Lung volume recruitment in a preterm pig model of lung immaturity

Running head: Lung volume recruitment after preterm birth

Esmond L. Arrindell, Jr.†, Ramesh Krishnan†, Marie van der Merwe², Frank Caminita³, Scott C Howard² Jie Zhang⁴, and Randal K Buddington²*

1 Pediatrics, University of Tennessee HSC, Memphis, TN, USA
2 School of Health Studies, University of Memphis, Memphis, TN, USA
3 Dräger Medical Inc., Telford, PA, USA
4 Pathology, University of Tennessee HSC, Memphis, TN, USA
† Co-first authors

*Corresponding author: Randal K. Buddington, School of Health Studies, 495 Zach Curlin Way, University of Memphis, Memphis, TN 39152
Phone: 901 678 4743; fax: 901 678 3591; Email: rbddngtn@memphis.edu
Abstract

A translational preterm pig model analogous to infants born at 28 weeks of gestation revealed that continuous positive airway pressure results in limited lung recruitment but does not prevent respiratory distress syndrome (RDS); whereas, assist-control + volume guarantee (AC+VG) ventilation improves recruitment, but can cause injury, highlighting the need for improved ventilation strategies. We determined whether airway pressure release ventilation (APRV) can be used to recruit the immature lungs of preterm pigs without injury. Spontaneously breathing pigs delivered at 89% of term (model for 28 week infants) were randomized to 24 hours of APRV (n=9) versus AC+VG with a tidal volume of 5ml/kg (n=10). Control pigs (n=36) were provided with supplemental oxygen by an open mask. Nutrition and fluid support was provided throughout the 24-hour period. All pigs supported with APRV and AC+VG survived 24 hours, compared to 62% of control pigs. APRV resulted in improved lung volume recruitment compared with AC+VG based on radiographs, lower PCO2 levels (44±2.9 vs 53±2.7 mm Hg, p=0.009) and lower FiO2 requirements (36±6 vs 44±11 %, p<0.001), and higher oxygenation index (5.1±1.5 vs 2.9±1.1, p=0.001). There were no differences between APRV and AC+VG pigs for heart rate, wet/dry lung mass, pro-inflammatory cytokines, or histopathologic markers of lung injury. Lung protective ventilation with APRV improved recruitment of alveoli of preterm lungs, enhanced development and maintenance of functional residual capacity without injury, and improved clinical outcomes relative to AC+VG. Long-term consequences of lung volume recruitment using APRV should be evaluated.

Key words: prematurity, pulmonary, animal model, development
Introduction

Respiratory distress syndrome (RDS) remains the leading cause of morbidity and mortality for preterm infants [16] and improving neonatal resuscitation remains a priority [30]. Rapid airway clearance and lung recruitment following preterm birth are crucial [10]. Presently, preterm infants may be intubated for administration of surfactant, but because of concern of causing ventilator induced lung injury (VILI) are then extubated for the initiation of non-invasive ventilation, generally using continuous positive airway pressure (CPAP), despite concerns about its efficacy, safety [3], and limited lung recruitment [2].

We hypothesized lung protective modes of MV can be adapted to recruit functional reserve capacity (FRC) immediately after birth without surfactant. We compared the commonly used assist-control + volume guarantee (AC+VG) mode with airway pressure release ventilation (APRV), also known as biphasic positive airway pressure. APRV is a pressure-limited, time-cycled, time-triggered mode of ventilation [19] that recruits FRC using a defined peak pressure ($P_{\text{high}}$) that is extended over a prolonged period ($T_{\text{high}}$). Ventilation occurs when $P_{\text{high}}$ is reduced ($P_{\text{low}}$) for a period of time ($T_{\text{low}}$) that allows for expiration, but is short enough to prevent alveolar collapse and de-recruitment. Although APRV improves oxygenation, decreases physiologic dead space ventilation, and reduces alveolar edema, surfactant inactivation, histopathologic damage, and the incidence and severity of RDS [17,21], the use of APRV for preterm neonates has not been systematically studied, except for a few case studies after onset of RDS[8,14]. To determine the efficacy of proactive APRV initiated soon after delivery, we used a preterm pig model of lung immaturity and surfactant deficiency, since it closely mimics respiratory issues in preterm humans, and therefore provides information that can readily be translated to clinical care [2].
Methods

Source of preterm pigs. The caesarian section, care, and sampling of preterm pigs followed our established protocol [2] and were approved by the Institutional Animal Care and Use Committees of the University of Tennessee Health Science Center (location of caesarian section) and the University of Memphis (site of critical care). Preterm pigs were delivered from four specific pathogen-free, artificially inseminated sows of a defined genetic lineage without antenatal steroids on gestation day 102 (89% of 115-day term), when lung development is similar to that of 28 week preterm infants. After the airway was suctioned and cleared and spontaneous breathing was established the pigs were transported to a facility established for chronic intensive care.

Processing and intensive care. Pigs with birth weight > 600 grams were intubated with 2.5 French endotracheal tubes, but were not provided surfactant. Within each litter those of similar body weights were randomized to either APRV (n=9) or AC+VG (n=10) using Dräger VN500 ventilators (Dräger Medical, Incorporated, Dräger, Telford, PA). Pigs with significant tracheal trauma (e.g. perforation) caused by placement of the endotracheal tube were removed from the study (n=7), regardless of the ventilator strategy to which they had been assigned. Supplemental oxygen was provided to the remaining pigs in each litter using an open mask. The initial settings for APRV were a $P_{\text{high}}$ of 16, $P_{\text{low}}$ of 0 to minimize expiratory resistance, $T_{\text{high}}$ of 2 seconds, and $T_{\text{low}}$ set for termination of expiration at 75% peak expiratory flow rate. The initial AC+VG settings of 40 breaths per minute, positive end expiratory pressure (PEEP) of 5 cm H$_2$O, inhalation time of 0.35 seconds, tidal volume of 5 ml/kg, and $FiO_2$ of 40% are considered to be lung protective [18]. All pigs were allowed to breathe spontaneously.

Caffeine or doxapram were not provided during the 24 h of MV. Pigs were repositioned each
hour to avoid dependent edema and lung atelectasis and sedated as needed (Ketamine Bioniche Teoranta, Galway, Ireland; 2 mg/kg/dose) via an umbilical catheter (3.5Fr Argyle TM, Covidien, Massachusetts, USA), which was also used for arterial blood sampling, parenteral nutrition, single prophylactic doses of maternal plasma (5 ml/kg) for passive immunity, antibiotic (Cefazolin; 50 mg/kg/dose), and supplemental lactated Ringers as needed to maintain tissue perfusion (usually 3-4 ml/kg/hour).

Heart rate and oxygen saturation were monitored continuously (Masimo Radical 7, Masimo TM, USA). Arterial blood gas measurements (iSTAT ® and CG 4 cartridges; Abaxis, Union City, CA) were performed every 3 hours or after adjustment of ventilator settings to maintain a pH of 7.25 to 7.35, pCO₂ of 40 to 55 mmHg, and oxygen levels within a range of 90-95% saturation.

**Radiography.** A chest x-ray (CXR) was obtained after insertion of the endotracheal tube to confirm and if necessary adjust placement and as a baseline measure of lung volume recruitment (Duoview high Resolution Digital Radiography System, Revo², Kennesaw, GA). A second CXR after 24 hours was used to assess lung volume recruitment.

**Necropsy.** After 24 h of MV the pigs were euthanized (Euthasol; Virbac AH, Inc. Fort Worth, TX, 1 ml/kg; IV), the lungs were removed en bloc, inflated using the endotracheal tube and a NeoPuff™ (Fisher & Paykel Healthcare, Irvine, CA, USA) to a pressure of 20 cm H₂O, and the trachea was clamped. The right lower lobe was tied off, excised, and submerged in formalin for histopathology. Bronchoalveolar lavage fluid (BALF) was collected from the right middle lobe for analysis of cytokines. The weight of the left lung was recorded before and after drying (50°C for 48 h) to determine the ratio of dry weight relative to wet weight as an indicator of pulmonary edema.
**Histologic analysis.** The formalin fixed tissues were processed into paraffin, sectioned, and stained with hematoxylin and eosin. A pediatric pathologist, blinded to the study protocol, reviewed the sections for inflammation, hemorrhage, edema, necrosis and atelectasis using a Likert scale that ranged from 0 (no injury), 1 (1% to 25% injury), 2 (26% to 50% injury), 3 (51% to 75% injury), to 4 (76% to 100% injury).

**Cytokine analysis.** Interleukin (IL)-1α, IL-1β, IL-4, IL-6, IL-8, IFN-γ, and TNF-α were measured in the BALF using Millipore Milliplex Map Porcine Cytokine and Chemokine Magnetic Bead Panel (EMD, Millipore Corporation, Billerica, MA, USA).

**Statistical analysis.** Data were analyzed using Student’s t-test and categorical variables were compared using Fischer’s exact test. The selected level of significance was a two-sided p<0.05.

**Results**

Preterm pigs are challenging to intubate and 7 pigs were excluded because of substantial trauma or damage to the trachea or a bronchus. The APRV and AC+VG pigs within each litter had similar birth weights, with both larger than controls (Table 1). Litters 1 and 2 provided six pigs each that were large enough for successful intubation. Litters 3 and 4 provided fewer pigs >600 g. All 19 of the successfully intubated pigs provided MV survived the 24 hours period of mechanical ventilation, whereas 12/36 (33%) of the control pigs died suddenly or required euthanasia prior to 24 hours after developing symptoms consistent with RDS. Risk of death for control pigs <600 g was similar to pigs >600 g (P=0.75).

**Physiology.** During the first 60 min of APRV, the $P_{\text{high}}$ (18.7 cm H$_2$O±0.2) exceeded the PIP required to achieve the targeted 5 ml/kg volume of the AC+VG protocol (13.7±1.1 ml/kg; P<0.01) and yielded an average ventilation volume (Vt) of 6.3 ml (+0.4). At 3 hours the Vt had
increased to 7.7 (±0.4; P=0.008) despite gradually decreasing P\textsubscript{high} in response to blood gases and oxygenation (Figure 1). Even though P\textsubscript{high} remained relatively stable after 3 hours, lung recruitment continued and the V\textsubscript{t} for the last hour of the study (9.1±0.4) was higher compared to 3 hours (P=0.02). As expected, adjusting P\textsubscript{high} changed ventilation volumes.

The PIP declined from 13.6 cm H\textsubscript{2}O (±0.4) for the first 60 minutes to 11.6 cm H\textsubscript{2}O (±0.3) for the 3\textsuperscript{rd} hour of AC+VG (Figure 1; P=0.002). During the last 2 hours of ventilation the PIP increased in 3 of the 10 AC+VG pigs and averaged >25 cm H\textsubscript{2}O and FiO\textsubscript{2} was increased to as high as 0.9 to maintain targeted oxygen saturation. At necropsy, examination of the lungs revealed findings consistent with early RDS.

Pigs provided APRV required a lower FiO\textsubscript{2} (P<0.001) compared with pigs assigned to AC+VG (Table 2). PCO\textsubscript{2} was in the normal range for both groups, but was higher among AC+VG pigs. There were no differences for pH, PaO\textsubscript{2} and oxygen saturation, as expected since ventilator settings were adjusted to maintain these values within the targeted range. The oxygenation index (FiO\textsubscript{2} x mean airway pressure/PaO\textsubscript{2}) was higher for the APRV pigs (P<0.001), indicating improved gas exchange. The heart rate was similar for both groups of ventilated pigs.

**Radiography and gross pathology.** The lungs were consolidated at the start of MV. After 24 hours, lung expansion was significantly improved with APRV compared to AC+VG (Figure 2). Inflation of the lungs to a pressure of 20 cm H\textsubscript{2}O revealed the AC+VG pigs had extensive areas of atelectasis, patchy expansion, and focal sites of hemorrhage that were not evident in the APRV pigs (Figure 2). As before [2], lung volume recruitment was limited among control pigs that survived for 24 hours.

The wet/dry lung ratio did not differ between the APRV and AC+VG groups (86.1% wet mass ±0.6% vs 86.2% ±0.4%, respectively, p=0.8).
Histopathology. There were no significant differences in hemorrhage, atelectasis, edema, necrosis, or inflammation, with all scores averaging <1 for both groups (all P>0.1). The lungs of APRV pigs had regions with alveolar septal thinning whereas the lungs of AC+VG pigs exhibited areas of microatelectasis (Figure 2).

Inflammation. None of the pro-inflammatory cytokines IL-1α, IL-1β, IL-6, and IL-8 measured in the lung lavage samples differed between the groups of ventilated pigs. IL-10, IFN-γ, and TNF-α were below the limits of detection and IL-4 was detected at low levels in 1 of the 9 APRV and 1 of the 10 AC+VG pigs.

Discussion

Concern for VILI underlies the preference for non-invasive ventilation such as CPAP to provide the positive pressure ventilation support recommended to recruit FRC after preterm delivery [23]. Using lung protective modes of MV proactively as an alternative is novel and clinically relevant. The improved recruitment of FRC for the APRV pigs compared with AC+VG based on CXR, gross pathology, lower FiO₂ to meet targeted oxygen saturation, and higher OI are consistent with the limited clinical data that suggest APRV as a rescue mode for preterm infants with RDS results in improved gas exchange without causing excessive lung injury [8,14].

The prolonged T_{high} and short T_{low} of APRV results in a higher MAP without wide pressure swings that effectively provides a continuous distending pressure that enhances the replacement of fluid with air without allowing de-recruitment [9,21] and causes a progressive and even distribution of lung recruitment in both the compliant and non-compliant regions of the lung. The continuous, but lower pressure provided by CPAP results in minimal lung recruitment and lower survival (2) and is consistent with concerns of efficacy for use with preterm infants
<28 weeks gestation (3). Conventional tidal ventilation, such as AC+VG causes wider pressure fluctuations with fluid expulsion limited to the inspiration phase [12]. Pressure during the expiration phase must remain high enough to prevent the collapse of the distal airways and de-recruitment [11,25]. Even though the 5 cm H2O pressure used for the PEEP is a common setting in clinical practice (18), the longer expiratory period of AC+VG results in a larger magnitude of pressure fluctuations and may increase the risk of alveolar collapse and de-recruitment of lung volume, contributing to regional variability in lung recruitment [1] and requiring more time and a larger number of inflations to reach a full FRC. It is possible that a higher PEEP would enhance the efficacy of the AC+VG and decreased the risk of alveolar collapse [11].

The similar wet/dry lung mass for AC+VG and APRV pigs is not surprising based on the limited differences in lung recruitment after 24 hours. The proactive use of either model of MV for 24 hours did not result in VILI by histologic criteria, even with the APRV protocol causing lung expansion to the 11th rib, which is indicative of over distension. However, the heterogeneous, patchy pattern of lung recruitment and hemorrhage seen in the AC+VG pigs and the increasing PIP values recorded during the final hours for 30% of the AC+VG pigs are suggestive of a decrease in lung compliance and de-recruitment and coincided with increased FiO2 to maintain targeted oxygen saturation levels.

The APRV protocol is a gentler method than sustained lung inflation protocols that use high pressure or volume for neonatal resuscitation and rapid lung recruitment [6,19,27]. Rapid, large scale lung distension can cause histopathologic damage, increase BALF protein concentrations, trigger rapid (<30 min) inflammatory responses [29], increase the expression of inflammatory cytokines, and acute phase proteins [10], and alter expression of genes associated with alveolar development and vascularization of newborn lungs [4,15,28].
One concern is the potential impact of APRV on cardiac function in neonates due to increased intrathoracic pressure that could impede venous return. A small case series in children demonstrated that APRV does not decrease blood pressure or urine output [13], but this needs to be investigated in preterm neonates. Though not specifically addressed in this study, there were no gross signs at necropsy of intracranial hemorrhage or compromised bowel. The possible increased translocation of bacteria from the lungs to the blood caused by the effectively higher PEEP of APRV [5] needs to be evaluated.

**Perspectives**

The present study is limited by the use of preterm pigs that are relevant to 28-week preterm infants that with surfactant often do well without intense ventilation support [2]. The potential benefits and limitations of APRV need to be evaluated at earlier stages of preterm lung development, and the optimal initial $P_{\text{high}}$ to effectively and safely recruit lung volume must be determined. Although the short-term benefits of proactive APRV are evident, the longer-term pulmonary outcomes, including assessing the impact on survival, time to extubation, and subsequent lung development need to be evaluated.

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study successful. This study was supported in part by a grant from the LeBonheur Children’s Hospital of Memphis, TN and by the University of Memphis.
References


Figure 1. Changes in $P_{\text{High}}$ (solid circles) and ventilation volumes (open squares) during 24 h of providing APRV to preterm pigs (top graph) and PIP while providing 24 h of AC+VG to preterm pigs (bottom graph). Values are means and standard errors calculated from the data collected by the Dräger VN500 ventilators. Each data point for APRV is the mean of the 9 preterm pigs and for AC+VG as the mean of 10 preterm pigs. Values for individual pigs were averaged from 4 recordings (total of 20 min).

Figure 2. Representative chest x-rays, lungs, and histology (10X magnification) after providing 24 h of ventilation support to preterm pigs using APRV (A, C, E) or AC+VG (B, D, F). Expansion of the lungs with APRV was to the 10th to 11th ribs and with AC+VG to the 8th to 9th ribs. The resected lungs were inflated to a pressure of 20 cm H2O using a Neopuff®. Lungs from APRV pigs had uniform recruitment, minimal or no signs of hemorrhage, and a greater proportion of expanded alveoli and less microatelectasis than lungs of AC+VG pigs with patchy, heterogeneous recruitment, extensive areas of atelectasis and focal points of hemorrhage.
Table 1. Birth weights (means and SE) of preterm pigs assigned for APRV, AC+VG, and as controls. Treatment groups with different superscripts are significantly different (P<0.05).

<table>
<thead>
<tr>
<th>Litter</th>
<th>All (n, females, Intubation failures)</th>
<th>APRV (n, females)</th>
<th>AC-VG (n; females)</th>
<th>Control (n, females)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grams</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>917 ± 68 (14,9,0)</td>
<td>1076 ± 33^a (3,2)</td>
<td>1177 ± 49^a (3,1)</td>
<td>760 ± 77^b (8,6)</td>
</tr>
<tr>
<td>2</td>
<td>895 ± 36 (16,9,0)</td>
<td>1029 ± 18^a (3,2)</td>
<td>1038 ± 20^a (3,3)</td>
<td>813 ± 38^b (10,4)</td>
</tr>
<tr>
<td>3</td>
<td>513 ± 42 (15,9,3)</td>
<td>644 ± 190^a (1,1)</td>
<td>655 ± 83^a (2,2)</td>
<td>422 ± 40^b (9,6)</td>
</tr>
<tr>
<td>4</td>
<td>882 ± 73 (15,4,2)</td>
<td>991 ± 25^a (2,0)</td>
<td>1093 ± 101^a (2,1)</td>
<td>780 ± 112^b (9,3)</td>
</tr>
<tr>
<td>All</td>
<td>802 ± 35 (60,31,5)</td>
<td>1019 ± 27^a (9,5)</td>
<td>1005 ± 78^a (10,7)</td>
<td>695 ± 42^b (36,19)</td>
</tr>
</tbody>
</table>
Table 2. Responses of preterm pigs to 24 h of APRV or AC+VG. Values are means and (ranges).

<table>
<thead>
<tr>
<th></th>
<th>APRV</th>
<th>AC+VG</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>9 of 9</td>
<td>10 of 10</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 (7.1-7.5)</td>
<td>7.3 (7.0-7.5)</td>
<td>NS</td>
</tr>
<tr>
<td>pCO₂ (mm Hg)</td>
<td>44 (31-74</td>
<td>53 (30-79)</td>
<td>0.009</td>
</tr>
<tr>
<td>paO₂ (mm Hg)</td>
<td>105 (54-173)</td>
<td>100 (56-180)</td>
<td>NS</td>
</tr>
<tr>
<td>Oxygenation index</td>
<td>5.1 (1.9-12.9)</td>
<td>2.9 (1.2-7.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Fraction of inspired oxygen (FiO₂ %)</td>
<td>36 (30-50)</td>
<td>44 (30-90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dynamic compliance</td>
<td>1.3 (1.1-1.5)</td>
<td>1.5 (0.9-1.7)</td>
<td>NS</td>
</tr>
<tr>
<td>(Cdyn ml/cmH₂O)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen saturation (%)</td>
<td>97 (75-100)</td>
<td>96 (73-100)</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beat per minute)</td>
<td>160 (129-196)</td>
<td>167 (109-199)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean airway pressure (cm H₂O)</td>
<td>13 (9.4-18)</td>
<td>7.4 (4.4-20)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Result of Student’s t-test test for continuous variables and Fishers exact test for categorical variables.