Original Article
Ultrasound reaeration scores to assess lung recruitment in rabbits with acute respiratory distress syndrome

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Abstract: Objective: The aim of this study was to establish rabbit models of acute respiratory distress syndrome (ARDS) and evaluate positive end-expiratory pressure (PEEP)-induced lung recruitment by ultrasound reaeration scores (US-RAS). Methods: Twenty rabbits were randomly assigned into two groups, a maximum oxygenation (OXY) group and lung ultrasound score (LUS) group. Both groups used the stepwise incremental PEEP method of lung recruitment but with different recruitment endpoints. The endpoint defined by OXY group was the sum of PaO₂ and PaCO₂ greater than 400 mmHg, while the LUS group endpoint was a new ultrasound reaeration score calculated by measuring ultrasound changes. The hemodynamics, respiratory mechanics indexes, ultrasound reaeration scores, Smith pathologic score and wet/dry ratio were compared between the groups. Results: Both groups achieved the endpoint of lung recruitment. PEEP (19.5 ± 1.9 mmHg VS. 14.6 ± 1.2 mmHg, p<0.05), arterial oxygen pressure (512 ± 47.6 mmHg VS. 424 ± 57.6 mmHg, p<0.05), peak airway pressure (24.6 ± 2.1 cmH₂O VS. 17.4 ± 1.7 cmH₂O, p<0.05), and US-RAS (14.8 ± 2.4 points VS. 7.1 ± 1.7 points, p<0.05), at the endpoint of lung recruitment, were higher in the LUS group than the OXY group. However, pathological scores and wet/dry ratios were not significantly different between the two groups. Conclusion: A small comet tail artifact was the earliest ultrasonic manifestation of ARDS. PEEP-induced lung recruitment can be adequately assessed by ultrasound. Compared with the maximum oxygenation-guided method, ultrasound is used to guide the choice of higher lung opening pressure to improve oxygenation levels and pulmonary ventilation.

Keywords: Acute respiratory distress syndrome, lung ultrasonography, lung recruitment, ultrasound reaeration scores, positive end expiratory pressure (PEEP)

Introduction

According to the Berlin Definition [1], acute respiratory distress syndrome is an acute diffuse pulmonary inflammatory response that can increase pulmonary vascular permeability and reduce ventilated lung tissue. Pathological characteristics of the acute phase of ARDS include diffuse alveolar injury, such as alveolar edema, as well as inflammatory response, hyaline membrane formation, and bleeding. ARDS is a common disease with high morality in intensive care units (ICU) due to multiple risk factors. Currently, respiratory support is the main therapy. Recent “protective pulmonary ventilation” strategies, including low tidal volume and positive end expiratory pressure (PEEP), have aimed to recruit collapsed alveoli, decrease pulmonary edema, correct refractory hypoxemia, and improve alveolar oxygenation. However, inappropriate mechanical ventilation may exacerbate lung injuries, thus, maintaining appropriate PEEP is crucial. Clinically, methods for evaluating lung recruitment guided by PEEP include pulmonary CT and static P-V curve. However, these methods are limited by transfer or deep sedation and muscular relaxation [2]. Maximal oxygenation-guided recruitment is commonly used with PaO₂ + PaCO₂ ≥ 400 mmHg as the endpoint of adequate recruitment [3]. Although the maximum oxygenation method can measure arterial oxygenation, it is limited by factors other than alveolar recruitment, such as cardiac output, vasoactive agents, and hypoxic pulmonary vasoconstriction. Most importantly, the maximum oxygenation method cannot evaluate alveolar morphology and respiratory mechanics.

In the past, gas-rich lungs were thought to be a restricted area for ultrasound. However, the lungs are an organ composed of a combination
of gas and liquid. Normal alveoli are filled with gas but the gas-liquid content in lung tissue changes with lung disease, making it possible to observe lung disease using ultrasound. Lung ultrasound is a useful technique for diagnosis and evaluation of disease based on different ultrasound signs caused by real-time changes in gas content in the lungs. Gardelli first reported monitoring of lung recruitment via ultrasound in 2009 [4]. In 2011, Bouhemad proposed that ultrasound reaeration scores (US-RAS), under the guidance of PEEP, could be evaluated by different pulmonary ultrasonic manifestations [5].

This present study sought to monitor and evaluate the process of PEEP-induced lung recruitment using a real-time lung ultrasound reaeration scoring method to aid in selecting the best recruitment endpoint. This study aimed to provide a theoretical basis for clinical application of ultrasound-guided lung recruitment.

Materials and methods

Rabbits

Twenty healthy adult rabbits (n = 20) were raised in the First Affiliated Hospital of Harbin Medical University. There were 8 males and 12 females, with weights ranging from 2.0 to 3.5 kg. All rabbits had access to rabbit food ad libitum. Ultrasonography showed no abnormalities before establishment of the rabbit model of ARDS. Rabbits were weighed and 1% sodium pentobarbital, at 20 mg/kg, was injected into the ear vein. After anesthesia, the rabbits were made to remain in the supine position. After skin shaving of the neck and chest, tracheotomy was performed. A 3.5# trachea cannula was inserted and connected to an animal ventilator (RWD Lift Science Co, RWD407). Tidal volume (VT) was 6 ml/kg, respiratory rate (RR) was 40 bpm, inspiration expiration ratio (I:E) was 1:1, fraction of inspired oxygen (FIO₂) was 1.0, and positive end expiratory pressure (PEEP) was 0 cmH₂O. An indwelling catheter for blood gas analysis was inserted in the right internal carotid artery and closed with a heparin cap. Extremities were connected to an electrocardiogram (ECG) monitor. Sodium pentobarbital and pancuronium bromide were provided to maintain sedation and muscular relaxation.

Table 1. Lung injury scores

<table>
<thead>
<tr>
<th>Abnormal ultrasonic manifestation</th>
<th>Ultrasound lung injury scores (points)</th>
<th>Anterior Region</th>
<th>Middle Region</th>
<th>Posterior Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

B1: sparse B lines (moderate loss of lung aeration); B2: diffuse B lines (severe loss of lung aeration); C: lung consolidation; N: normal lung manifestations (normal lung aeration). Score of each animal is the sum of scores of the 12 regions.

Table 2. Lung reaeration scores

<table>
<thead>
<tr>
<th>1 point</th>
<th>3 points</th>
<th>5 points</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1→N</td>
<td>B2→N</td>
<td>C→N</td>
</tr>
<tr>
<td>B2→B1</td>
<td>C→B1</td>
<td>C→B2</td>
</tr>
</tbody>
</table>

B1: sparse B lines; B2: diffuse B lines; C: lung consolidation; N: normal lung manifestations. The left and right sides of the (→) represent the ultrasonic manifestation before and after lung recruitment. Each lung region was scored according to the worst signs of gasification and the final score was the sum of scores of the 12 lung regions.
Rabbit model of ARDS

Oleic acid (0.1 ml/kg) was injected into each rabbit’s ear vein at a slow and constant rate. Timing began after injection of oleic acid. Pulmonary manifestations were observed via ultrasonography. Blood gas analysis (GEM PREMIRE3000, US) was performed every 30 minutes. The rabbit model of ARDS was established successfully by injection of oleic acid if oxygenation index (OI: PaO₂/FiO₂) was lower than 100 mmHg.

Ultrasoundography

An ultrasound instrument (MyLab30, ESAOTE S.P.A.) with a 3.5-MHz probe was used for this study. The sagittal plane of the chest was divided into left and right parts. Each part was divided into 3 regions from anterior to posterior and each region was further divided into 2 regions (superior and inferior), resulting in 12 regions (Figure 1). According to lung ultrasound injury scores (Table 1) and lung ultrasound reaeration scores (US-RAS) (Table 2), each lung region was examined by ultrasound. Ultrasonic manifestations of the lungs were defined according to four types: (1) B1: sparse B lines; (2) B2: diffuse B lines; (3) C: lung consolidation; and (4) N: normal lung manifestations. Each lung region was scored according to the worst signs of gasification. The final score was the sum of scores of the 12 lung regions.

Methods

Animals were randomly divided into two groups according to different lung recruitment maneuvers: maximal oxygenation guided lung recruitment group (OXY group) and ultrasound reaeration score guided group (LUS group).

In the OXY group, the PEEP value of the ventilator was increased from 5 cmH₂O with an increment of 2 cmH₂O each time. After 10 minutes of stabilization, arterial blood gas and lung US-RASs were analyzed. When results of blood gas analysis showed that PaO₂ plus PaCO₂ was greater than 400 mmHg, the collapsed alveoli were considered to be recovered, achieving lung recruitment endpoint. In the LUS group, the same incremental PEEP method, described above, was used with an increase in the ventilator from 5 cmH₂O and an increment of 2 cmH₂O each time. After 10 minutes of stabilization,
arterial blood gas and lung US-RASs were analyzed. Ultrasonic manifestation at different PEEP levels was compared with that before recruitment (PEEP = 0 cmH\textsubscript{2}O, ZEEP). According to scores shown in Table 2, when lung reaeration scores no longer increased, the lungs were considered to be fully recruited, achieving lung recruitment endpoint.

For this experiment, hemodynamics and respiratory mechanics indexes were recorded at different PEEP levels. In addition, ultrasonic manifestation and reaeration scores of each lung region of the rabbits were recorded under different PEEP levels.

Pathological examination

After the two groups of rabbits reached endpoint of recruitment, they were maintained for 20 minutes. The rabbits were killed, the left lung was removed, and surface water was drained and weighed with an electronic balance. This was recorded as the wet weight. Next, the left lung was placed in an oven until constant weight was achieved. This was recorded as the dry weight, to calculate the wet/dry ratio of the left lung. Smith pathological scoring system was used to evaluate pathological injuries of the right lung. The right lower lobe tissue was divided into three lung regions from anterior to posterior, fixed with 10\% formaldehyde solution, embedded in paraffin, and stained with hematoxylin-eosin (HE). Semi-quantitative scores were derived based on pulmonary edema, interstitial inflammation, alveolar hemorrhage, pulmonary atelectasis, and hyaline membrane formation. For each lung region, 10 high-magnification images were evaluated. The average value was calculated as the final score [6] (Table 3).
US-RAS to assess lung recruitment in rabbits with ARDS

shows ultrasonic manifestations in 216 regions. At the endpoint of lung recruitment, the two groups were able to meet corresponding recruitment endpoints. The PEEP level of the LUS group reached 19.5 ± 1.9 mmH₂O while that of the OXY group was 14.6 ± 1.2 mmH₂O. After lung recruitment, peak airway pressure (LUS group: 24.6 ± 2.1 cmH₂O, OXY group: 17.4 ± 1.7 cmH₂O, p <0.05) and oxygen partial pressure (LUS group: 512 ± 47.6 mmHg, OXY group: 424 ± 57.6 mmHg, p <0.05) in the LUS group were significantly higher than those in the OXY group (Figure 3A, 3B). There were also significant differences in lung reaeration scores (LUS group: 14.8 ± 2.4 points, OXY group: 7.1 ± 1.7 points, p <0.05) between the two groups. With an increase in PEEP levels, the two groups were able to get higher ultrasound reaeration scores (Figure 3C, 3D). Comparisons of other indexes are shown in Table 4. As shown in Figure 4, a statistically significant correlation was found between lung reaeration scores and oxygenation index (PaO₂/FiO₂).

Statistical analysis

SPSS 19.0 software was used for statistical analyses. Measurement data are presented as mean ± SD. Data was analyzed by Student’s t-test or one-way analysis of variance (ANOVA) to determine any differences between groups. P values <0.05 were considered statistically significant.

Results

Establishment of an ARDS model

Two rabbits died during anesthesia, due to rapid injection of anesthetics, while 18 rabbits met the criteria for establishment of an ARDS model. The normal A line became a small comet tail artifact perpendicular to the pleura in 18 rabbits during initial injection of oleic acid. This occurred first in the posterior lung region at a mean time of 5.87 ± 2.32 minutes. Subsequently, sparse comet tail artifacts (B1) and diffuse comet tail artifacts (B2) gradually appeared. After establishment of the ARDS model, mean lung injury score of the 18 rabbits was 26.38 ± 2.34 points. Anterior lung regions, in rabbits, are mainly characterized by B1-B2 lines, indicating moderate-severe loss of lung aeration. Middle and posterior lung regions are characterized by diffuse B2 lines and lung consolidation, indicating very severe loss of lung aeration. Figure 2 shows ultrasonic manifestations in 216 regions.

At the endpoint of lung recruitment

After using PEEP to begin lung recruitment, the two groups were able to meet corresponding recruitment endpoints. The PEEP level of the LUS group reached 19.5 ± 1.9 mmH₂O while that of the OXY group was 14.6 ± 1.2 mmH₂O. After lung recruitment, peak airway pressure (LUS group: 24.6 ± 2.1 cmH₂O, OXY group: 17.4 ± 1.7 cmH₂O, p <0.05) and oxygen partial pressure (LUS group: 512 ± 47.6 mmHg, OXY group: 424 ± 57.6 mmHg, p <0.05) in the LUS group were significantly higher than those in the OXY group (Figure 3A, 3B). There were also significant differences in lung reaeration scores (LUS group: 14.8 ± 2.4 points, OXY group: 7.1 ± 1.7 points, p <0.05) between the two groups. With an increase in PEEP levels, the two groups were able to get higher ultrasound reaeration scores (Figure 3C, 3D). Comparisons of other indexes are shown in Table 4. As shown in Figure 4, a statistically significant correlation was found between lung reaeration scores and oxygenation index (PaO₂/FiO₂).

Ultrasonic manifestations

In terms of ultrasonic images, the major improvement in the OXY group was that the diffuse B2 line in the anterior and middle lung regions improved to the B1 line, indicating mod-

### Table 4. Hematology and respiratory mechanics parameters in the OXY and LUS groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ZEEP (cmH₂O)</th>
<th>OXY (cmH₂O)</th>
<th>LUS (cmH₂O)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEEP</td>
<td>0.0 ± 0.0</td>
<td>14.6 ± 1.2</td>
<td>19.5 ± 1.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>VT (ml)</td>
<td>20.6 ± 1.9</td>
<td>16.4 ± 2.6</td>
<td>17.8 ± 1.8</td>
<td>0.25</td>
</tr>
<tr>
<td>Ppeak (cmH₂O)</td>
<td>13.8 ± 2.6</td>
<td>17.4 ± 1.7</td>
<td>24.6 ± 2.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>203 ± 19</td>
<td>187 ± 23</td>
<td>191 ± 20</td>
<td>0.58</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>76 ± 11.2</td>
<td>83 ± 17</td>
<td>81 ± 13</td>
<td>0.43</td>
</tr>
<tr>
<td>EtCO₂ (mmHg)</td>
<td>17.6 ± 2.4</td>
<td>24.5 ± 2.8</td>
<td>21 ± 1.9</td>
<td>0.67</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>46.7 ± 4.6</td>
<td>45.4 ± 4.8</td>
<td>50.3 ± 5.3</td>
<td>0.48</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>57.9 ± 19.4</td>
<td>424 ± 57.6</td>
<td>512 ± 47.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>US-RAS (point)</td>
<td>2.3 ± 1.4</td>
<td>7.1 ± 1.7</td>
<td>14.8 ± 2.4</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

RM: recruitment maneuver; ZEEP: before recruitment maneuver, PEEP = 0 cmHg; PEEP: positive end expiratory pressure; VT: tidal volume; Ppeak: Peak airway pressure; HR: heart rate; MAP: mean arterial pressure; EtCO₂: end-tidal partial pressure of carbon dioxide; PaCO₂: arterial carbon dioxide partial pressure. PaO₂: arterial oxygen pressure; US-RAS: ultrasound re-aeration score; *Data was statistically significant.
US-RAS to assess lung recruitment in rabbits with ARDS

Rationale.

Lung recruitment is necessary to improve oxygenation and prevent ventilation/perfusion mismatch, whereas conventional mechanical ventilation cannot always achieve this goal. Therefore, new methods for lung recruitment are needed.

Methods.

In this study, rabbits were randomly divided into the LUS group and the ZEEP group. Ultrasound was used to identify lung recruitment, and pathological examination was used to evaluate lung condition.

Results.

The LUS group showed better A1 line and B1 line than the ZEEP group. Furthermore, pathological examination showed that the LUS group had better lung condition than the ZEEP group.

Discussion.

Lung recruitment is necessary to improve oxygenation and prevent ventilation/perfusion mismatch. Ultrasound can be used to identify lung recruitment, and pathological examination can be used to evaluate lung condition. The LUS group showed better A1 line and B1 line than the ZEEP group. Furthermore, pathological examination showed that the LUS group had better lung condition than the ZEEP group.
scores of pulmonary reaeration, based on changes of air and water content in alveoli, could be used to evaluate the effects of PEEP on lung recruitment [15, 16].

Intravenous injection of oleic acid is currently the recognized method for inducing exogenous ARDS [17]. Oleic acid can increase the permeability of endothelial cells and block Na⁺ channels to promote accumulation of fluid in the alveoli and alveolar space, leading to ARDS [18]. After injection of oleic acid, the small comet tail artifact appears first from the hyper-echoic arc perpendicular to the pleura and stops in the first horizontal artifact behind the pleura. This study showed that the small comet tail artifact appeared 5 minutes after injection of oleic acid, first in the posterior lower lung region. The small comet tail artifact was the first ultrasonic manifestation in ARDS and could serve as the early ultrasonic manifestation of ARDS [19]. During establishment of ARDS, the comet tail artifact in the rabbit lungs progressed from anterior to posterior and from upper to lower with decreased oxygenation levels. Lung consolidation also appeared [20].

This dynamic process was associated with decreased oxygenation levels and consistent with heterogeneous distribution of pathological injuries in ARDS. Thus, it was dependent on gravity [21]. During lung recruitment, ultrasonography showed heterogeneous pulmonary injuries of ARDS. Disappearance of B lines was mainly in anterior and upper region, while consolidation in posterior region was less impacted by PEEP. This indicates that recruitment was easier in anterior and upper regions than in posterior or lower regions, which were impacted by gravity. This was consistent with CT evaluation of lung recruitment in ARDS [22] and also consistent with the fact that lung injuries, in dif-

Figure 6. Example of dynamic changes in lung ultrasound manifestations in one posterior lower lung region during recruitment. The upper panel shows normal A line was changed into “insect eroding” lung consolidation after intravenous injection of oleic acid. The lower panel shows that the area of lung consolidation was decreased gradually and became B2 line with increased PEEP. Arrowhead indicates the small comet tail artifact.

Figure 7. Pathological manifestations of the anterior, middle, and posterior lung regions of an ARDS model rabbit of LUS group. A. Anterior lung region. Smith score: 2 points. B. Middle lung region. Smith score: 5 points. C. Posterior lung region. Smith score: 8 points.
ferent regions, progressed from anterior to posterior regions in both groups.

Animals in both groups underwent a lung recruitment maneuver with increasing PEEP guided by maximum oxygenation or lung ultrasonography. All animals achieved the endpoint of recruitment. PEEP at the endpoint of recruitment was lower in the OXY group. Thus, lung ventilation was not significantly improved while many diffuse B line manifestations were seen in the middle and posterior regions. In contrast, higher PEEP at the endpoint of recruitment under guidance of lung ultrasonography allowed further recruitment of collapsed alveolus by higher airway pressure. In particular, diffuse B lines were significantly less in the middle and posterior regions. Most regions showed normal ultrasonic manifestations or sparse B lines, indicating significantly improved lung ventilation and reaeration scores compared to the OXY group. PaO$_2$ and PaO$_2$ + PaCO$_2$ at the endpoint of recruitment were significantly different between the two groups. However, both Smith pathological scores and wet/dry ratios were not significantly different between the groups. This indicates that higher lung opening pressure under guidance of lung ultrasonography further increased ventilation in ARDS. However, higher lung opening pressure did not cause significant differences in lung compliance or circulatory parameters. In other words, higher PEEPs, under the guidance of ultrasound, would not result in alveolar hyperinflation or further lung injury.

Although the lung ultrasound reaeration score method evaluates lung recruitment in rabbits with ARDS via change in alveolar ventilation, this method was limited by the following: 1) Lung reaeration scores are a semi-quantitative scoring system, progressive scoring was not sufficiently refined, and changes in lung consolidation area during recruitment could not be graded; 2) This study establishes an exogenous ARDS model by intravenous injection of oleic acid and did not include different ARDS models caused by lavage of warm saline, endotoxin, or chest strike. Future studies are necessary for more straightforward, precise, and clinically oriented ultrasonic evaluation.

In conclusion, ultrasonography is a real-time, noninvasive, and reproducible method. This method demonstrates changes in lung ventilation during ARDS and evaluates alveolar reaeration during lung recruitment. This method could further increase lung opening pressure and oxygenation levels, as well as improve lung ventilation, compared to the maximum oxygenation method.

Disclosure of conflict of interest

None.

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