0031-3998/05/5804-0689
PEDIATRIC RESEARCH
Vol. 58, No. 4, 2005
Printed in U.S.A.

Fetal Tracheal Occlusion in Lambs with Congenital Diaphragmatic Hernia: Role of Exogenous Surfactant at Birth

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ABSTRACT

Fetal tracheal occlusion (TO) has been used to reverse the lung hypoplasia associated with congenital diaphragmatic hernia (CDH). However, TO has a detrimental effect on type II pneumocyte function and surfactant production. Previously, we have shown that in surgically created CDH lambs, TO improved markedly the response to resuscitation even though the lungs remain surfactant deficient. The goal of this investigation was to assess the effects of exogenous surfactant administered at birth to CDH lambs with or without fetal TO during 8 h of resuscitation. Lambs were divided into five groups: CDH, CDH+surfactant (SURF), CDH+TO, CDH+TO+SURF, and nonoperated controls. A left-sided CDH was created in fetal lambs at 80 d gestation. TO was performed at 108 d, and the lambs were delivered by hysterotomy at 136 d. Bovine lipid extract surfactant was administered before the first breath and again at 4 h of life. All CDH+SURF lambs, but only three of five CDH lambs, survived up to 8 h. When compared with the corresponding nonsurfactant-treated group, surfactant-treated CDH and CDH+TO lambs did not demonstrate improved alveolar-arterial oxygen gradients, pH, or P_{CO_2}. In fact, in the CDH+TO group, surfactant treatment significantly worsened ventilation efficiency as measured by the ventilation efficiency index. The observed improvement in pulmonary compliance secondary to surfactant treatment was not significant. This investigation demonstrates that prophylactic surfactant treatment at birth does not improve gas exchange or ventilation efficiency in CDH lambs with or without TO. (***Pediatr Res* 58: 689–694, 2005**)

Abbreviations

$AaDO_2$, alveolar-arterial oxygen gradient
CDH, congenital diaphragmatic hernia
$Paco_2$, partial pressure of arterial carbon dioxide
Pao_2, partial pressure of arterial oxygen
SURF, surfactant
TO, tracheal occlusion
VEI, ventilation efficiency index

The high mortality rate associated with CDH is related to its complex pathophysiology. Pulmonary hypoplasia, pulmonary hypertension, decreased pulmonary compliance, and surfactant deficiency may all contribute to hypoxemia, hypercarbia, and acidosis. These factors stimulate pulmonary artery vasoconstriction, creating a cycle of worsening pulmonary hypertension, right-to-left shunting, and further physiologic deterioration of blood gases (1–3).

Both the surgically created CDH animal model as well as human neonates with CDH, have significantly increased lung growth after fetal TO compared with their CDH-only counterparts (4–6). In addition, this fetal surgical intervention prevents excess pulmonary artery muscularization in fetal CDH lambs by thinning the medial area of small pulmonary arteries (7). These structural changes decrease pulmonary hypertension and improve gas exchange, ventilation, and compliance. However, TO in intact fetal lungs is associated with a dramatic decrease in the number and function of type II pneumocytes, the cells that produce lung surfactant (8–11).

Both CDH lambs and rats are believed to be surfactant deficient (4,12,13). Although the hypoplastic lungs of lambs, induced by lung liquid drainage, are associated with a higher density of type II pneumocytes (14), the function of these pneumocytes at birth appears impaired. Moreover, surfactant

Received January 10, 2005; accepted February 3, 2005.
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This work was supported by the Canadian Institute of Health Research, the Foundation for Research into Children's Diseases, and BLES Biochemical Inc. BP was supported by an award from the Fonds de Recherche en Santé du Québec.

DOI: 10.1203/01.PDR.0000180534.42731.95

protein mRNA expression in lung homogenates is decreased (15). This observation is consistent with data showing a decrease in the concentration of phospholipids and surfactant proteins recovered by bronchoalveolar lavage (BAL) (4). There is controversy concerning the surfactant status of human newborn infants with CDH. Surfactant deficiency in CDH infants was suggested by Glick *et al.* (16) when they showed clinical improvement in three patients after exogenous surfactant administration. However, this result is confounded because two out of the three infants were premature. In contrast, Ijsselstijn *et al.* (17) showed that CDH patients have a similar surfactant phospholipid concentration as various control patients. However, an alternate report from Asabe *et al.* (18) suggests that the protein content of surfactant is abnormal in human CDH patients.

Antenatal steroid administration benefits lung development and maturation in both lambs with surgically created CDH (19,20) and rats with pharmacologically induced CDH (21). However, the beneficial effect of steroids may be independent from their effect on surfactant (22–27). Indeed, we have shown that despite antenatal treatment with steroids, lambs with CDH or CDH+TO remain markedly surfactant deficient at birth (4). However, we have also observed that CDH lambs with TO have a marked and sustained improvement in oxygenation and VEI over an 8-h resuscitation period (28). In contrast, complete recovery of the expression of mRNA for surfactant proteins B and C in lambs with CDH, but not in lambs with CDH and TO, was observed after a short period of resuscitation (28,29).

Exogenous surfactant can be administered to neonates prophylactically (before the first breath) or as "rescue therapy" (after the appearance of respiratory distress symptoms). In lambs and humans with CDH, prophylactic surfactant administration has been shown to significantly improve gas exchange and lung mechanics (16,30). However, the absence of control groups combined with the short duration of the experiments in lambs, and the presence of prematurity along with the lack of appropriate controls in the small human study, make the significance of these changes difficult to assess. In contrast, rescue surfactant therapy does not improve gas exchange or compliance in either animals or humans with CDH (31,32). Prophylactic surfactant is thought to distribute itself more evenly throughout the lung as aeration has not yet taken place (33,34).

Permissive hypercapnia and other modalities have increased the survival of neonates with CDH in the past decade (35). Further improvement will require a combination of various treatment strategies. The goal of this study was to assess the benefit of prophylactic surfactant treatment in CDH lambs with and without TO over an 8-h resuscitation period.

MATERIALS AND METHODS

Ethics approval for all animal experiments was obtained from the McGill University Animal Care Committee. Methods for this study, with the exception of the exogenous surfactant portion, were similar to those previously published by our group (28). The CDH, CDH+TO, and control groups were the same animals used in our previous study (28).

Fetal lamb interventions. A left-sided diaphragmatic hernia was created in fetal lambs at 80 d gestation as described previously (36). Fetal tracheoscopy (2.7 mm Semi Flexible Mini-Endoscope, Karl Storz GmbH & Co., Tuttlingen Germany) was used in combination with a detachable balloon system (GVB12 Latex Goldvalve Balloon, diameter 14 mm, length 22.5 mm, volume 2.5 mL; CCOXLS co-axial catheters) to achieve TO at 108 d gestation (37). At 129 d gestation, all ewes, including the control group, received 250 mg medroxyprogesterone intramuscularly (i.m.) to decrease the incidence of preterm labor (38); and at 135 d gestation, they received 0.5 mg/kg betamethasone i.m. to accelerate lung development and maturation (39). Careful assessment of lung growth, lung development, surfactant content, and vascular remodeling in lambs killed at birth following a similar antenatal protocol was published previously (4,7).

Ex-utero *intrapartum treatment (EXIT).* While under maternal inhalational anesthesia with the placental circulation maintained, the fetal lambs underwent a limited neck dissection to permit cannulation of both the right common carotid artery (preductal) and internal jugular vein. A tracheostomy was performed through which a 4-mm uncuffed endotracheal tube was inserted. The fetus was then disconnected from placental circulation and ventilated for up to 8 h.

Surfactant supplementation. The first dose of of BLES (bovine lipid extract surfactant) (15 mL, approximately 5 mL/kg) (BLES Biochemical Inc, London, ON, Canada) was given through an 8F feeding tube positioned just above the carina during the EXIT procedure when the lamb was still connected to its placental circulation. Then, the endotracheal tube was occluded until delivery. A second dose of BLES (5 mL/kg) was given at 4 h of life. Given that CDH lambs with TO were still profoundly surfactant deficient after 8 h of resuscitation as measured by their surfactant protein C content (28) and given the known deleterious effects that ventilation and hyperoxia have on surfactant (40–43), we decided to administer this second dose of surfactant to maximize the chance of obtaining a sustained improvement. The lamb was disconnected from the ventilator and manually ventilated for 30 s. Surfactant was administered in three aliquots with the lamb in the following positions: on the left side, on the right side, and supine. After delivery of each aliquot, the lamb was manually ventilated for 1 min. Upon completion of surfactant delivery, the lamb's endotracheal tube was reconnected to the ventilator.

Eight-hour resuscitation. Sedation was achieved with ketamine 2 mg/kg/h (i.v.), paralysis with pancuronium 0.1 mg/kg/h (i.v.), and alkalosis with sodium bicarbonate 0.5 mmol/kg/h (i.v.). We used the following initial ventilator settings (Sechrist Infant Ventilator Model IV-100B, Sechrist Industries, Anaheim, CA) with the permitted range in parentheses: peak inspiratory pressure (PIP) 25 cm H_2O (15–30); peak end-expiratory pressure (PEEP) 5 cm H_2O (3–7); Fio_2 1.0 (0.21–1.0); respiratory rate (RR) 120 breaths per minute (10–120); minimum inspiratory time 0.25 s; inspiratory time to expiratory time ratio (I:E) 1:1. Ventilator settings were changed accordingly if $Paco_2$ >65 mm Hg or $Paco_2$ <40 mm Hg; if Pao_2 <40 mm Hg or Pao_2 >100 mm Hg; and if pH <7.4 or pH >7.5. We calculated the correction necessary to bring the pH up to 7.4 and gave boluses of 2 mmol/kg of $NaHCO_3$ (i.v.) to increase the pH by 0.1 unit. Tension pneumothoraces were treated with chest tubes, including subxiphoid incisions. Preductal arterial blood gases were analyzed using a portable clinical analyzer and EG7+ cartridges (i-STAT, Sensor Devices Inc., Waukesha, WI).

Outcome measures. Oxygenation and ventilation parameters were calculated as follows: $AaDO_2 = [((713 \times Fio_2) - Paco_2)/0.8] - Pao_2$; $VEI = 3800/[(PIP - PEEP) \times \text{respiratory rate} \times Paco_2]$ (44,45).

Statistical analysis. Five groups were compared: CDH ($n = 5$), CDH+SURF ($n = 4$), CDH+TO ($n = 5$), CDH+TO+SURF ($n = 6$), and nonoperated controls (Cont) ($n = 4$). For longitudinal data, a repeated measure analysis procedure (SAS, SAS Institute, Cary, NC) was used to assess the effect of time between groups (treatment by time interaction). In the case of missing values, we used the previous value. For Pco_2, pH, $AaDO_2$, and VEI, the amount of missing values was <4%, which included the missing values for the lambs that died before the end of the 8-h resuscitation protocol. For the compliance data, due to technical difficulties, the amount of missing values was 10%, including three lambs for which no measure of compliance was available (two CDH and one control). For nonlongitudinal data, a one-way ANOVA test with treatment as a factor was used. Four post hoc comparisons were performed. The effects of surfactant treatment on CDH were assessed by comparing i) CDH with CDH+SURF, and ii) CDH+SURF with controls. The effects of surfactant treatment in CDH+TO were assessed by comparing iii) CDH+TO with CDH+TO+SURF, and iv) CDH+TO+SURF with controls. For comparison between CDH, CDH+TO, and controls, the reader should refer to our previous publication (28). The Bonferroni procedure was used in the case of multiple comparisons. Data are presented as the mean ± SEM.

RESULTS

The *in utero* mortality rates for both sets of experiments were ≤35% in the different experimental groups. This is less than the commonly reported rate of 50% (12).

Only animals with a diaphragmatic defect and herniated viscera in the left chest at the time of autopsy were considered as CDH+TO+SURF lambs. One animal was excluded due to inadequate CDH.

All 10 lambs in both SURF groups survived the 8-h resuscitation period. In contrast, only three of the five CDH-only lambs survived (Table 1). The need for chest tube placement to treat tension pneumothoraces was also recorded (Table 1). None of the control animals required chest tubes. In contrast, all CDH-only animals required chest tubes, and three of five CDH+TO lambs had chest tubes in place. The addition of exogenous surfactant appeared to decrease the incidence of pneumothoraces, although this could not be shown as being statistically significant due to the small numbers of animals per group. Three of the four CDH+SURF lambs required chest tubes whereas only two of the six CDH+TO+SURF animals required chest tubes.

Both CDH and CDH+SURF lungs were hypoplastic with wet lung weight/body weight (LW/BW) ratios of 1.11 ± 0.12% and 0.99 ± 0.14%, respectively (Fig. 1). TO+SURF significantly increased the LW/BW ratio (CDH+TO: 2.39 ± 0.42% and CDH+TO+SURF: 2.14 ± 0.23%) of CDH animals to values comparable to those of controls (1.73 ± 0.04%) (Fig. 1). More complete studies looking at lung growth, lung development, surfactant content, and vascular remodeling in lambs killed at birth after a similar antenatal protocol have been published previously (4,7).

Arterial pH, but not $Paco_2$, significantly improved after surfactant administration in the CDH-only group (Fig. 2, *A* and *B*, Table 2). In contrast, pH and Pco_2 failed to improve in the group of lambs with CDH+TO+SURF when compared with CDH+TO (Fig. 2*B*, Table 2). In fact, after 180 min of resuscitation, both pH and Pco_2 had worsened with surfactant supplementation after TO; however, this difference was not significant ($p > 0.05$) (Fig. 2, *A* and *B*, Table 2).

Oxygenation, as calculated by $AaDO_2$, did not improve with surfactant treatment in any of the two groups (Fig. 2*C*, Table 2). Ease of ventilation, as measured by an increased VEI, was not improved with the addition of surfactant. In fact, the VEI was significantly worse in the CDH+TO+SURF group when compared with the CDH+TO group (Fig. 2*D*, Table 2). Pulmonary compliance was lowest for the CDH-only group throughout the resuscitation period (Fig. 3). Surfactant administration appears to improve compliance. The CDH+SURF group maintained higher compliance than the CDH group, reaching levels similar to the control group. CDH+TO+SURF lambs had the highest compliance. However, none of these differences proved to be statistically significant owing to the large differences among individuals of the same group and the limited number of animals in some groups.

Table 1. *Survival and Barotrauma*

Experimental Group	Survived 8 hours	Age at death (hours)	Chest tube
CDH	3/5	5, 7	5/5
CDH + SURF	4/4	n/a	3/4
CDH + TO	5/5	n/a	3/5
CDH + TO + SURF	6/6	n/a	2/6
Control	4/4	n/a	0/4

$p > 0.05$ for all comparisons, n/a: not applicable.

DISCUSSION

In this investigation, we demonstrate that prophylactic delivery of exogenous surfactant at birth significantly worsens the physiologic/clinical response to resuscitation in lambs with CDH+TO. However, our results do suggest that surfactant treatment does marginally improve the response to respiratory gas exchange in lambs with CDH.

We have shown previously that fetal TO induces lung growth that reverses pulmonary hypoplasia associated with CDH (4). In addition, TO prevents excess pulmonary muscularization, which is associated with pulmonary hypertension at birth (7). The combination of these effects on lung growth and vascular remodeling leads to improved gas exchange and ventilation (28).

Unfortunately, TO accelerates lung growth at the expense of type II cell accumulation (8–11). We have shown that release of TO combined with antenatal steroid treatment can prevent this decrease in type II pneumocyte density in fetal lambs with normal lungs (46,47). However, the situation with hypoplastic lungs in CDH cases is more complex. These lungs have a higher density of type II pneumocytes, even though the phospholipid content of the BAL or levels of surfactant proteins and mRNA expression in lung tissue are decreased at birth (4,15). In CDH lambs, TO decreases the density of type II pneumocytes to control levels (intact fetuses) but worsens the abnormalities in surfactant production despite the use of antenatal steroids (4). However, after short-term resuscitation, recovery of mRNA expression of surfactant proteins B and C was observed in lambs with CDH and CDH+TO with release of tracheal (TR) occlusion 1 wk before delivery, but not in lambs with CDH+TO only (without release) (28,29). However, in our previous study, the CDH+TO group did significantly better for the VEI and $AaDO_2$ than the CDH+TO+TR in spite of similar lung growths (28). In fact, the CDH+TO lambs did as well as the controls for those parameters. However, all of the lambs were delivered at 137 d gestation (term = 145 d) and, therefore, even the control lambs may have had insufficient surfactant to cope with air breathing because of their relatively young gestational age. Indeed, a relatively elevated $AaDO_2$ gradient in the control group was observed.

In the present investigation, we have demonstrated that prophylactic surfactant fails to improve both gas exchange and ventilation over an 8-h resuscitation period in CDH lambs with TO. In fact, by 240 min, marked improvement of the VEI was observed in the CDH+TO group and in untouched control lambs, whereas the CDH+TO+SURF group fared as badly as the CDH alone. This may be a consequence of both the volume of surfactant given and its method of administration. The second dose of surfactant was calculated based on lamb body weight rather than on lung weight and, thus, was an overestimation of the amount of surfactant required. Consequently, this second dose may have "drowned" the lungs, rendering gas exchange and ventilation more difficult. In addition, the latter dose of surfactant required manual bagging and changing the

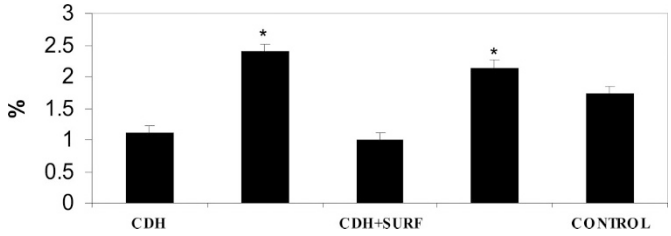

Figure 1. Lung weight/body weight. Five groups were compared: congenital diaphragmatic hernia (CDH, $n = 5$), CDH+surfactant (CDH+SURF, $n = 4$), CDH+tracheal occlusion (CDH+TO, $n = 5$), CDH+TO+SURF $n = 6$), and non-operated controls (Control, $n = 4$). Data is presented as mean ± SEM where * = different from CDH±SURF ($p < 0.05$).

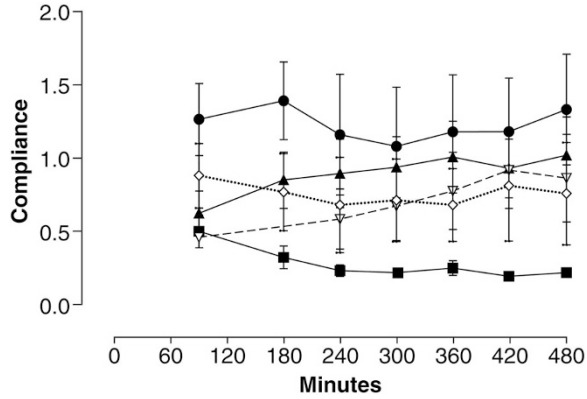

Figure 3. Response of compliance to resuscitation over an 8 hours period. Five groups were compared: congenital diaphragmatic hernia (CDH, $n = 3$), CDH+surfactant (CDH+SURF, $n = 4$), CDH+tracheal occlusion (CDH+TO, $n = 5$), CDH+TO+SURF ($n = 6$), and non-operated controls (Cont, $n = 3$). Data is presented as mean ± SEM. Differences are not statistically significant.

position of the animal. Even though this dose was administered rapidly over 3–5 min, the lungs of these CDH lambs may be very sensitive to any manipulation. CDH+TO+SURF lambs continued to demonstrate high $Paco_2$ levels for the remaining 4 h of resuscitation whereas the control group showed marked improvement. In contrast, the CDH+TO group had a notable decrease of $Paco_2$ levels after 240 min, similar to the one observed in control lambs (28). Thus, the addition of exogenous surfactant to TO appeared detrimental with regard to hypercarbia in this model.

Our study failed to demonstrate significant positive effects of surfactant treatment on the compliance. The absence of significant differences could be explained by the relatively small groups and the high variation of the measured values within each group (SEM). In addition, the total respiratory compliance measurements may underestimate changes in lung compliance due to interference with other elements of the respiratory system, such as thoracic rigidity and the presence of intestines in the thoracic cavity. However, the apparent reduction in the mortality rate and the decreased incidence of pneumothoraces in the CDH+SURF group *versus* CDH lambs, even though not statistically significant, suggests that exogenous surfactant may have some beneficial effects on CDH.

Our results are in accordance with a previous study that examined the role of surfactant supplementation in CDH (30) even though the beneficial effects appear modest when compared with the effects of tracheal occlusion. As published previously, pH is improved significantly by surfactant treatment at birth in the CDH lamb model (30). Our inability to demonstrate a decrease in Pco_2 after exogenous surfactant may be related to a different ventilation strategy. In addition, Wilcox's study had a resuscitation period of only 4 h, and no control groups were used for comparison (30). Our study shows that the limited effect of exogenous surfactant failed to translate into significant beneficial effects with respect to ease of ventilation or oxygen diffusion over 8 h. It is possible that a longer observation period is needed to show any beneficial effect.

Overall, the current data suggest that fetal TO continues to yield the best results in terms of overall postnatal lung function. The data further support the notion that this outcome is likely due to surfactant independent mechanisms. These mechanisms include reversal of pulmonary hypoplasia along with lung and pulmonary artery remodeling. The associated lung remodeling, by altering the collagen:elastin ratio and decreasing alveolar wall thickness, results in greater alveolar distension, which leads to increased lung compliance. In addition, the decreased area of the media of small arteries observed in CDH+TO (7) may decrease the ventilation/perfusion mismatch. In this study, accelerated prenatal lung growth, along with lung and artery remodeling, rather than repletion of surfactant levels at birth, appears more important in improving postnatal lung function in lambs with a surgically created CDH.

Fetal intervention is not without risk for either the fetus or mother (35,48). The rationale for any prenatal intervention for a fetus with CDH is to improve postnatal outcome with the respect to the present success of conservative management (49). This can potentially be achieved either by selecting a

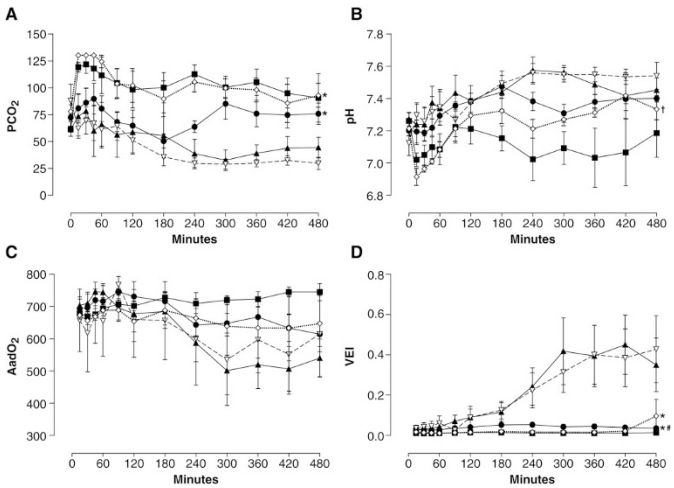

Figure 2. Response of pCO_2, pH, $AaDO_2$ and VEI to resuscitation over an 8 hours period. Evolution of the A) arterial partial pressure of CO_2 (pCO_2, B) pH, C) $AaDO_2$ and D) ventilation efficient index (VEI). Five groups were compared: congenital diaphragmatic hernia (CDH, ■, $n = 5$), CDH+surfactant (CDH+SURF, —, $n = 4$), CDH+tracheal occlusion (CDH+TO, ▲, $n = 5$), CDH+TO+SURF (●, $n = 6$), and non-operated controls (Cont, s, $n = 4$). Data is presented as mean ± SEM where * = different from controls over time, # = different from CDH+TO over time and + = different from CDH over time ($p < 0.05$).

Table 2. *Statistical comparisons for longitudinal data presented in figure 2*

	CDH vs CDH + SURF	CDH + TO vs CDH + TO + SURF	Controls vs CDH + SURF	Controls vs CDH + TO + SURF
pH	**0,003 (<)**	0,320	0,076	0,180
PCO_2	0,965	0,157	**0,011 (<)**	**0,012 (<)**
$AaDO_2$	0,417	0,417	0,974	0,988
VEI	0,279	**<0,001 (>)**	**0,001 (>)**	**<0,001 (>)**
Compliance	0,746	0,746	0,746	0,877

p value for each comparison. For each parameter, four comparisons were made and the Bonferroni procedure was used to correct for the level of significance: for an $\alpha \leq 0{,}05$, p should be $\leq 0{,}012$. Significant comparisons are highlighted in bold. (>) or (<) indicates if the first-listed group is significantly higher (>) or lower (<) than the second group.

subgroup with an extremely high risk of mortality (50,51) and/or by improving the technical aspects of the proposed intervention. Given the nature of this disease and the complexity of its treatment, it is essential to pursue studies in animal models to assess both the short-term mortality and the long-term morbidity.

Acknowledgments. The authors thank Lucile Turcot and Tommy Seaborn for their help with the statistical analysis.

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