Value of lung ultrasound score for evaluation of blast lung injury in goats

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A B S T R A C T

Purpose: To establish a severe blast lung injury model of goats and investigate the feasibility of lung ultrasound score in the evaluation of blast lung injury.

Methods: Twenty female healthy goats were randomly divided into three groups by different driving pressures: 4.0 MPa group (n = 4), 4.5 MPa group (n = 12) and 5.0 MPa group (n = 4). The severe blast lung injury model of goats was established using a BST-I bio-shock tube. Vital signs (respiration, heart rate and blood pressure), lung ultrasound score (LUS), PaO2/FiO2 and extravascular lung water (EVLW) were measured before injury (0 h) and at 0.5 h, 3 h, 6 h, 9 h, 12 h after injury. Computed tomography scan was performed before injury (0 h) and at 12 h after injury for dynamic monitoring of blast lung injury and measurement of lung volume. The correlation of LUS with PaO2/FiO2, EVLW, and lung injury ratio (lesion volume/total lung volume*100%) was analyzed. All animals were sacrificed at 12 h after injury for gross observation of lung injury and histopathological examination. Statistical analysis was performed by the SPSS 22.0 software. The measurement data were expressed as mean ± standard deviation. The means of two samples were compared using independent-sample t-test. Pearson correlation analysis was conducted.

Results: (1) At 12 h after injury, the mortality of goats was 0, 41.67% and 100% in the 4.0 Mpa, 4.5 MPa and 5.0 MPa groups, respectively; the area of pulmonary hemorrhage was 20.00% ± 15.33% in the 4.5 MPa group and 42.14% ± 15.33% in the 4.5 MPa group. A severe lung shock injury model was established under the driving pressure of 4.5 MPa. (2) The respiratory rate, heart rate, LUS and EVLW were significantly increased, while PaO2/FiO2 was significantly reduced immediately after injury, and then they gradually recovered and became stabilized at 3 h after injury. (3) LUS was positively correlated with EVLW (3 h: r = 0.698, 9 h: r = 0.729; p < 0.05) and lung injury ratio (12 h: r = 0.884, p < 0.05), negatively correlated with PaO2/FiO2 (3 h: r = -0.871, 6 h: r = -0.637, 9 h: r = -0.658; p < 0.05).

Conclusion: We established a severe blast lung injury model of goats using the BST-I bio-shock tube under the driving pressure of 4.5 MPa and confirmed that ultrasound can be used for quick evaluation and dynamic monitoring of blast lung injury.

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Introduction

In recent years, due to the increase of local conflicts, terrorist attacks and accidents in manufacturing and daily life (such as gas explosions at home or in coal mine), the incidence of explosions has increased annually. 1 The shock waves in explosion cause primary blast injuries, and the lungs are one of the most vulnerable target organs for these injuries. The incidence of blast lung injury (BLI) in the battlefield of Afghanistan from 2008 to 2013 was 11.2%. 2 The treatment of BLI is challenging in clinic due to its high occurrence and mortality, and lack of effective evaluation methods at the early stage. 3 BLI mainly manifests as pulmonary hemorrhage and pulmonary edema because the alveoli are affected by the overpressure of shock wave and the following negative pressure, leading to the rapid rupture of alveolar capillaries. 1 Animal experiments mostly focus on the pathological observation of organs immediately after injury and only a few studies focus on the dynamic changes and development of injury, but with inadequate parameters. It was believed that ultrasound has low diagnostic value for chest injury due to its low penetration in air. However, currently, lung ultrasound has been widely used in critically ill cases. By quick scanning of the...
chest, doctors can identify pulmonary edema, pneumothorax, pulmonary embolism or pleural effusion resulting in acute dyspnea and hypoxemia in a short time, so as to give appropriate treatments. In this study, we established a severe blast lung injury model of goats, observed dynamic change of trauma, detected related parameters including oxygenation index (PaO$_2$/FiO$_2$), extravascular lung water (EVLW) and computed tomography (CT) imaging and completed the feasibility analysis of ultrasound for BLI diagnosis. Our study is of military and social significance because it provides a novel convenient and accurate assessment for on-site to hospital treatment process and improves the efficacy in trauma rescue.

**Methods**

**Experimental animals**

A total of 20 healthy, female goats, 3–6 months old, weighing 15 kg, were provided by the Animal Experimental Center of Institute of Surgery, Daping Hospital, Army Medical University. The BST-I bio-shock tube at a length of 39.4 m, produced by the Institute of Surgery, Army Medical University (Fig. 1), was adopted to induce injury. The driving segment was separated from the experimental segment by two 3.9 mm semi-hard aluminum membranes. The compressed air was used to produce the driving pressure of 4.0 MPa, 4.5 MPa, and 5.0 MPa, respectively.

**Establishment of blast injury model**

The goats were fasted for 24 h before the experiment and no water was given 8 h before the experiment. Then 1 mg/kg of midazolam (Nhwa Pharma Co. China) was intramuscularly injected for anesthesia, and 3% pentobarbital sodium of 15 mg/kg (Shanghai Pharmaceutical Co., imported sub-package) was intravenously injected when the animals began reviving for maintenance. A midline incision was made on the neck and a double-chamber central venous catheter (7 Fr, Duo V730, Braun, Germany) was inserted in the external jugular vein by a needle. It was inserted for about 15–20 cm, and fixed at the anterior vena cava, 2 cm from the right atrium; then the trachea was exposed for tracheal intubation of T-shaped incision (6.0, ET6014C, Tampa Co. China), connected to the ventilator (Babylog 8000, Germany). The ventilator parameters were as follows: IPPV (intermittent positive pressure ventilation) mode, VT (tidal volume): 8 ml/kg; R (respiratory frequency): 15 times/min; I/E (inspiratory/expiratory): 1: 1.5 (TI: 1.6s; TE: 2.5s); V (flow rate): 7.2 L/min; FiO$_2$ (oxygen concentration): 21%; PEEP (positive end-expiratory pressure): 3.9 cmH$_2$O; Pinsp (inspiratory pressure): 9–12 cmH$_2$O. The medial incision in the right inguinal region was made, and a picco thermodilution catheter (4 Fr, PV2014L16N, PULSION, Germany) was placed in the femoral artery under the guidance of a needle, and inserted for 16 cm. After operation, the animal was fixed in the standing position on the frame, which was placed in the shock tube, 37.5 m from the explosion source, with the explosion center on the right side (Fig. 2). According to the driving pressure, the animals were randomly divided into three groups: 4.0 MPa group (n = 4), 4.5 MPa group (n = 12) and 5.0 MPa group (n = 4). Immediately after the injury, the animals were removed from the shock tube and the bloody secretions in the trachea were cleared. If apnea occurred, artificial ventilation was performed immediately.

**General condition of animals**

The traumatic condition was observed and recorded immediately after the injury and within 12 h after injury, and the mortality...
of each group was recorded accordingly. The bed-side monitor (N12, Mindray, China) was connected for real-time monitoring of heart rate (HR), respiratory rate (RR), blood pressure (BP), etc., once an hour.

**Ultrasound examination**

The D236 portable wireless color ultrasound device (Youkey Bio-Medical Electronics Co., China), with a micro-convex probe (5.0–9.0 MHz), was used for evaluation by a trained sonographer. According to the classical Lung Ultrasound Scoring (LUS) standard based on the conditions of animals, the 66-partition method was adopted for ultrasonography, 33 partitions on the left and 33 partitions on the right. The scanning was conducted from the tenth intercostal space to the first intercostal space, from the spine to the sternum. Scoring criteria: 0, normal A line; 1, multiple separated B lines or mixed B lines <50%; 2, diffuse mixed B lines; 3, signs of lung consolidation.

**CT**

Chest CT (light speed 64-row CT, GE, US) scanning was performed layer by layer before injury (0 h) and at 12 h after injury (scanning parameters: current: 200 mAs; voltage: 100 kV; rotation time: 0.4 s by a radiologist). Using the Philips EBW workstation, the threshold value of lung injury was set by the radiologist and three-dimensional volume analysis of the whole lung and the injured part was made respectively to calculate the volume ratio of lung injury, i.e., lesion volume/total lung volume* 100%.

**PaO2/FiO2**

At 0 h before injury, 0.5, 3, 6, 9 and 12 h after injury, 0.3 ml arterial blood was taken through the arterial catheter and quickly added to the blood gas biochemical test chart (CG4+, Abbott, USA). Blood gas analysis was performed using a portable blood gas analyzer (i-STAT 300G, Abbott, USA) to calculate PaO2/FiO2.

**EVLW**

At 0 h before injury, 0.5, 3, 6, 9 and 12 h after injury, all sensors were connected to the picco module of the monitor. All the sensors were placed flush with the heart, and marked zero at the arteries and veins. During the measurement, 10 ml of iced physiological saline was injected rapidly and constantly through the tube of temperature sensor connected to the end of the intravenous catheter. The parameters were detected three times to be averaged. The EVLW index was recorded.

**Gross anatomy and pathological observation**

At 12 h after operation, the animals were anesthetized with 3% sodium pentobarbital and sacrificed by exsanguinations. The lung tissues were dissected. The gross anatomical analysis was accomplished and the injury was evaluated according to the Yelverton et al’s scoring system and previous evaluation criteria in the laboratory. The scale was as follows:

- No injury: no obvious damage.
- Minor injury: scattered hemorrhagic lesions, the total area of ecchymosis less than 10% of the total lung area.
- Moderate injury: the area of ecchymosis or hepatization ≤30% of the total lung area;
- Severe injury: the area of ecchymosis or hepatization ≤50% of the total lung area;
- Extremely severe injury: the area of ecchymosis or hepatization >50% of the total lung area.

![Fig. 3. The partitions (A) and scoring (B, C, D, E corresponded to 0, 1, 2, and 3 of lung ultrasound score respectively) for ultrasound examination on the body surface of a goat. The first to third intercostal space is in axillary area, and the fourth to tenth intercostal space is from top to bottom. The rectangular square refers to the probe scanning area, and the spine is on the left (A).](image-url)
The samples were taken from the lung tissues with pulmonary ultrasound score of 0–3 and HE staining was performed for pathological observation.

**Statistical analysis**

Statistical analysis was performed using the SPSS 22.0 software. The measurement data were expressed as mean ± standard deviation (X±S). The means of each group were tested for normal distribution, and the means of two samples were compared using independent-sample t-test. Pearson correlation analysis was conducted. p < 0.05 was considered significantly different.

**Results**

**General data after injury**

The mortality immediately after injury was 0 in the 4.0 MPa group, 16.67% (2/12) in the 4.5 MPa group, and 100% (4/4) in the 5.0 MPa group. The mortality at 12 h after injury was 0 in the 4.0 MPa group, 41.67% (5/12) in the 4.5 MPa group, and 100% (4/4) in the 5.0 MPa group. Immediately after injury, the bloody fluid was found in the tracheas of animals, and hemoptysis and apnea were observed in some animals, but no fractures and hemopneumothorax occurred. The changes in vital signs of goats in each group over time are shown in **Table 1**. The respiratory rate was rapidly increased immediately after injury (presented a significant difference compared with pre-injury value). The heart rate was then gradually stabilized. The blood pressure was decreased in the 4.5 MPa group compared with pre-injury value. The heart rate was increased at 3 h after injury and began to increase at 3 h after injury, and recovered to pre-injury level at 12 h.

**Ultrasound examination**

The LUS immediately after injury was 70.08 ± 16.23 and 30.00 ± 16.52 in the 4.5 MPa group and the 4.0 MPa group, respectively, suggesting a significant increase in the 4.5 MPa group (p < 0.05) and no significant increase in the 4.0 MPa group compared with pre-injury score. Then LUS was increased and stabilized at 3 h after injury, significantly higher than pre-injury score (p < 0.05). And the 4.5 MPa group presented a significantly higher LUS than the 4.0 MPa group (p < 0.05) (Fig. 4). It demonstrated that different injuries can be classified and evaluated by LUS.

**Correlation analysis between LUS and conventional lung injury parameters**

**PaO2/FiO2, EVLW and lung injury ratio**

PaO2/FiO2 was decreased significantly after injury in each group, and began to increase at 3 h after injury and became stabilized; the oxygenation index in the 4.0 MPa group was superior to that in 4.5 MPa group immediately after injury (0 h) and at 3 h after injury (p < 0.05), while it presented no significant difference at 6 h, 9 h and 12 h after injury. EVLW was increased immediately after injury, and continued to increase in the 4.0 MPa group, but decreased and gradually stabilized in the 4.5 MPa group at 12 h after injury, which was slightly higher than that in the 4.0 MPa group, with no statistically significant difference. The lung injury ratio at 12 h after injury was significantly higher than that before injury, and the ratio in the 4.5 MPa group was significantly higher than that in the 4.0 MPa group (p < 0.05) (Table 2).

**Correlation analysis between LUS and PaO2/FiO2, EVLW and lung injury ratio**

Pearson correlation analysis showed that there was no significant correlation between LUS and PaO2/FiO2 at 0 h before injury (r = 0.176, p = 0.530), but a negative correlation was observed at

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**Table 1**

Changes in vital signs of goats over time under different driving pressures.

| Vital signs | Time (h) | 0  | 0.5 | 3 | 6 | 9 | 12 |
|-------------|----------|----|-----|---|---|---|----|
| 4.0 MPa     |          |    |     |   |   |   |    |
| RR (times/min) | 15.00 ± 1.00 | 24.50 ± 0.71* | 17.67 ± 4.62 | 17.67 ± 4.62 | 17.67 ± 2.31 | 18.67 ± 4.04 |
| HR (times/min) | 120.67 ± 12.50 | 129.00 ± 16.64 | 143.67 ± 27.43 | 125.67 ± 25.58 | 127.00 ± 39.00 | 123.33 ± 19.50 |
| MAP (mmHg) | 85.67 ± 9.45 | 80.33 ± 8.74 | 82.00 ± 8.89 | 76.87 ± 9.61 | 91.33 ± 18.93 | 86.00 ± 9.54 |
| 4.5 MPa     |          |    |     |   |   |   |    |
| RR (times/min) | 15.67 ± 1.83 | 21.50 ± 6.00** | 22.20 ± 7.50 | 21.00 ± 4.87* | 19.50 ± 4.17* | 19.14 ± 6.26 |
| HR (times/min) | 122.25 ± 23.10 | 122.92 ± 46.79 | 139.70 ± 23.20 | 135.33 ± 24.67 | 132.13 ± 12.28 | 116.71 ± 44.34 |
| MAP (mmHg) | 98.08 ± 17.71 | 57.00 ± 14.33*** | 75.00 ± 12.16** | 70.67 ± 19.98** | 78.38 ± 17.67* | 85.00 ± 15.94 |

Note: RR: respiratory rate; HR: heart rate; MAP: mean arterial pressure.

* p < 0.05, comparison between two groups; **p < 0.01, comparison between two groups; ***p < 0.05, compared with pre-injury score; ****p < 0.01, compared with pre-injury score.
Table 2
The changes of PaO2/FiO2, ELWI and lung injury ratio over time under different pressures.

| Lung injury characters | Time (h) | 0 | 0.5 | 3 | 6 | 9 | 12 |
|------------------------|----------|---|-----|---|---|---|----|
| 4.0 MPa                |          |   |     |   |   |   |    |
| PaO2/FiO2 (mmHg)       |          |   |     |   |   |   |    |
| EVLW (mL)              |          |   |     |   |   |   |    |
| Lung injury ratio (%)  |          |   |     |   |   |   |    |
| LUS                    |          |   |     |   |   |   |    |
| 4.5 MPa                |          |   |     |   |   |   |    |
| PaO2/FiO2 (mmHg)       |          |   |     |   |   |   |    |
| EVLW (mL)              |          |   |     |   |   |   |    |
| Lung injury ratio (%)  |          |   |     |   |   |   |    |
| LUS                    |          |   |     |   |   |   |    |

Note: PaO2/FiO2: oxygenation index; EVLW: extravascular lung water; LUS: lung ultrasound score.
*p < 0.05, comparison between two groups; **p < 0.01, comparison between two groups; *p < 0.05, compared with pre-injury score; **p < 0.01, compared with pre-injury score.

3 h, 6 h and 9 h after injury (3 h: r = −0.871, p = 0.031; 6 h: r = −0.637, p = 0.015; 9 h: r = −0.658, p = 0.039); then the correlation became nonsignificant statistically at 12 h after injury (r = −0.596, p = 0.091). There was no significant correlation between LUS and EVLW at 0 h before injury (r = −0.309, p = 0.283), but they were positively correlated at 3 h, 6 h and 9 h after injury (3 h: r = 0.597, p = 0.031; 6 h: r = 0.698, p = 0.012; 9 h: r = 0.729, p = 0.017), and then the correlation was not statistically significant at 12 h after injury (r = 0.687, p = 0.065). No significant correlation was observed between LUS and lung injury ratio at 0 h before injury (r = −0.212, p = 0.531), but a positive correlation was noted at 12 h after injury (r = 0.884, p = 0.002) (Fig. 5).

Pathological results

At 12 h after injury, the anatomical results showed that the area of pulmonary hemorrhage in the 4.0 MPa group was 20.00% ± 13.14%, mainly regarded as moderate injury; the area of pulmonary hemorrhage in the 4.5 MPa group was 42.14% ± 15.33%, mainly regarded as severe injury. Therefore, the injury condition under the pressure of 4.5 MPa met the requirements of severe injury. The pathological results showed that LUS 0’: there were generally pink lung tissues and the corresponding tissue slices indicated normal alveoli with no obvious hemorrhage but a small amount of protein fluid effusion in the corresponding tissue slices; LUS 1’: there were generally red-spotted or scattered hemorrhagic foci, and the corresponding tissue slices indicated the alveolar and interstitial edema, mild damage on alveolar structure and a few red blood cells and inflammatory cells in the alveolar interstitium and alveolar spaces; LUS 2’: there was brightly red hemorrhagic area and the corresponding tissue slices indicated remarkable alveolar and interstitial edema, severe damage on alveolar structure and plenty of red blood cells and inflammatory cells in the alveolar interstitium and alveolar spaces; LUS 3’: there was diffuse dark red hemorrhagic area, hepatization and palpable solid tissue and the corresponding tissue slices indicated the rupture of alveolar wall, no intact alveolar structure, and diffuse hemorrhage and exudation (Fig. 6).

Discussion

BLI has the characteristics of mild manifestation, severe damage and rapid development, so early injury assessment and real-time dynamic monitoring are of great significance for patient delivery, treatment and prognosis prediction. Studies have shown that the “butterfly-like” or “bat-like” shadow is gradually expanded from the hilum to the periphery within 2 h after injury, usually not at the border of the lungs according to X-ray and CT images. However, the chest X-ray examination has the disadvantages of radiation, unstable diagnostic value which can be easily affected by respiration and position of critically ill patients and X-ray projection, and no availability on-site or in pre-hospital transfer. Although chest CT can accurately assess lung tissue lesions, its disadvantages such as increased risk in transfer, time-consuming, radioactive exposure and the need for special large equipments also limit its application in dynamic assessment for critically ill patients. Due to its convenience, rapidity, accuracy, non-invasiveness and reproducibility, bedside ultrasound has been widely used for quick evaluation and treatment in critically ill patients at home and abroad. Moreover, with the advancement of ultrasound technology, more portable
and intelligent handheld ultrasound devices are developed, which are more convenient for pre-hospital or critical treatment. Among them, pulmonary ultrasound can identify the cause of acute dyspnea and hypoxemia, and provide guidance for treatment; besides, LUS can be used for semi-quantitative evaluation of extravascular lung water and dynamic monitoring of lung injury. This study aimed to investigate the feasibility of bedside lung ultrasound for the diagnosis of blast injuries.

Based on previous data on blast injury in laboratory, this study used the BST-I bio-shock tube which utilized compressed air to simulate the explosion, selected the drive pressures of 4.0 MPa, 4.5 MPa and 5.0 MPa, and established a severe blast lung injury model of goats under a driving pressure of 4.5 MPa according to the mortality and anatomical features of injury. The animals in this study had no fractures or hemopneumothorax; the heart rate and respiratory rate were significantly increased, while blood pressure was significantly decreased within 3 h after severe injury; then all parameters were gradually recovered and stabilized 12 h after injury, presenting no significant difference compared with pre-injury values. It indicated that animals had a stress response after injury and there was a short-term shock compensatory phase. As for the respiratory function, the change of PaO₂/FiO₂ showed that the animals developed ARDS immediately after injury, and remained in mild ARDS (acute respiratory distress syndrome) state in spite of the recovery 6 h after injury (with reference to the latest definition of ARDS in Berlin), indicating that severe lung injury caused grave damage to lung function, which was irreversible in short term. EVLW was increased immediately after injury and remained a trend of elevation within 3 h, but decreased 3 h after injury, suggesting the possibility of metabolism and absorption of lung water. However, the studies have shown that chest X-ray, blood oxygen and lung compliance are not always related to pulmonary edema, and it shows that changes in tidal volume (VT) and PEEP can affect hemodynamic parameters such as EVLW. The increase of VT and PEEP leads to a slight but significant reduction in EVLW, which may explain why the change of EVLW is inconsistent with that of PaO₂/FiO₂. Whether other influencing factors are involved in this process still needs further studies. According to the imaging evidences, lung injury ratio was significantly increased at 12 h after injury; LUS was significantly increased immediately after injury, then remained an increasing trend, and became stabilized at 3 h after injury. It proved that ultrasound can achieve timely assessment and dynamic monitoring of BLI immediately after injury.

The results of correlation analysis have showed that LUS has a good correlation with conventional parameters. There was no significant correlation between LUS and PaO₂/FiO₂ and EVLW immediately after injury, but a negative correlation between LUS and PaO₂/FiO₂, and a positive correlation between LUS and EVLW were observed at 3–9 h after injury, suggesting that ultrasonography has gradually become valuable and feasible with the development of the injury despite the low sensitivity immediately after injury. LUS showed no significant correlation with PaO₂/FiO₂ and EVLW at 12 h after injury, which may be due to the small sample size. LUS was highly correlated with lung injury scores at 12 h after injury in our study, so the ultrasonography is a potential alternative to CT in the frontline treatment of war wound since the devices are portable and convenient. In addition, the difference in LUS between the two groups was more significant than that in conventional parameters, suggesting that ultrasound may have a higher sensitivity for BLI.

There are some limitations in this study. On the one hand, due to the operational difficulty and high cost of large animal experiments, the sample size is small; on the other hand, the individual difference in large animals and the difference from real patients reduce the validity of our experiment, so further clinical study is needed.

In conclusion, this study established a severe blast lung injury model of goats, and investigated the value of ultrasound in the early diagnosis and dynamic monitoring of BLI. The results have showed that ultrasound can reflect the changes in lung function timely and objectively, which is a quick and easy way to assess lung injury, worthy of clinical application.

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Ethical Statement

This study was approved by the Medical Ethics Committee of Amy Medical University.

Declaration of Competing Interest

All authors state that there is no conflict of interest.

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