracic cavity in 5 out of 6 animals a fulminant lung edema (excluded from analysis and replaced), while no animal was lost in the gradual brain death induction model, expansion of the fogarty catheter over a period of 30 min. with a pump; HR– heart rate (beats/min.); MAP– mean arterial pressure; Paw– pulmonary airway pressure; the mean arterial pressure in the explosive model was significantly higher during twenty minutes before end of brain death induction compared to the gradual model. During the initial phase of hemodynamic stabilization was the MAP higher in the explosive model, while there was no difference between groups after four hours of stabilization (Figure 1). In contrast to this the need of total volume administration in the explosive model was higher compared to the gradual model (1.2 ± 1.1 ml (EM) vs. 0.6 ± 0.5 ml (GM); p < 0.05). Nevertheless, there were no relevant differences in Wet-Dry ratio between the groups including the control group.

Physiological data

Of the three groups, only the two brain death groups were ventilated and stabilized during which time physiological data were collected. The parameters pulmonary inspiratory pressure, heart rate and mean arterial pressure of each brain death model were taken together as available for the given time points and analyzed (Table 2). There was no difference in pulmonary inspiratory pressure between the models but the heart rate was elevated in the explosive brain death model.
The time of brain death induction does not influence the investigated proinflammatory gene expression during 4 hrs. of brain death

In both brain death models the pro-inflammatory cytokine gene expression of Tnf and Il6 was induced, but levels did not reach significance between the two models. Gene expression of the Il8-like Cinc1 was unaffected by groups and time. For Mcp1 and Vcam1 an increase in a quadratic regression was noted over time with the time centered at 1.5 hrs. For Gapdh a trend towards significance (p < 0.053) was found between groups for that reason different housekeeping genes were chosen for further analysis (Table 3).

Table 3. Changes in pro-inflammatory cytokine gene expression after different durations of brain death.

<table>
<thead>
<tr>
<th>RTD-PCR</th>
<th>BD 0.5h</th>
<th>BD 1h</th>
<th>BD 2h</th>
<th>BD 4h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>EM</td>
<td>GM</td>
<td>EM</td>
</tr>
<tr>
<td>Tnf (n=8)</td>
<td>0.02 ± 0.36</td>
<td>0.60 ± 0.32</td>
<td>0.73 ± 0.19</td>
<td>0.75 ± 0.32</td>
</tr>
<tr>
<td>Il6 (n=8)</td>
<td>0.01 ± 0.36</td>
<td>0.72 ± 0.40</td>
<td>0.75 ± 0.40</td>
<td>1.17 ± 2.78</td>
</tr>
<tr>
<td>Cinc1 (n=8)</td>
<td>0.03 ± 0.97</td>
<td>0.41 ± 0.33</td>
<td>0.92 ± 0.33</td>
<td>1.67 ± 1.36</td>
</tr>
<tr>
<td>Mcp1 (n=8)</td>
<td>0.02 ± 0.71</td>
<td>0.35 ± 0.06</td>
<td>0.10 ± 0.21</td>
<td>0.2 ± 0.83</td>
</tr>
<tr>
<td>Vcam1 (n=8)</td>
<td>0.14 ± 0.25</td>
<td>0.55 ± 0.26</td>
<td>0.98 ± 0.36</td>
<td>1.33 ± 0.36</td>
</tr>
<tr>
<td>GAPDH (n=8)</td>
<td>0.82 ± 0.54</td>
<td>0.81 ± 0.24</td>
<td>1.26 ± 0.24</td>
<td>1.12 ± 0.52</td>
</tr>
</tbody>
</table>

The values presented are mean ± standard deviation (SD).

Explosive brain death induction results in more severe lung injury then gradual brain death induction on a histological level.

Total lung injury score was higher in the explosive brain death model than in the gradual brain death model, as well as changes are significant over time. This is the result of a more pronounced hemorrhagic infarcted lung tissue, edema and pleural infarction in the explosive model compared to the gradual brain death model (Figure 2 + Table 4).

Figure 2. Histological total lung injury score. Formalin fixed, paraffin embedded and haematoxylin-eosin stained left lung lobes slices were scored for the extent of intra- and extra-alveolar hemorrhage, intra-alveolar edema, inflammatory infiltration of the interalveolar septa and airspace and over-inflation. The pathological findings were graded as negative = 0, slight = 1, moderate = 2, high = 3, and severe = 4. Deviating from the published original scoring scheme erythrocyte accumulation below the pleura was scored as 1 = present or 0 = not present. The sum of variables gave the total lung injury score. Morphological examination was performed in a blinded fashion by 2 investigators, using a conventional light microscope at a magnification of 200 across 10 random, non-incidentional microscopic fields. All values are presented as mean.

Control: no intervention; EM: explosive brain death induction within 1 minute by expansion of the intracranial foggity catheter; GM: gradual brain death induction over a period of 30 minutes, 0.5-30 minutes of donor stabilization, 1/2/4- hours of donor management.

Table 4. Histological lung injury score.

<table>
<thead>
<tr>
<th>Histological Score</th>
<th>BD 0.5h</th>
<th>BD 1h</th>
<th>BD 2h</th>
<th>BD 4h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>0.2 ± 0.1</td>
<td>1.5 ± 1.8</td>
<td>0.1 ± 0.2</td>
<td>0.7 ± 1.5</td>
</tr>
<tr>
<td>Hemorrhage (n=8)</td>
<td>0.0 ± 0.0</td>
<td>0.5 ± 0.5</td>
<td>0.3 ± 0.0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Edema (n=8)</td>
<td>1.6 ± 0.4</td>
<td>2.3 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>Inflammation (n=8)</td>
<td>0.6 ± 0.2</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Over-inflation (n=8)</td>
<td>0.0 ± 0.0</td>
<td>0.9 ± 0.9</td>
<td>0.0 ± 0.0</td>
<td>0.9 ± 0.9</td>
</tr>
<tr>
<td>Pleura Infarction (n=8)</td>
<td>0.0 ± 0.0</td>
<td>0.5 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>0.9 ± 0.9</td>
</tr>
<tr>
<td>Total (n=8)</td>
<td>2.0 ± 0.3</td>
<td>5.2 ± 5.2</td>
<td>2.8 ± 2.8</td>
<td>6.5 ± 6.5</td>
</tr>
</tbody>
</table>

The values presented are mean ± standard deviation (SD).
**Neurogenic shock**

To prevent neurogenic shock during the acute phase of brain death, we administered 0.01 mg/ml noradrenalin (NA) as needed with a perfusor. This was to ensure sufficient oxygenation of the solid organs, particularly the kidneys, and to maintain a stable heart rate and blood pressure. However, it is important to note that the effects of neurogenic shock can be variable and may require close monitoring and adjustment of treatment as needed.

**Table 3**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>EM</th>
<th>GM</th>
<th>BD 0.5h</th>
<th>BD 1h</th>
<th>BD 2h</th>
<th>BD 4h</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>0.8 ± 0.0</td>
<td>0.6 ± 0.1</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.0</td>
<td>0.8 ± 0.1</td>
<td>0.1 ± 0.2</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>CK-MB</td>
<td>409 ± 175</td>
<td>678 ± 84</td>
<td>731 ± 109</td>
<td>830 ± 300</td>
<td>225 ± 258</td>
<td>670 ± 66</td>
<td>227 ± 90</td>
</tr>
<tr>
<td>Troponin</td>
<td>22 ± 39</td>
<td>31 ± 11</td>
<td>28 ± 17</td>
<td>47 ± 14</td>
<td>23 ± 16</td>
<td>33 ± 13</td>
<td>126 ± 12</td>
</tr>
</tbody>
</table>

**DISCUSSION**

This is to our knowledge, the first study investigating the effect of explanting and gradual brain death induction models on lung graft quality over different time periods in rats. Both models resulted in the decrease of graft quality over time, however, more pronounced in the explanting brain death induction model during the initial phase. This difference was observed in higher mortality, increase of pulmonary inspiratory pressure, and more need of hemodynamic support and pronounced patho-histological changes. In contrast, this was not observed in the investigated proinflammatory gene expression.

Since in kidney and heart transplantation the cause of brain death seems to have substantial influence on the early outcome after transplantation, a number of studies have investigated the effect of cause of brain death in lung transplantation. While they failed to show a correlation between cause of brain death and outcome, it was noted that donors with traumatic brain injury had a higher incidence in severity of rejection episodes though this had no effect on survival. However, these studies did not analyse whether the cause of brain death affects the availability of donor organs.

In a comparative study, with emphasis on the heart, it has been shown that the two brain death induction models differ in the development of mean arterial pressure during brain death induction. The acute increase in mean arterial pressure in the explanting brain death induction model is compared to a more tempered development in the gradual model, and is considered to be the result of a more pronounced sympathetic discharge, followed by a more profound hypotension. Sudden increase in mean arterial pressure causes the rupture of the capillary-venous membrane and disruption of the barrier integrity. Preventing the hypertensive response to explanting brain death induction limited the inflammatory immune response and prevented changes in capillary-venous membrane integrity. This could explain, why acute cerebral insult and brain death have been associated with the onset of pulmonary edema, while it is rare in subarachnoid hemorrhage, which causes a gradual increase of intracranial pressure. Additionally, the loss of capillary integrity is accompanied by hemorrhage. The hemodynamic pattern during the initial phase between our two brain death induction models respectively differs and may explain the difference we found in loss of animals during the initial phase after brain death induction. After the induction phase the mean arterial pressure was stabilized in our set up by utilization of HAES and noradrenalin. Administration of exogenous noradrenalin has been associated with graft deterioration in other solid organ systems, however in the lung the prevention of hypotensive collapse using noradrenalin correlated with reduced edema and inflammation. In this setting intrinsic catecholamine levels up on brain death induction were not determined nor histological myocardial changes. However it is expected that the increase in Troponin and CK-MB are the results of more pronounced intrinsic catecholamine release. While there seems to be a correlation between hemodynamic changes and the inflammatory immune response during brain death, it is not the only reason for the inflammatory immune response found in brain dead donor lungs. Exposing living controls to blood withdrawn from brain dead donors in the absence of hemodynamic changes causes an inflammatory response. The origin of this systemic inflammation has not been identified yet.

In conclusion, the results of this study suggest that donor lungs suffer more morphological injury after explanting onset of brain death. As a consequence, less lungs from donors with acute onset of brain death might be considered suitable for lung transplantation however this has not been investigated in this study, nor to our knowledge clinically. However, findings in gene expression lead us to conclude that the outcome after transplantation should be independent of the etiology of brain death.
REFERENCES


