ORIGINAL ARTICLE

WHAT IS HAPPENING IN THE ARDS PIGLET LUNGS: - THE ORIGIN OF B-LINES ON ULTRASONOGRAPHY -

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Abstract  Background: B-lines are the important signs of pulmonary edema in acute respiratory distress syndrome (ARDS) detected with ultrasonography. We studied the mechanism of the origin and disappearance of B-lines in ARDS piglets using ultrasonography and microscopy.

Methods: ARDS was induced by intratracheal administration of 0.1M Hydrochloric Acid (HCl) in 23 anesthetized, ventilated piglets. The occurrence of B-lines was evaluated using transthoracic echography (TTE) and transesophageal echography (TEE). Changes in B-lines were recorded after a saline infusion into the pleural space, and after pulmonary consolidation developed and reached the pleura. Left lower lobe was excised and lung sections were examined by light microscopy.

Results: Following the saline infusion into the pleural space, we observed B-lines fanning out from the visceral pleura. As pulmonary consolidation developed, B-lines disappeared. The structure that B-lines fanned out from was the complex of thickened pleura and sub-pleural interlobular septa surrounded by gas. In the tissue where B-lines disappeared, the thickened sub-pleural interlobular septa were surrounded by cells and liquid.

Conclusions: We found that B-lines derive from the complex of thickened pleura and sub-pleural interlobular septa surrounded by gas. B-lines disappeared when the sub-pleural thickened interlobular septa were surrounded by cells and liquid.

Key words: B-lines; ARDS; TTE; TEE.

Introduction

Among the different forms of respiratory or circulatory distress, there are several clinical statuses that need immediate intensive care. Early diagnosis being essential for favorable, survival rates, prompt and reliable imaging methods are required. Ultrasonography has several advantages as an imaging tool. It is a radiation-free, less-invasive and easy-to-use technology which can be used at bedside. At present, ultrasonography is used to evaluate thoracic abnormalities such as pleural effusion, pneumothorax, and acute respiratory distress syndrome (ARDS).

B-lines are considered important signs of ARDS detected with ultrasonography. B-lines are defined as discrete laser-like vertical hyperechoic reverberation artifacts that arise from the pleural line (previously described as "comet-tail artifact"), extend to the bottom of the screen without fading, and move synchronously with lung sliding\(^1\). The detection of B-lines is clinically important because they can show the locality and severity of pulmonary edema in ARDS patients at the bed side\(^2\). The presence of B-lines on ultrasonography prompts clinicians to initiate therapy to alleviate pulmonary edema. Use of ultrasonography in diagnosis of interstitial syndrome (pulmonary edema caused by ARDS included this syndrome) is likely to improve the care of patients in whom this

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diagnosis is possible\(^1\).

The origin of B-lines has been investigated with computed tomography (CT) and chest radiography. It is believed that the B-lines come from water-thickened interlobular septa\(^3\). However, the ability of CT and chest radiography to analyze small amounts of tissues is limited and B-lines originate from the complex of pleura and interlobular septa in our observation using an ARDS animal model. B-lines are well known to correlate with pulmonary artery wedge pressure, thoracic bioelectrical impedance, B type natriuretic peptides, and lung weight and density obtained from CT\(^4\).

The consolidated region of the lung is visualized with lung ultrasound as an echo-poor or tissue like image, depending on the extent of air loss and fluid predominance, which is clearly different from the normal pattern\(^1\). B-lines disappeared after the development of consolidation. There is no precise explanation for the disappearance of B-lines. Therefore, we studied their origin and the effects of consolidation on B-lines with ultrasonography and light microscopy.

Positive end-expiratory pressure (PEEP) is employed to improve oxygenation in patients with ARDS and other abnormalities. PEEP has the potential to reduce the size of complexes of thickened pleura and subpleural interlobular septa. If the number of B-lines observed decreases after PEEP application, this supports the assumption that the complex of thickened pleura and sub-pleural interlobular septa is involved in the production of B-lines. We also evaluated the change in extravascular lung water volume (EVLW) during PEEP application.

**Methods**

The experiment was performed in accordance with Guidelines for Animal Experimentation, Hirosaki University. A total of 23 piglets of both sexes weighing 25–30 kg were used in this study. Figure 1 shows the allocation of the 23 piglets to each examination. The animals were anesthetized with intramuscular ketamine 20 mg/kg and intubated with a 7 mm tracheal tube. Anesthesia was maintained with propofol and pancuronium. Ringer’s lactate was intravenously infused at a rate of 7 mL/kg/h. Ventilation was begun (Servo-D respirator, Siemens-Elema, Stockholm, Sweden) at a tidal volume of 10 mL/kg, respiratory rate 20–25 breaths/min, F\(_{1O2}\) 0.4 and PEEP 0 cm H\(_2\)O.

Ultrasonography was performed with the SONOS 7500 ultrasound system (Philips, Amsterdam, The Netherlands) equipped with a 5 MHz 64-element transesophageal multiplanar probe and a 3.5 MHz phased array transthoracic probe. Two-dimensional images were used to observe B-lines. Vertical artifacts that fanned out from the pleural line spreading to the edge of the screen were considered B-lines when observed by both transesophageal echography (TEE) and transthoracic echography (TTE).

We found two windows for B-lines detection with TEE. One was the superior vena cava for observing the right pleura, and the other was the descending aorta for observing the left pleura. To study the effect of PEEP, we used two windows of the descending aorta at the mitral position and the superior vena cava. To study the effect of saline infusion and development of consolidation, we used the window of the descending aorta at the mitral position to observe the left lung. In TTE observation, we observed the change of the number of B-lines from the left anterior axillar. In both TEE and TTE we observed the number of B-lines on one screen.

A dose of 0.1M HCl (1 ml/kg) was administered through the endotracheal tube with a fiberoptic bronchoscope as a guide. One hour later, after B-lines were observed, saline solution (10 mL/kg) was injected into the left pleural space
(n=7) to clarify the role of the parietal and visceral pleura in the formation of B-lines. In another five piglets, B-lines were observed until consolidation developed and reached the pleura (n=5). Because pulmonary consolidation replaces gas, this procedure was designed to show the effect of gas and fluid on B-lines.

Histological examination of the left lower lung was performed in a control group (n=2), in another group after B-lines development (n=2) and in a third group after consolidation developed (n=5). Specimens were placed into 10% neutral buffered formalin for 24 hours at 4°C. The excised specimens were stained with hematoxylin and eosin using the standard protocol.

Next, we evaluated the effect of PEEP on the formation of B-lines using another seven piglets (n=7), employing the commercially available single thermal indicator-dilution technique system (PiCCO, Pulsion Medical Systems AG, Munich, Germany) to determine EVLW and cardiac output (CO). A central venous catheter and a 4 Fr femoral arterial catheter with a thermistor tip were inserted for measurement. Cold saline (8°C, 10 ml) was injected into the central venous catheter. The change in downstream temperature in the abdominal aorta was measured by the thermistor.

PEEP (5, 10, 15 and 20 cm H$_2$O) was applied every three minutes under controlled mechanical ventilation in seven 0.1 M HCl-exposed piglets after B-lines appeared. Changes in the number of B-lines within the scanned area were investigated using a 5 MHz 64-element transesophageal multiplanar probe and 3.5 MHz transthoracic echo probe. Changes in systolic blood pressure, CO and EVLW were compared at PEEP 0 cm H$_2$O and 20 cm H$_2$O.

A paired t-test was used for statistical analysis of the change in the number of B-lines before and after saline infusion in the pleural space. It was also used to analyze changes in blood pressure, CO and EVLW between PEEP 0 cm H$_2$O and PEEP 20 cm H$_2$O. One-way analysis of variance and the Newman–Keuls
post hoc test for multiple comparisons were used for other analyses. P < 0.05 was considered statistically significant. All values are expressed as the mean ± SD with their corresponding 95% confidence intervals (CIs). The power of this study was calculated using the GPower analysis program (http://www.gpower.hhu.de/). The power was above 0.8.

Two observer determined the presence of B-lines. Observers were blinded to the experimental stage of the animal. Interobserver and intraobserver variability were evaluated in 60 randomly selected observation. Bland-Altman analysis was used to compare observers’ measurement. The mean difference between the repeated measurements on the same subjects by the same observer was small (mean 0.119, 95% CI: 0.055, 0.200). The mean difference between the repeated measurements on the same subjects by the different observer was also small (mean 0.118, 95% CI: 0.063, 0.280).

**Results**

After HCl instillation, both TEE and TTE detected the appearance of B-lines. We found two windows for B-lines detection with TEE: the superior vena cava for observing the right pleura, and the descending aorta for observing the left pleura. TEE images of the pleura were similar to, but clearer than the TTE images.

**Impact of saline-induced pleural effusion on B-lines:**

With TEE, through the descending aorta window the saline solution can be seen separating the pleural layers into parietal and visceral components. We found B-lines fanning out from the visceral pleura. The number of B-lines decreased from 3.86 ± 0.26 (before saline infusion) to 0.71 ± 0.28 after saline infusion (p < 0.001, 95% CI, 2.31 to 3.91). After the consolidation developed and reached the
pleura, B-lines disappeared (Figure 2).

**Histologic findings:**

In the lung section in which B-lines were seen to be fanning out, the only structure that could produce B-lines was the complex of thickened pleura and sub-pleural interlobular septa surrounded by gas. The thickness of the complex increased from 0.7 mm to more than 1.0 mm. The lung section showing that the B-lines had disappeared after consolidation had reached the sub-pleural parenchyma, also showed that the thickened interlobular septa were surrounded by cells and fluid (Figure 3).

**Impact of PEEP on the number of B-lines, hemodynamic indices and EVLW:**

Increasing PEEP from 0 cm H₂O to 20 cm H₂O changed the number of B-lines, hemodynamic indices and EVLW (Table 1). Systolic blood pressure decreased from 121.9 ± 3.5 mmHg to 87.3 ± 4.7 mmHg ($p < 0.001$, 95% CI, 29.71 to 39.41); CO decreased from 3.34 ± 0.19 L/min to 2.61 ± 0.24 L/min ($p < 0.001$, 95% CI, 0.57 to 1.00); and EVLW increased from 321.1 ± 13.1 ml to 351.1 ± 10.6 ml ($p < 0.01$, 95% CI, -47.40 to 17.59). However, PaO₂ and P/F ratio were 128.6 ± 38.7, 321.6 ± 96.9 at PEEP 0 cm H₂O and 125.4 ± 46.1, 313.5 ± 115.4 at PEEP 20 cm H₂O respectively. PaO₂ and P/F ratio did not change significantly during PEEP application. When PEEP increased, the number of B-lines decreased. In the descending aorta window, the number decreased from 3.42 ± 0.53 to 0.28 ± 0.48 ($p < 0.05$, 95% CI, 2.79 to 3.48) and in the superior vena cava window they decreased from 2.55 ± 0.30 to 0.33 ± 0.51 ($p < 0.05$, 95% CI, 1.06 to 2.94) (Figure 4). TTE imaging through the left lateral chest wall indicated that the number of B-lines decreased from 3.57 ± 0.53 to 0.71 ± 0.48 ($p < 0.05$, 95% CI, 2.50 to 3.20).
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<th>PEEP (cm H₂O)</th>
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<tr>
<td>Number of B-lines (TEE: Descending Aorta)</td>
<td>3.42 ± 0.53</td>
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<td>Number of B-lines (TEE: SVC)</td>
<td>2.55 ± 0.30</td>
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<tr>
<td>Number of B-lines (TEE: Left lateral chest wall)</td>
<td>3.57 ± 0.53</td>
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<tr>
<td>Systolic Blood pressure (mmHg)</td>
<td>121.9 ± 3.53</td>
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<tr>
<td>Cardiac Output (L/min)</td>
<td>3.34 ± 0.19</td>
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<tr>
<td>EVLW (ml)</td>
<td>321.1 ± 13.1</td>
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*p<0.05  
**p<0.01

**Figure 4** Effects of PEEP on the number of B-lines observed with TEE. The graph and pictures show the changes of the number of B-lines observed with TEE from the SVC window or the descending aorta window. The number of B-lines decreased as PEEP increased. Arrows indicate B-lines observed with TEE. TEE: transesophageal echography, SVC: superior vena cava.

**Discussion**

B-lines are defined as discrete laser-like vertical hyperechoic reverberation artifacts that arise from the pleural line (previously described as “comet-tail artifact”), extend to the bottom of the screen without fading, and move synchronously with lung sliding. B-lines move with the lung sliding, spreading to the edge of the screen without fading. Reverberation occurs when there is a marked difference in acoustic impedance between a thick object and its surroundings. However, the thickness of the structure that creates B-lines is unknown. Lichtenstein et al suggested the diseased septum has a width of 0.7 mm, which remains under the power of ultrasonography but allows the generation of B-lines.

Based on CT analysis, Lichtenstein et al. reported that B-lines derive from thickened interlobular septa. Generally, the smallest size visible on CT ranges from 0.3 to 0.5 mm in
diameter. Normal sub-pleural interlobular septa have a thickness of about 0.1 mm. Therefore, CT does not seem to be an appropriate tool to investigate the origin of B-lines. One report explains the origin of B-lines using microscopic imaging. The illustration provided did not contain the pleura, however, which is one of the most important factors in the creation of the artifacts.

Smargiassi et al. suggested the possible “generator” of B-lines as follows: 1) single or multiple interlobular and/ or intra lobular septa thickening; 2) increase of lung parenchymal water content (increased density); 3) increase of “tissue” content of the lung parenchyma (increased density); 4) diminution of ventilation and porosity due to any cause (increased density); 5) micronodules, pleural irregularities and sub-pleural microconsolidations. In the lung section where B-lines could be seen to be fanning out, the only structure that could have produced B-lines was the complex of thickened pleura and sub-pleural interlobar septa surrounded by gas. These thickened pleura and sub-pleural interlobar septa were connected and appeared to be the same tissue that was observed with microscopy. After an injection of saline, B-lines were found to have derived from the sub-pleural area under the visceral pleura. However, the number of B-lines had decreased. Although the parietal pleura is not essential for B-lines formation, a thickened parietal pleura increases the number of B-lines by increasing the thickness of the pleural complex.

After consolidation development reaches the sub-pleural parenchyma, B-lines disappear. Consolidation occurs when the permeability of the membrane increases because of direct or indirect lung injury, resulting in a marked increase in the amount of cells, liquid and protein leaving the vascular space. Smargiassi et al. used a contrast medium and classified the consolidation pattern as 1) inflammatory consolidation, 2) consolidation associated with pulmonary embolism, 3) atelectasis, 4) neoplasm. Our observed consolidation corresponded to inflammatory consolidation. Without gas in the alveoli, there is no difference in acoustic impedance between the alveoli and the septa. After consolidation reaches the sub-pleura, the parenchyma becomes dense, and the complex of thickened pleura and sub-pleural interlobar septa can no longer produce B-lines.

TEE is used to observe the heart and vessels. It is also used to investigate pulmonary diseases such as pleural effusion, atelectasis, and ARDS. TEE can examine the mediastinal pleura, which cannot be observed by TTE. By combining TTE and TEE, it is possible to observe a wider area of the pleural membrane. In this study using piglets, major vessels appeared to be intact and did not affect the number of B-lines. However, in clinical situations, the vessels themselves have been reported to cause reverberation because of calcification in the aortic wall or insertion of vascular catheters.

In this study, PEEP decreased the number of B-lines that could be seen in the scanned area. The number of B-lines began to decrease within one second of PEEP application. EVLVW measured with the PiCCO system increased after PEEP 20cmH2O application. Therefore, one possibility is that increased EVLW reduced the number of these complexes surrounded by gas and reduced the number of B-lines. This supports the involvement of the complex of thickened pleura and sub-pleural interlobar septa surrounded by gas in producing the artifacts.

**Limitations**

In both TEE and TTE, we observed the number of B-lines appearing on one screen. Observing the number of B-lines on one screen lacks the information provided by three dimen-
sional viewing. Volpicelli et al. and Rademacher et al. recommended scanning eight regions of the thorax to observe the number of B-lines in humans and dogs, respectively, with TTE\(^2\). However, scanning many regions was not adequate to observe the rapid change of the number of B-lines caused by PEEP in our experiment. In addition, no method has yet been recommended to observe the number of B-lines with TEE.

CO is one of the major factors for calculating EVLW using the PiCCO system. Various studies have found good correlation in CO measurement with the PiCCO system and pulmonary artery catheter. However, Schöglohofer et al. reported that CO measurement based on PiCCO shows only limited agreement with intermittent bolus thermodilution during hemodynamic instability\(^3\). Therefore, there is a possibility that EVLW measured by PiCCO during PEEP application does not truly reflect EVLW.

**Conclusion**

This study performed ultrasonic, hemodynamic and histo-pathological studies to determine origin of B-lines. B-lines consist of the complex of thickened pleura and sub-pleural interlobular septa surrounded by gas. Consolidation erases B-lines by eliminating the gas around the interlobular septa. PEEP thus reduces the number of B-lines.

**References**


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