Lung inhomogeneities and time course of Ventilator-Induced mechanical injuries

Supplemental Digital Content 1

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Additional Methods

Study design

Figure 1 – Flowchart of the study

Induction of general anesthesia ➔ Cannulation of an auricolar vein, endotracheal intubation, gastric emptying and esophageal balloon positioning

Surgical preparation (in supine position) ➔ Insertion of arterial, central venous and bladder catheter

Recruitment maneuver (in prone position) ➔ Pressure controlled mode: FiO2 0.5, inspiratory pressure 45 cmH20, PEEP 5, I:E 1:1, RR 10 for 2 minutes

Baseline CT scan for the determination of FRC ➔ Strain (=VT/FRC) higher than 2.5

Determination of lethal VT ➔ Volume controlled mode: FiO2 0.5, lethal VT, PEEP 0, I:E 1:2

Start of lethal ventilation ➔ Every 3 hours (or more frequently if clinical variation):
- CT scan: end-inspiration and end-expiration

Data collection ➔ Every 6 hours (or more frequently if clinical variation):
- Respiratory system mechanics: Peak, plateau pressure, intrinsic PEEP, esophageal pressure – derivation of ERS, ECW and EL – espiratory VT and VE.
- Haemodynamics: arterial pressure, CVP, HR, urinary output and fluid balance.
- Arterial and central venous EGA

End of lethal ventilation ➔ After 54 hours or when whole-lung edema developed

Sacrifice and autopsy ➔ Samples collection for Wet/Dry ratio and histological analysis
Anesthesia and surgical preparation

Anesthesia was induced with an intramuscular injection of medetomidine 0.025 mg/kg and tiletamine/zolazepam 5 mg/kg. An auricular vein was cannulated. The animal was kept in prone position and, after preoxygenation, an endotracheal tube was inserted in prone position and mechanical ventilation started. Anesthesia was maintained with propofol 5-10 mg/kg/h, pancuronium bromide 0.3-0.5 mg/kg/h and medetomidine 2.5-10.0 µg/kg/h. Normal saline (NaCl 0.9%) was administered at 100 ml/h during surgery and 50 ml/h thereafter. Ceftriaxone 1 g i.v. and Tramadol 50 mg i.v. were administered preoperatively and every 12 hours thereafter. Low molecular weight heparin 2000 IU was given subcutaneously once a day.

Surgical preparation was carried out under sterile conditions, with piglets under general anesthesia and in supine position. During the procedure, mechanical ventilation was set as follows:

- Volume-controlled mode
- Fraction of inspired oxygen (FiO₂) = 0.5
- Tidal Volume (V_T) = 10 ml/kg
- Respiratory rate (RR) = 20-22 breaths per minute
- Positive end-expiratory pressure (PEEP) = 3-5 cmH₂O
- Inspiratory to expiratory time ratio (I:E) = 1:2

Right carotid artery was exposed and cannulated. A three lumen central venous catheter was inserted through the right internal jugular vein. A bladder catheter was positioned via cistostomy. Once the surgical procedure was completed, the animal was turned prone. Stomach emptying was performed by gastric suction. A latex thin wall, 5 cm long, esophageal balloon was advanced in
the inferior third of the esophagus and filled-in with 1.5 ml of room air. Proper positioning of endovascular catheters and esophageal balloon was later verified on thorax computed tomography. Pressure transducers were connected to the endotracheal tube, the esophageal balloon and the endovascular catheters, zeroed at room air at heart level, as appropriate. Data were recorded and analyzed using a dedicated software (Colligo, Italy, www.elekton.it).

*Lung computed tomography*

Before the beginning of the study, a baseline CT scan (Lightspeed, General Electric) was obtained. Each piglet underwent a recruitment maneuver, defined as two minutes of pressure-controlled ventilation with the following settings:

- \( \text{FiO}_2 = 0.5 \)
- Inspiratory pressure = 45 cmH\(_2\)O
- \( \text{RR} = 10 \) breaths per minute
- \( \text{PEEP} = 5 \) cmH\(_2\)O
- \( \text{I:E} = 1:1 \)

Lung CT scan was performed with the following settings:

- Collimation width: 32 x 2 x 0.6 mm
- Spiral pitch factor: 1.2
- Slice thickness: 5 mm
- Reconstruction interval: 5 mm
- Data collection FOV: 500 mm
Reconstruction FOV: 300 mm
- KVP: 120
- X-Ray Tube Current: 110 mA
- Pixel dimensions: 0.585938/0.585938
- Acquisition matrix: 512 x 512

**Study ventilatory settings**

Volume-controlled mechanical ventilation was set as follows:

- **FiO_2** = 0.5
- **V_T** corresponding to a strain (**V_T*/FRC, see above) greater than 2.5, which corresponds to 750 ml in piglets of about 20-25 Kg, or to 38-40 ml/Kg.
- Respiratory rate = 15 breaths per minute
- PEEP = 0 cmH_2O
- I:E = 1:2

Strain was defined as the ratio between **V_T** and functional residual capacity (FRC). The value of 2.5 was chosen because it is known to be lethal within 54 hours\(^1\). The animals were ventilated with no PEEP in order to avoid any of its potential protective effects and to facilitate the onset of lung lesions.

**Hemodynamic protocol**

To maintain hemodynamic stability, a target mean arterial pressure (MAP) was set between 60 and 70 mmHg, with continuous saline infusion (50 ml/h). A fall in MAP below 60
mmHg was corrected with saline bolus (100-150 ml) and increases in saline infusion, up to 75-100 ml/h. If these were not sufficient to restore the target MAP, norepinephrine was administered, from a minimum of 0.1 μg/kg/min to a maximum of 1.0 μg/kg/min. If MAP rose above 70 mmHg, hemodynamic support was deescalated. Cumulative fluid intake was computed as the sum of intravenous fluids infused but not including drugs. Fluid balance was computed as cumulative fluid intake minus total urinary output.

*Data collection*

A complete data collection was performed at least every 3 hours or more often if respiratory or hemodynamic variables changed. Prior to data collection, tracheal suctioning was performed. \( V_T \), airways pressure (Paw) and esophageal pressure (Pes) were recorded during end-inspiratory and end-expiratory pauses.

Transpulmonary pressure was computed at end-inspiration as:

\[
\Delta\text{Transpulmonary pressure (cmH}_2\text{O)} = \Delta\text{Airway pressure (cmH}_2\text{O)} - \Delta\text{esophageal pressure (cmH}_2\text{O)}
\]

\[
\Delta\text{Airway pressure (cmH}_2\text{O)} = \text{Plateau airway pressure (cmH}_2\text{O)} - \text{End expiratory pause airway pressure (cmH}_2\text{O)}
\]

\[
\Delta\text{Esophageal pressure (cmH}_2\text{O)} = \text{Plateau esophageal pressure (cmH}_2\text{O)} - \text{End expiratory pause esophageal pressure (cmH}_2\text{O)}
\]

Plateau airway and esophageal pressure were measured during an end-inspiratory pause.

Respiratory system (\( E_{RS} \)), lung (\( E_{L} \)) and chest wall (\( E_{CW} \)) elastance were calculated:

\[
E_{RS} = \Delta\text{Paw}/V_T
\]
\[ E_L = \Delta(P_{aw}-P_{es})/V_T = \Delta P_L/V_T \]

\[ E_{CW} = \Delta P_{es}/V_T \]

\( \Delta P_L \) and \( \Delta P_{es} \) were defined as the difference between end-inspiratory (plateau) and end-expiratory transpulmonary and esophageal pressures, respectively. Arterial and central venous blood gases were analyzed (ABL825FLEX, Radiometer, Copenhagen, Denmark®). Variables describing systemic hemodynamics were recorded; central venous pressure (CVP) was measured during an end-expiratory pause. Urine output was recorded and fluid balance over a 6 hours period was calculated. Rectal temperature was measured. Every 3 hours, 2 CT scans were performed. The former was obtained during an end-inspiratory pause, the latter during an end-expiratory pause.

**Sacrifice and autopsy**

After the scheduled 54 hours of the study, or before if whole lung edema developed, piglets were sacrificed with a bolus injection of KCl 40 mEq i.v. under deep sedation, obtained with a 50 mg bolus dose of propofol. After sacrifice, tracheal tube was clamped during end-inspiratory pause. Chest was opened and trachea was clamped and cut. Lungs were excised and weighed, keeping the trachea clamped.

**Light microscopy**

Each lung was divided in 4 regions, as shown in Figure 2.
Figure 2: Lung regions for histological samples

Lung fragments were obtained from each region of both lungs: three samples from subpleural regions taken at the tips of the lobes (1, 2, 3, 5, 6, 7 in Figure 2) and one sample from the internal part of the lung (4, 8 in Figure 2). Fragments were immediately processed for morphological procedures by fixation in 4% formalin in 0.1M phosphate buffered saline (PBS), pH 7.4. After fixation lung fragments were routinely dehydrated, paraffin embedded, and serially cut (thickness 5 μm). For each specimen and for each staining we analysed three slides obtained at a 100 μm distance. Sections were stained with freshly made hematoxylin-eosin to evaluate cells and tissue morphology. To obtain specific stain for fibrillary collagen, sections were deparaffinised and immersed for 30 minutes in saturated aqueous picric acid containing 0.1% Sirius red F3BA (Sigma, Milan, Italy)\(^2\). To analyze elastic fibers, sections were stained in blue-black by Weigert’s resorcin-fuchsins\(^3,4\). Briefly, after bringing sections to water, they were incubated for 2 minutes in potassium permanganate, washed in water and immersed in 1% Oxalic acid for 1 minute. After washing, slides were stained o.n. in Weigert’s resorcin-fuchsins at room
temperature. Hematoxylin-eosin stained sections for each lung region were analysed at light microscope in blind by two independent operators using a semi quantitative grading scale to assess various features of the tissue. The variables included in the scale for the analysis of lung structure and damage were: hyaline membranes formation, diffusion and severity of interstitial and septal infiltrate, vascular congestion and intra-alveolar haemorrhaging, alveoli rupturing and basophilic material deposition. Overall injury was expressed by a scoring system from 0 to 4: 0 - no alterations, 1 - 25% of field involved; 2 - 50% of field involved; 3 - 75% of field involved; 4 - 100% of field involved. For elastin and collagen content analysis slides were photographed by a digital camera connected to a Nikon Eclipse 80i microscope. At least 8 fields per section per area were analyzed with Photoshop (Adobe, USA) allowing to detect resorcin-fuchsin and Sirius red stained tissue. Collagen and elastin content were expressed as a percent of the stained area relative to the lung tissue. For this purpose, we considered the effective lung parenchyma excluding the areas occupied by lung alveoli lumen, and we excluded perivascular and peribronchiolar regions.

**Wet to dry ratio**

Three samples from each lung (upper, medium and lower lobe) were collected. They were immediately weighed and, after being dried for 24 hours at 50 °C, were weighed again. Wet to dry ratio was determined as the ratio between the two measurements.
Figure 3: New densities classification

New densities classified as: subpleural (red line); peribronchial (green line); parenchymal (yellow line).
CT scan: baseline lung densities

An example of an end-inspiratory density is shown in the figure below:

**Figure 4**

![CT Scan Example](image_url)

Before the beginning of the study, two CT scans were performed, the former at end-inspiration and the latter at end-expiration. A recruitment maneuver (see above) preceded the CT scan. On the basis of these scans, two groups of animals were identified: the first group included pigs with non-recruitable baseline lung lesions; the second group included pigs without pulmonary infiltrates. Baseline lesions were manually delineated and were kept delineated in end-expiration and in end-inspiration scans along the study, until whole lung edema developed.

**Quantitative analysis of CT scan**

For each CT scan obtained during the study, lung profiles were manually drawn. Analysis was performed using a dedicated software (SoftEFilm, Elekton, Italy, [www.softefilm.eu](http://www.softefilm.eu)). The density of lung parenchyma was assumed to be close to the density of water (0 HU) and each voxel was assumed
to be formed by two compartments: lung tissue (including blood, 0 HU) and air (-1000 HU).

Gas fraction was computed in each voxel as:

\[
\text{Volume gas} \div (\text{volume gas} + \text{volume tissue}) = \frac{\text{mean CT number observed}}{(\text{CT number gas} - \text{CT number tissue})}
\]

Rearranging:

\[
\text{Gas fraction} = \frac{\text{voxel density (Hounsfield units)}}{-1000}
\]

\[
\text{Tissue fraction} = 1 - \text{gas fraction}
\]

Consequently, gas and tissue volumes can be defined as:

\[
\text{Gas volume} = \text{gas fraction} \times \text{voxel volume}
\]

\[
\text{Tissue volume} = \text{tissue fraction} \times \text{voxel volume}
\]

Voxel weight is equal to the tissue volume, assuming that tissue density is 1.

Aeration of lung parenchyma was classified in four subsets:

- Not inflated tissue: density > -100 HU
- Poorly inflated tissue: density between -100 and -500 HU
- Well inflated tissue: density between -500 and -900 HU
- Over inflated tissue: density < -900 HU

We defined “new density” a region of at least 6 mm (inner diameter of tracheal tube) of maximal diameter with a density corresponding to poorly or not inflated tissue, not present in the previous CT scan and distinguishable from the surrounding parenchyma. For seek of clarity the new densities are defined in the manuscript as “densities”. The densities were classified by 3 independent observers (M.G., C.C. and M.L.). New densities were classified on the basis of location in the lung. New
densities under the visceral pleura were classified as sub pleural; new densities near bronchi were classified as peribronchial; all the remaining densities were classified as parenchymal. New densities were also classified dividing lungs into 6 fields: dependent vs non dependent regions (lesions respectively above or under an ideal line through the trachea or the centre of the lung) and apex/hilum/basis (if new densities are respectively above the carina, between carina and diaphragm appearance and under diaphragm appearance level). Note that new densities could be classified as peribronchial only if a bronchogram was clearly identifiable, while many parenchymal new densities were located near a blood vessel but, since the vessel was not clearly identifiable without contrast medium these lesions could not be counted as “perivascular”.

Piglet lung acinus size determination

As the ratio between airway space dimensions and animal weight follows a logarithmic scale\(^5\) we estimated the acinar volume of piglets from the data presented by Sapoval and Weibel\(^6\) reporting the acinus size in mouse, rat, rabbit and humans. For humans we used the 1/8 subacinus since, as detailed by the authors, this 1/8 subacinus is more comparable to acini in other species\(^7\) and computed an acinar volume of 12.1 mm\(^3\) corresponding to a radius of 1.42 mm.
Figure 5 presents the relationship between log10 of animal weight and log10 of acinar volume in mouse, rat, rabbit and human as described in reference 6. For the human acinus the size of 1/8 subacinus was used. \[ \log_{10}(\text{acinus size}) = 0.39363 + 0.49331 \log_{10}(\text{animal weight in Kg}), \quad r^2=0.99, \quad p<0.01 \]. The computed log10 of the acinar size of 25 Kg piglet is reported with a red dot.
Lung inhomogeneities determinations

Since CT scan images are composed by voxels whose dimensions depend both on the CT scan hardware and on the setting for image reconstruction, we decided to produce a lung inhomogeneities map with dimensions 1:1 to the original CT scan map, but using as a “basic dimension” the acinar volume and filtering the map with a gaussian filter with a radius equal to the radius of the acinus. In this way, we obtained a CT value of each voxel which was dependent to the CT value of the neighboring voxels. Around each voxel we defined a spherical crust starting at distance of one acinar radius from the voxel center and of ½ acinar radius thickness. The ratio of the surrounding voxel gas to the central voxel gas fraction indicates homogeneity if equal to 1, inhomogeneity when greater than 1. We computed a vector of lung inhomogeneities dividing the filtered gas fraction in each of the voxels included, at least partially, in the spherical crust and the filtered value of the central voxel and we wrote the maximum of the vector in the lung inhomogeneities map. It must be stressed that while average is a square filter and takes into the same account near and far voxels, gaussian filters exponentially decreases weight of far voxels. We considered as stress raisers those points causing inhomogeneities greater than 95th percentile of the values observed in our normal piglets at baseline resulting in a threshold of 1.685. Lung inhomogeneity was expressed both as intensity (average ratio) and extent (fraction of lung volume with inhomogeneities greater than 1.685). The pressure multiplication factor by the stress raiser can be estimated according to Mead et al.\textsuperscript{9} as the volume/gas ratio between two lung regions elevated to the 2/3 power (factor scale from volumes to surfaces).
**Study time-points**

In order to study the development of VILI in our piglets, we decided to synchronize our analysis establishing 6 time-points. Every time-point corresponds to a defined CT image pattern. The time-points are the following:

- **Time 0** – the baseline CT scan
- **Time 1** – the last CT scan without new densities
- **Time 2** – the first CT scan with at least a new density
- **Time 3** – the last CT scan in which the new densities were distinguishable
- **Time 4** – the first CT scan with one-field edema, that is a pattern in which increase of density occupies at least 1 lung field previously described
- **Time 5** – the first CT scan with all-field edema, that is a pattern in which increase of density occupies all the 6 lung field previously described

In 5 piglets some of the above time-points were coincident, because of a rapid evolution of CT scan pattern (see Table 1). As shown, this phenomenon occurred mainly in piglets with baseline lesions.

**Table 1**

<table>
<thead>
<tr>
<th>Pig code</th>
<th>Baseline lesions</th>
<th>Coincident time-points</th>
</tr>
</thead>
<tbody>
<tr>
<td>KT</td>
<td>YES</td>
<td>T0-T1-T2-T3</td>
</tr>
<tr>
<td>RB</td>
<td>NO</td>
<td>T0-T1 and T4-T5</td>
</tr>
<tr>
<td>MN</td>
<td>YES</td>
<td>T0-T1 and T2-T3</td>
</tr>
<tr>
<td>DLC</td>
<td>YES</td>
<td>T4-T5</td>
</tr>
<tr>
<td>JNN</td>
<td>YES</td>
<td>T4-T5</td>
</tr>
</tbody>
</table>
Lung recruitability (y axis) as a function of not inflated tissue (g) at end-expiration (x axis). (Lung recruitability (g) = -25 + Not inflated tissue at end-expiration (g) * 0.90, $r^2 = 0.97$, $p < 0.0001$).
Figure 7 – Time-course of not-inflated, poorly inflated and well-inflated tissue

Time course of not inflated (black dots), poorly inflated (white dots) and well inflated (triangles) tissue ± SD at the 6 time points (x axis).

(Not inflated tissue (g) = 42 + 0.005*e^{0.52*hours}, r^2 = 0.99, p<0.0001;)
Poorly inflated tissue (g) = 197 + 0.051*e^{0.42*hours}, r^2 = 0.99, p=0.0016;
Well inflated tissue (g) = 172 - 0.042*e^{0.41*hours}, r^2 = 0.97, p=0.0054).
Pre-study physiological variables in pigs with and without non recruitable end-inspiratory densities

Piglets with and without recruitable baseline lung densities presented similar gas exchange variable before starting the study (i.e. with a $V_T$ of approximately 10 ml/Kg) while the $E_{RS}$ resulted to be significantly different despite a similar plateau pressure.

Table 2 – Comparison of pre-study variables between piglets with and without baseline densities

<table>
<thead>
<tr>
<th></th>
<th>Piglets with recruitable baseline lung densities (n=6)</th>
<th>Piglets without recruitable baseline lung densities (n=6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (Kg)</td>
<td>21±2.5</td>
<td>22±6.3</td>
<td>0.60</td>
</tr>
<tr>
<td>$V_T$ (ml)</td>
<td>285±50</td>
<td>308±34</td>
<td>0.37</td>
</tr>
<tr>
<td>PaO$_2$/FiO$_2$</td>
<td>482±82</td>
<td>487±106</td>
<td>0.92</td>
</tr>
<tr>
<td>PaCO$_2$ (mmHg)</td>
<td>41±7.3</td>
<td>43±3.3</td>
<td>0.60</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.4±0.062</td>
<td>7.4±0.088</td>
<td>0.25</td>
</tr>
<tr>
<td>Plateau pressure (cmH$_2$O)</td>
<td>16±3.2</td>
<td>15±1</td>
<td>0.24</td>
</tr>
<tr>
<td>$E_{RS}$ (cmH$_2$O/l)</td>
<td>58±5.2</td>
<td>48±6</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>78±8.8</td>
<td>82±9.3</td>
<td>0.57</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>77±16</td>
<td>96±18</td>
<td>0.15</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>6.5±5</td>
<td>7.5±5.7</td>
<td>0.78</td>
</tr>
<tr>
<td>ScvO$_2$ (%)</td>
<td>78±8.8</td>
<td>82±9.3</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Blood gas and hemodynamic variables were compared with unpaired t-test.
**Pre-study CT-scan variables in piglets with and without non-recruitable end-inspiratory densities**

CT scan data showed a trend toward increased total lung weight, increased fraction of not inflated tissue and a trend toward a reduced fraction of well inflated tissue and significantly greater lung inhomogeneities extent. The total volume of baseline lesions was 61±54 ml and their average CT number was -115±77 HU. Note that the lung inhomogeneities extent is computed on the pre-study CT scan and is different from the Baseline one reported in Table 2, which is recorded after starting the study.

**Table 3 – Comparison of pre-study variables between piglets with and without baseline densities**

<table>
<thead>
<tr>
<th></th>
<th>Pigs with recruitable baseline lung densities (n=6)</th>
<th>Pigs without recruitable baseline lung densities (n=6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tissue (g)</td>
<td>459±65</td>
<td>361±83</td>
<td>0.06</td>
</tr>
<tr>
<td>Total gas (g)</td>
<td>333±98</td>
<td>405±132</td>
<td>0.34</td>
</tr>
<tr>
<td>Not inflated tissue (%)</td>
<td>15±6.2</td>
<td>5±2.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Poorly inflated tissue (%)</td>
<td>56±20</td>
<td>40±22</td>
<td>0.23</td>
</tr>
<tr>
<td>Well inflated tissue (%)</td>
<td>29±23</td>
<td>55±22</td>
<td>0.08</td>
</tr>
<tr>
<td>Over inflated tissue (%)</td>
<td>0.0083±0.0053</td>
<td>0.032±0.023</td>
<td>0.06</td>
</tr>
<tr>
<td>Extent of lung inhomogeneities (% of lung volume)</td>
<td>9.6±3.9</td>
<td>5.2±1.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Average lung inhomogeneities</td>
<td>2.5±0.22</td>
<td>2.6±0.28</td>
<td>0.48</td>
</tr>
</tbody>
</table>

CT-scan variables were compared with unpaired t-test.
**Length of mechanical ventilation**

Piglets with baseline densities tended to deteriorate more rapidly than piglets without baseline densities.

**Table 4 – Comparison of length of mechanical ventilation in piglets with and without baseline densities**

<table>
<thead>
<tr>
<th></th>
<th>Pigs with recruitable baseline lung densities (n=6)</th>
<th>Pigs without recruitable baseline lung densities (n=6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 1 (hours)</td>
<td>4.3±4.3</td>
<td>7±8.3</td>
<td>0.50</td>
</tr>
<tr>
<td>Time 2 (hours)</td>
<td>6.8±5.4</td>
<td>10±7.1</td>
<td>0.41</td>
</tr>
<tr>
<td>Time 3 (hours)</td>
<td>10±8.4</td>
<td>20±13</td>
<td>0.13</td>
</tr>
<tr>
<td>Time 4 (hours)</td>
<td>13±7.6</td>
<td>22±13</td>
<td>0.18</td>
</tr>
<tr>
<td>Time 5 (hours)</td>
<td>15±6.9</td>
<td>24±13</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Times were compared with unpaired t-test.
Microscopic anatomy

Figure 8 - Microphotographs of lung sections stained with Sirius red for collagen

Figure 9 - Microphotographs of lung sections stained with Weigert’s resorcin-fuchsin for elastin

Histological analysis were available in 7 pigs. Figure 8 shows a representative coloration of collagen and Figure 9 shows a representative coloration of elastin. The percentage of lung parenchyma
which stained for elastin was greater than the one which stained for collagen (9.45% [6.43 – 13.47] for elastin vs 4.58% [2.89 – 7.11] for collagen, p<0.0001). When the fraction of parenchyma which stained for elastin were compared between the peripheral and central lung regions, no significant difference was found (median 9.45% [6.93 – 15.51] of lung parenchyma in the central regions vs 8.89% [7.52 – 10.78] in the peripheral lung regions compared to the central lung regions, p=0.22); while we found a slightly greater area of stained with collagen in the peripheral lung regions compared to the central ones (median 3.80% [3.14 – 5.35] of lung parenchyma in the central regions compared to 4.48% [3.58 – 6.60] of lung parenchyma in the peripheral ones, p=0.047).
Microphotographs showing lung sections stained with H&E. Lung damage ranged from moderate to severe. Panel B shows the presence of a connectival septum which separates, in the same field, two
different diseases presentation. Original magnification: 4x (A), 10x (B, C). We observed, within the parenchyma, septa of connective tissue, which delimit the different area of damage (ranging from moderate to severe). We may speculate that also those intrinsic inhomogeneities of parenchyma could act as pressure multipliers. However, this is an anatomical feature of the animal in question, which then finds no analogy with the ultra structural features of the human lung.
Figure 11 – Ratio between intra and extra-alveolar infiltrate

Figure presents the ratio of intra to extra-alveolar infiltrate as a function of lung recruitability. Lung recruitability (% of total lung tissue) = 0.194 + 1.271 * Intralveolar/Extralveolar, infiltrate ratio, $r^2 = 0.5$, $p=0.07$
Table 5 – Dependent/non dependent distribution of new densities

<table>
<thead>
<tr>
<th></th>
<th>Non Dependent</th>
<th>Dependent</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subpleural</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[4.75 - 14]</td>
<td>[2.75 - 12.00]</td>
<td></td>
</tr>
<tr>
<td><strong>Parenchymal</strong></td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[0 - 3.25]</td>
<td>[1.5 - 7]</td>
<td></td>
</tr>
<tr>
<td><strong>Peribronchial</strong></td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[0 - 3.25]</td>
<td>[0.75 - 5.25]</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>12</td>
<td>15.5</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>[5.50 - 20.75]</td>
<td>[7.25 - 24.75]</td>
<td></td>
</tr>
</tbody>
</table>

Table summarizes the number of new densities in the dependent and non dependent lung regions expressed as median – interquartile range. The total number of new lesions was analyzed with a mixed model on the ranked number of lesions using as fixed effects the density localization (subpleural/parenchymal/peribronchial) and the spatial localization as dependent/non dependent and apex/hilum/base. The subpleural/parenchymal/peribronchial localization was highly significant (p<0.0001) as it was the apex/hilum/base localization while the dependent/non dependent localization was not significant (p=0.11), but the interaction term between subpleural/parenchymal/peribronchial was significant (p=0.0057); the other interaction terms were not significant.
Table 6 – Hemodynamic variables

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Baseline CT-scan (Time 0)</th>
<th>Last CT-scan without new densities (Time 1)</th>
<th>First CT-scan with new densities (Time 2)</th>
<th>Last CT-scan with distinguishable densities (Time 3)</th>
<th>First CT-scan with one-field edema (Time 4)</th>
<th>First CT-scan with all-field edema (Time 5)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (hours)</td>
<td>0±0</td>
<td>5.7±6.5</td>
<td>8.4±6.3</td>
<td>15±12</td>
<td>18±11</td>
<td>20±11</td>
<td></td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>92±19</td>
<td>87±17</td>
<td>93±16</td>
<td>87±22</td>
<td>81±23</td>
<td>82±25</td>
<td>0.34</td>
</tr>
<tr>
<td>Central Venous Pressure (mmHg)</td>
<td>6.3±4.5</td>
<td>6.6±4</td>
<td>7.3±3.6</td>
<td>7.3±3.6</td>
<td>8±3.3</td>
<td>8.1±4.1</td>
<td>0.10</td>
</tr>
<tr>
<td>SvO₂ (%)</td>
<td>75±14</td>
<td>70±13</td>
<td>65±11*</td>
<td>63±8.2*</td>
<td>59±12*</td>
<td>52±14*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arterial-venous oxygen difference (ml/dl)</td>
<td>4±1.6</td>
<td>4.6±1.7</td>
<td>5.3±1.3</td>
<td>5.4±1.2*</td>
<td>5.7±1.2*</td>
<td>7.1±2.3*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Arterial lactates (mmol/l)</td>
<td>1.9±1</td>
<td>1.9±1.1</td>
<td>1.8±1.1</td>
<td>1.6±0.94</td>
<td>1.4±0.78</td>
<td>2.2±1.6</td>
<td>0.39</td>
</tr>
<tr>
<td>Cumulative fluid intake (ml)</td>
<td>1358±790</td>
<td>2134±1753</td>
<td>2451±1694*</td>
<td>3425±2312*</td>
<td>3812±2293*</td>
<td>4087±2382*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cumulative urinary output (ml)</td>
<td>788±823</td>
<td>1254±1283</td>
<td>1600±1301*</td>
<td>2451±1564*</td>
<td>2669±1556*</td>
<td>3091±2064*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cumulative fluid balance (ml)</td>
<td>570±507</td>
<td>880±730</td>
<td>851±720</td>
<td>974±974</td>
<td>1143±1109</td>
<td>996±1032</td>
<td>0.10</td>
</tr>
<tr>
<td>Vasopressors (number of pigs/total number of pigs)</td>
<td>0/12</td>
<td>0/12</td>
<td>0/12</td>
<td>2/12</td>
<td>2/12</td>
<td>5/12</td>
<td>0.06 †</td>
</tr>
</tbody>
</table>

* p < 0.05 vs baseline (Time 0) † Fisher's exact test.
Figure 12 presents a CT scan taken at Time 4 with three images showing the 6 lung fields (apex dependent/non dependent, hilum dependent/non dependent and base dependent/non dependent). As shown at the basis lung fields are not inflated.
Reference List


