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TITLE:

Neonatal Pneumothorax Ultrasound Diagnosis and Ultrasound-guided Thoracentesis Based on International Expert Consensus

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SUMMARY:
Pneumothorax is a common emergency and critical disease in newborn infants that needs rapid, clear diagnosis and timely treatment. Diagnosis and treatment based on chest X-rays are

associated with delayed management and radiation damage. Lung ultrasound (US) provides useful guidance for rapid, accurate diagnosis and the precise thoracentesis of pneumothorax.

ABSTRACT:

Pneumothorax (PTX) represents accumulation of the air in the pleural space. A large or tension pneumothorax can collapse the lung and cause hemodynamic compromise, a life-threatening disorder. Traditionally, neonatal pneumothorax diagnosis has been based on clinical images, auscultation, transillumination, and chest X-ray findings. This approach may potentially lead to a delay in both diagnosis and treatment. The use of lung US in diagnosis of PTX together with US-guided thoracentesis results in earlier and more precise management. The recommendations presented in this publication are aimed at improving the application of lung US in guiding neonatal PTX diagnosis and management.

INTRODUCTION:

PTX is defined as the presence of air within the pleural space. It is a well-recognized medical emergency condition with high mortality rates, especially in neonates with associated risk factors[1-3]. The incidence of PTX is reported to be 1–2% in term infants and 6% in premature infants with respiratory distress[2-3]. In addition, lung US (LUS) performed on asymptomatic term infants show that the incidence of mild PTX in these patients can be as high as 10%[2,3]. Risk factors associated with increased incidence of PTX include meconium aspiration syndrome (MAS), respiratory distress syndrome (RDS), and persistent pulmonary hypertension of the newborn (PPHN)[4-7]. A 1 min Apgar score ≤ 7 was associated with a 2.67x increased risk of PTX (95% CI 1.14–6.25)[8]. Increasing peak inspiratory pressure (PIP) during conventional mechanical ventilation has been shown to be a risk factor for PTX, and a PIP increase of 1 cm H_2O increases the odds of PTX by 1.46 (95% CI 1.02–2.07)[8]. The incidence of PTX in infants with a <2,500 g birth weight (BW) increases almost 10x compared to those with a BW ≥ 2500 g[8]. Notably, PTX is associated with increased mortality, with an odds ratio of 5.27 (95% CI = 1.96–14.17)[7]. Apiliogullari et al. reported that aggregate mortality was as high as 30% in PTX patients while survivors also had an increased rate of bronchopulmonary dysplasia (4.28x vs. controls)[9]. Therefore, early and accurate diagnosis followed by adequate treatment is imperative[3-14]. Lately, less expensive US imaging systems have become readily available, and non-ionizing, fast, and repeatable LUS represents an ideal tool for the diagnosis of neonatal PTX.

PTX is traditionally diagnosed by clinical imaging, auscultation, transillumination, and chest X-ray findings. In some cases of non-tension PTX, watchful waiting is warranted. However, large PTX or tension PTX requires prompt evacuation of the air in the pleural space by thoracentesis. Obtaining a chest X-ray image can be time-consuming and prolong the diagnosis of tension PTX. For these reasons, in many neonatal intensive care units (NICUs), LUS is replacing chest X-rays in diagnosing PTX due to its superior sensitivity and specificity[15-17]. Moreover, LUS has been shown to be more accurate than chest X-rays even for small, non-tension PTX[18-27]. LUS signs of PTX were first studied and described in adult critical patients. Patients with suspected PTX were scanned with LUS and computed tomography (CT). LUS signs characteristic of PTX were abolition of lung sliding in the B-mode (corresponding to the stratosphere sign in the M-mode), presence of A-lines, and the lung point. In the same study, abolition of lung sliding alone has a sensitivity of 100%

and a specificity of 78% for PTX. Absent lung sliding together with the presence of A-lines had a sensitivity of 95% and a specificity of 94% while lung point alone had sensitivity of 79% and a specificity of 100%[18].

Similarly, the usefulness of LUS to diagnose PTX has been described in infants[19-24]. CT could not be used as the benchmark in neonatal patients, thus LUS was compared with chest X-ray and clinical exam findings. Most of the studies included infants with sudden deterioration of their respiratory status, where LUS was performed before or after chest X-ray. The diagnostic accuracy showed a sensitivity of 100%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 100%[16-19]. In cases characterized by large PTX, lung point was absent, which consequently decreased the sensitivity of this sign to 75–95%[21,22].The average time to perform the diagnostic tests in these studies was 5.3 ± 5.6 min for LUS versus 19 ± 11.7 min for a chest X-ray[19]. As expected, LUS showed better diagnostic accuracy than chest transilumination[19]. Keeping in mind that in infants with tension PTX the needle is blindly placed in the second intercostal space at the midclavicular line, it is not surprising to see treatment failure and/or complications[6]. On the other hand, PTX thoracentesis performed under LUS guidance has shown promising results in infants[28,29].

The neonatal Lung Ultrasound Training Base of China, Chinese College of Critical Ultrasound, as well as the World Interactive Network Focused On Critical Ultrasound China branch have organized this international expert panel that reviewed the latest literature related to neonatal PTX diagnosis and treatment aimed at the improvement in the application of LUS-based diagnosis and treatment of PTX.

Patients and timing of the examination

The LUS exam can be used on any neonate in respiratory distress. It is indicated in the following situations: 1) Suspicion of PTX in neonates with sudden deterioration of respiratory status; 2) Before and after thoracentesis.

Lung ultrasonography terminology used in PTX diagnosis

Frequently used ultrasound terms in diagnosis of PTX include: A-line, B-line, confluent B-lines, compact B-lines, alveolar-interstitial syndrome, pleural line, lung sliding, lung pulse, sandy beach sign, and stratosphere sign. The exact definitions of the terms used have been described in detail previously[30-34].

PROTOCOL:

This work was approved by the Research Ethics Committee of Beijing Chaoyang District Maternal and Child Healthcare Hospital & Beijing Chaoyang District Bureau of Science, Technology and Information. The study protocol follows the guidelines of the hospital's human research ethics committee.

1. Ultrasound exam preparation

1.1. Probe selection

1.1.1. Select a high-frequency linear probe (≥10.0 MHz) to scan the lungs.

1.2. Probe disinfection

1.2.1. Sterilize the transducer before and after each examination.

1.3. Preset selection

1.3.1. Select the **LUS** preset.

1.3.2. Optimize imaging settings for examination when no LUS preset is available.

1.3.2.1. Select one of the **Small Parts** presets.

1.3.2.2. Adjust depth to 4–5 cm using the **Depth** button.

1.3.2.3. Adjust the **Focus Zone** button to have 1 or 2 focuses.

1.3.2.4. Adjust the focus close to the pleural line.

1.3.2.5. Turn on the **SRI** (**Speckle Reduction Imaging**) by clicking the button and selecting a level of **2–3** to reduce the speckle noise.

1.3.2.6. Turn on the **CRI button** (**Crossbeam**) and select a level of **2** to improve the contrast resolution.

1.3.2.7. Select **Fundamental Imaging** for sharper A-lines or B-lines.

1.4. Using an ultrasound gel

1.4.1. Apply the appropriate volume of warm gel on the transducer to keep it in good contact with the skin surface.

2. Place the infant in a suitable position.

2.1. Keep the infant quiet. Use a pacifier when necessary.

2.2. Keep the infant in a supine, prone, or side position for examination.

3. Partitioning the lungs

3.1. Six regions: Divide each side of the lung into three regions along the anterior axillary and posterior axillary line. These are the anterior, lateral, and posterior areas. Thus, both lungs are divided into six regions.

3.2. Twelve regions: Divide each lung into upper and lower lung fields by the nipple connection line. Now there should be 12 regions on both lungs.

4. Procedure for LUS imaging

4.1. B-mode scanning

4.1.1. Press the **2D button** or **B** key to start B-mode scanning.

4.1.2. Place the transducer perpendicular to the ribs to start perpendicular scanning. Identify the presence of the pleural line, A-line, and B-lines.

4.1.3. On real-time US observe whether there is lung sliding or lung point.

4.1.4. Rotate the probe 90° to start parallel scanning.

NOTE: 1) The exam must cover the entire bilateral lung fields. Start at the highest part of the thorax, especially in emergency situations. Because newborns are usually placed in a supine position, this zone is usually located on both sides of the sternum; 2) Bilateral perpendicular scanning is the most important scanning method, while parallel scanning is helpful for diagnosing mild-to-moderate PTX.

4.2. M-mode scanning

4.2.1. Press the **M-button** to start M-mode scanning. Look for the presence of the stratosphere sign or lung point that indicate PTX.

NOTE: Experienced sonographers can detect PTX using only B-mode. M-mode scanning can be used to confirm the B-mode findings if an examiner is less experienced.

5. Identifying the presence of PTX

5.1. Observe if the pleural line, A-lines, and B-lines exist on B-mode.

5.2. Observe if lung sliding and lung point exist on real-time US.

5.3. Observe if the stratosphere sign is present on M-mode.

6. Identifying the degree of PTX

6.1. Identify the degree of PTX according to the LUS findings.

7. LUS-guided thoracentesis

7.1. Select an appropriate puncture needle (18-20 G needle or an angiocatheter connected to a 20 mL syringe and a three-way stopcock).

7.2. Body positioning

7.2.1. Keep the infant in a quiet state. Assure adequate pain control according to the local unit policy.

7.2.2. Place the infant in the supine, prone, or side position before thoracentesis, allowing the air on the affected side to rise.

7.2.3. Put on a pair of sterile gloves. Disinfect the puncture area.

7.3. Identify a suitable puncture point.

NOTE: When identifying a suitable puncture point, keep the following in mind: 1) Intercostal space where the pleural line and A-lines exist on B-mode; 2) Intercostal space that presents with a stratosphere sign in M-mode; 3) Intercostal space where lung sliding disappears on real-time US.

7.4. Thoracentesis

7.4.1. Keep the infant in a stable position.

7.4.2. Evacuate the pleural air by needle aspiration at the selected puncture point. Alternatively, a chest tube may be placed immediately.

NOTE: In general, thoracentesis achieves good results. Adequate pain control is strongly recommended (a local 1% lidocaine injection in the dose of 0.5–1.0 mg/kg or enteral pain control as per unit policy). The use of a pacifier is also encouraged. Larger or tension-PTX is at increased risk of having an underlying bronchopulmonary fistula. It may need a prolonged period of continuous chest tube drainage. Postprocedural LUS evaluation of the affected side is recommended. Cover the insertion site with petroleum gauze once thoracentesis is completed.

REPRESENTATIVE RESULTS:

The main purpose of these guidelines is to direct users on how to perform US-guided thoracentesis to treat PTX. Normal neonatal lung appears as a bamboo sign on B-mode US (**Figure 1A**) and as a seashore sign (**Figure 1B**) on M-mode US. Lung sliding is clearly evident under real-time US (see **Video 1** for lung sliding)[31-34].

PTX is diagnosed based on the following LUS imaging characteristics: 1) Disappearance of lung sliding. This is the most important sign in the US diagnosis of PTX; 2) Absence of B-lines; 3) Presence of the pleural line and A-lines; 4) On M-mode imaging a normal sandy beach sign is replaced by the stratosphere sign, which is highly specific for PTX; 5) Presence of the lung point in mild to moderate PTX. This sign may not be evident if PTX is large[30-34]. The PTX diagnostic flowchart is presented in **Figure 2**[34].

Identifying the degree of PTX

The severity of PTX can be identified by the several characteristics. 1) Mild PTX: LUS signs of PTX exist in the anterior chest areas only when an infant is in a supine position. The area where lung sliding disappears is approximately <50% of the whole lung field or the spared areas exist. Lung point is easily identifiable due to the normally expanded lung. The presence of a spared area generally suggests mild PTX; 2) Moderate PTX: LUS signs of PTX are evident in the anterior and lateral chest areas when the infant is in a supine position. The area where lung sliding disappears is >50% of the whole lung field. Identifying the transitional lung point area may be challenging; 3) Severe PTX: LUS signs of PTX exist in the anterior, lateral, and posterior lung areas. Lung sliding is absent in all lung areas. There is no identifiable lung point.

Thoracentesis under lung US guidance

The infant can be placed in the supine, prone, or side position. Slight elevation of the upper body helps obtain more complete air evacuation. If severe PTX is present, the thoracentesis must be performed immediately (**Figure 3, Video 2**). Place the patient in a prone position (**Figure 4A**), side position (**Figure 4B**), or supine position. In the case of tension PTX, continuous air drainage with a chest tube can be used with the infant in a supine position (**Figure 4C**). In moderate PTX, if thoracentesis is indicated, the site of needle insertion can be anywhere in the field where lung sliding is absent (**Figure 5, Video 3**). Mild PTX (**Figure 6, Figure 7, Video 4, Video 5, Video 6**) generally does not require thoracentesis. However, if the primary pulmonary disease of the infant is more severe and the infant presents with clinical deterioration, then thoracentesis may be indicated (**Figure 8, Video 7**).

FIGURE LEGENDS:

Figure 1: Normal neonatal LUS. (**A**) B-mode US: Pleural line and A-lines are smooth, regular, and straight hyperechoic lines, parallel and equidistant from each other. A-lines gradually diminish and finally disappear off the screen. (**B**) M-mode US: Above the pleural line are linear hyperechoic lines that correspond to the non-moving skin, and subcutaneous and muscle tissue. Below the pleural line is the normal lung tissue that moves with each respiration, leaving a grainy image. These M-mode findings create a seashore sign.

Figure 2: The PTX diagnostic flowchart program. This flowchart program shows that B-mode US is the most important method to diagnose PTX, while M-mode US is helpful to confirm the diagnosis. This figure is reproduced from Liu et al.[34].

Figure 3: Severe PTX. B-mode US (upper part): The pleural line and the A-lines are present, apparently normal LUS. M-mode US (lower part) shows a stratosphere sign as the lung below the

pleural line is displaced by PTX. The absence of lung movement under the pleural line cancels out the normal grainy image.

Figure 4: Body position. (**A**) Infant in prone position. (**B**) Infant in side position. An angiocatheter is used to evacuate the air from the pleural location. It is connected to a 20 mL syringe. (**C**) Chest tube under continuous suction.

Figure 5: Lung point in moderate PTX. B-mode US: Lung point with an area of disappeared lung sliding that is >50% of the whole field, suggesting moderate PTX. Evacuation of air is usually needed with this degree of PTX. The needle puncture site can be selected anywhere in the lung field without lung sliding.

Figure 6: Lung point in mild PTX. B-mode US: Lung point with an area of disappeared lung sliding that is <50% of the whole lung field suggests moderate PTX. Evacuation of air is rarely needed with this degree of PTX.

Figure 7: Spared area of normal lung in mild PTX. The pleural line and A-lines exist in the middle field of the lung while the significant B-lines exist in the upper and lower field of the lung. This kind of lung US sign in known as a spared area. You can find two lung points in this condition. The presence of a spared area generally suggests mild PTX (please also see **Video 6**). Air evacuation is usually not needed with this degree of PTX.

Figure 8: Spared area of normal lung in mild PTX. A male patient with a gestational age of 41 weeks and birth weight of 3,200 g. The patient was admitted to the NICU because of dyspnea 20 min after birth. LUS showed that spared areas existed in the left anterior chest only. B-mode LUS (**Figure 8**) and real-time US (**Video 7**) suggest the presence of mild PTX in the left chest together with pneumonia. Although the infant had only mild PTX, it was accompanied by severe dyspnea not alleviated with mechanical ventilation. Thus, the pleural puncture was performed. The infant's status significantly improved upon drainage of 15 mL of air from the left chest.

Video 1: Normal neonatal LUS. Positive lung sliding under real-time US appears as shimmering of the pleural line.

Video 2: Severe PTX. Absence of lung sliding under real-time US.

Video 3: Lung point in moderate PTX. Under real-time US the lung point presents as an alternate point of lung sliding emergence and disappearance.

Video 4: Lung point in mild PTX. Under real-time US the lung point presents as an alternate point of lung sliding emergence and disappearance.

Video 5: Spared area in mild PTX. Under real-time US, two alternating points of lung sliding emergence and disappearance, indicating two lung points and a spared lung field area.

Video 6: Spared area of in mild PTX. A spared area is present in the left anterior chest on real-time LUS.

Video 7: Spared area of in mild PTX. On real-time US in the middle field of the lung the lung sliding disappeared, but the pleural line and A-lines are there. In the upper and lower fields of the lung, lung sliding as well as significant B-lines exist. That is the spared area, suggesting mild PTX in the left chest.

DISCUSSION:

LUS for the diagnosis of neonatal PTX is a manageable and timely diagnostic modality[17,19-23,30,35-38]. Recent animal studies found that the LUS diagnosis of PTX is very accurate and reliable [39,40]. In one of these studies LUS and chest X-rays findings of PTX were compared to CT scans as a point of reference and confirmed that LUS is superior to chest X-rays in diagnosis of small PTXs[40]. In newborn infants with PTX, LUS sensitivity and specificity are also higher than that of chest X-rays[17,19-23,37,38], and recent meta-analysis further established that the sensitivity of LUS in diagnosing PTX is nearly 50% higher than the chest X-ray sensitivity[41,42].

Identification of the degree of PTX is very important for thoracentesis. However, absolutely accurate quantification of PTX volume by LUS is not easy. Finding the lung point effectively distinguishes the normal lung from the lung being separated from the chest wall by the presence of PTX. Similarly, LUS cannot ascertain the depth of air collection. Some studies have shown that semi-quantification of PTX volume is only reliable for small PTX[43]. Therefore, the comprehensive analysis of vital signs, physical exams, and LUS images are essential before making a decision as to whether or not to perform an invasive procedure such as thoracentesis or thoracostomy[44,45]. A study also showed certain variations among pediatric surgeons in the management of spontaneous PTX. The use of CT, timing of operation, and length of observation for air leak before performing surgery have not been adequately standardized[44]. Recent systemic reviews showed no significant difference between thoracentesis and chest tube placement with regards to safety and rates of immediate success. However, thoracentesis is associated with decreased pain and duration of hospital stay compared to chest tube thoracotomy[6]. Traditionally, thoracentesis is performed in the 2nd intercostal space at the midclavicular line or 4–5th intercostal space at the midaxillary line with the needle pointed toward the opposite shoulder with a repeat chest X-ray after the procedure. This technique may have several disadvantages. It may delay evacuation of the air because the needle may not always be located right above the PTX, rendering the evacuation incomplete. Evacuation drainage may be prolonged due to incomplete evacuation and the need to change the patient's body position. Also, repeated chest X-ray exposure is always needed. Finally, if the needle is not pointed in the right direction, major blood vessels can be pierced. LUS not only facilitates the needle aspiration by decreasing the risk of complications, but it also offers real-time observation of postprocedural PTX resolution and lung reexpansion[46]. In summary, compared to the traditional thoracentesis procedure there are several benefits of LUS-guided thoracentesis. These include 1) Convenience: There are no limitations to the infant's body position; 2) Accurate and real-time procedure performance: The procedure can be performed immediately after the LUS diagnosis, precisely aimed at the PTX with simultaneous follow-up of lung reexpansion; 3) Decreased risks of complications: LUS can guide the needle just above the

rib, avoiding the blood vessels and allowing the operator to visualize the needle as it enters the pleural space; 4) Pain reduction: Shortening the procedural time as well as accurate needle insertion may alleviate the infant's pain[47].

Critical steps within the protocol are to diagnose PTX and perform thoracentesis proficiently and accurately. The operator must be skilled in neonatal LUS examination as well as in the neonatal thoracentesis technique. Studies have shown that learning essential LUS skills requires short training programs with a relatively small number of supervised scans ranging between 20–80 LUS exams[34,35]. Several published guidelines should assist in developing and maintaining those skills[30-34].

Limitations to LUS-guided thoracentesis are: 1) Difficulty to accurately quantify the exact PTX volume; 2) Operator-dependent procedure; 3) Less experienced examiners may mistake PTX for diseases that are similar to it, such as bullae and some congenital pulmonary airway malformations[48,49].

For comprehensive neonatal LUS guidelines, including PTX diagnosis, one can also reference previous publications[30-34]. The diagnosis of PTX using LUS is relatively easy when guiding principles are followed. Formal LUS training allows trainees to quickly acquire these skills[50]. Thoracentesis remains a high-risk procedure, particularly in very low birth weight infants. US-guided thoracentesis offers several potential improvements over conventional landmark PTX management. Further, multicenter studies should aim to quantify the extent of this improvement. A detailed description of US-guided thoracentesis allows for a more standardized approach that should guide both clinical practice and research.

DISCLOSURES:

The authors have nothing to disclose.

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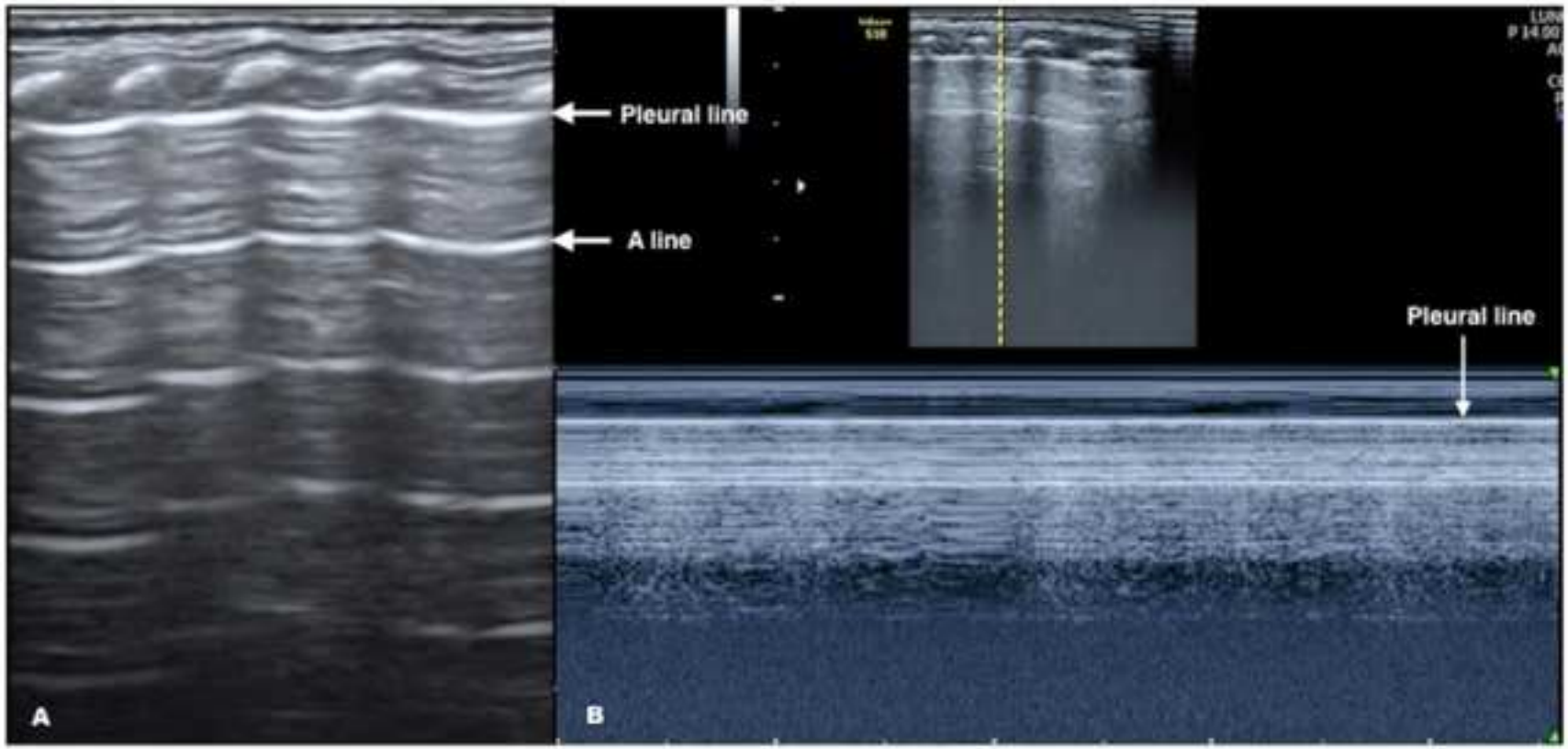
Figure-1

Click here to access/download;Figure;Figure 1 Normal lung imagine.001.tiff



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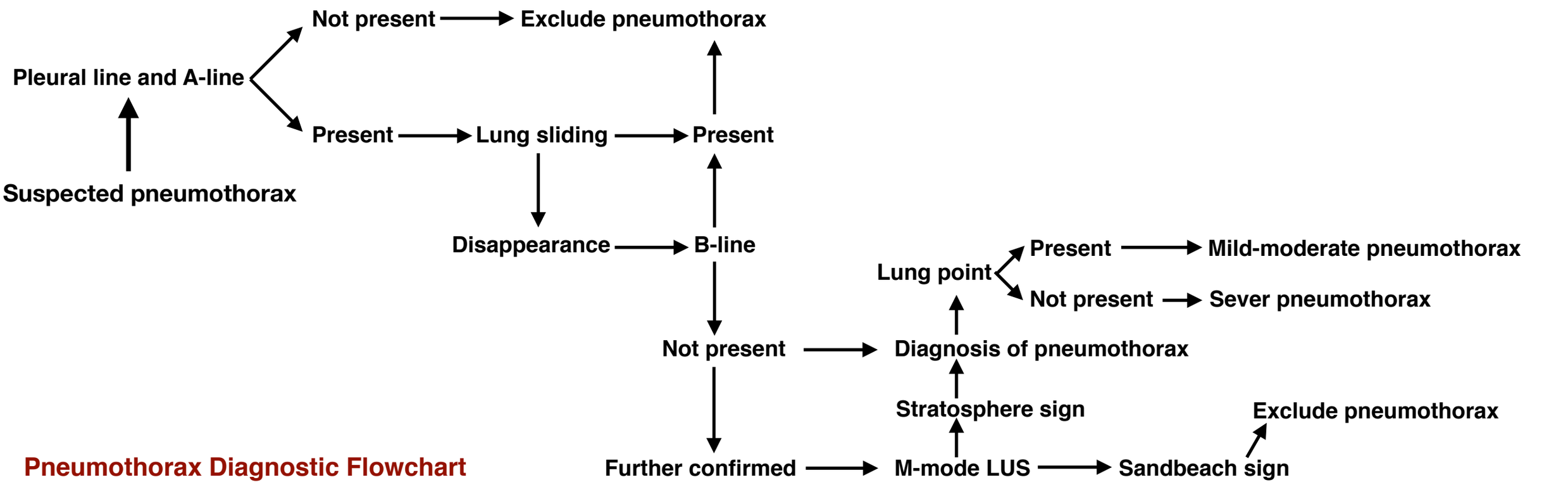

Figure-3

Click here to access/download;Figure;Figure 3 Severe Pneumothorax.001.tiff

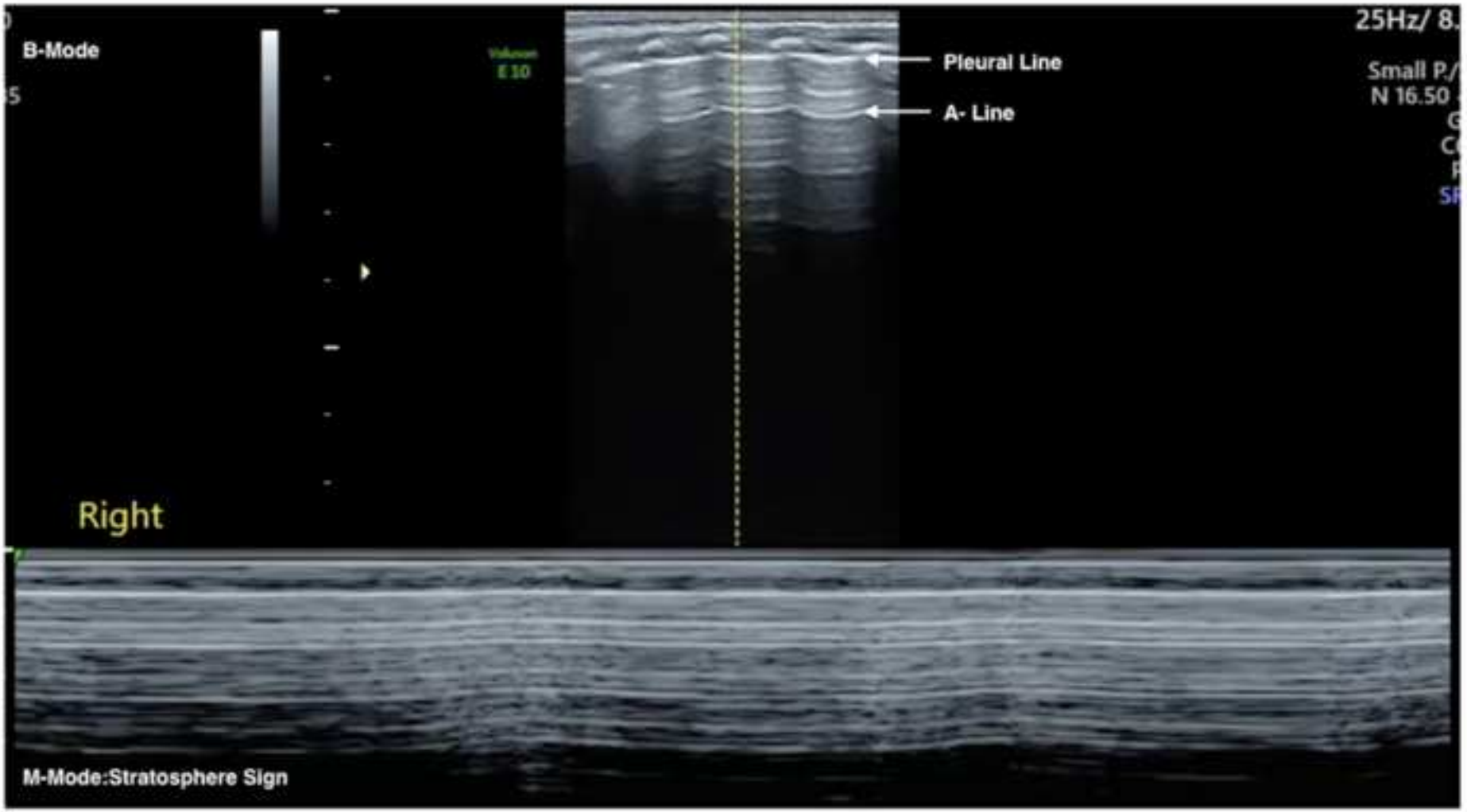

Figure 4

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Figure-5

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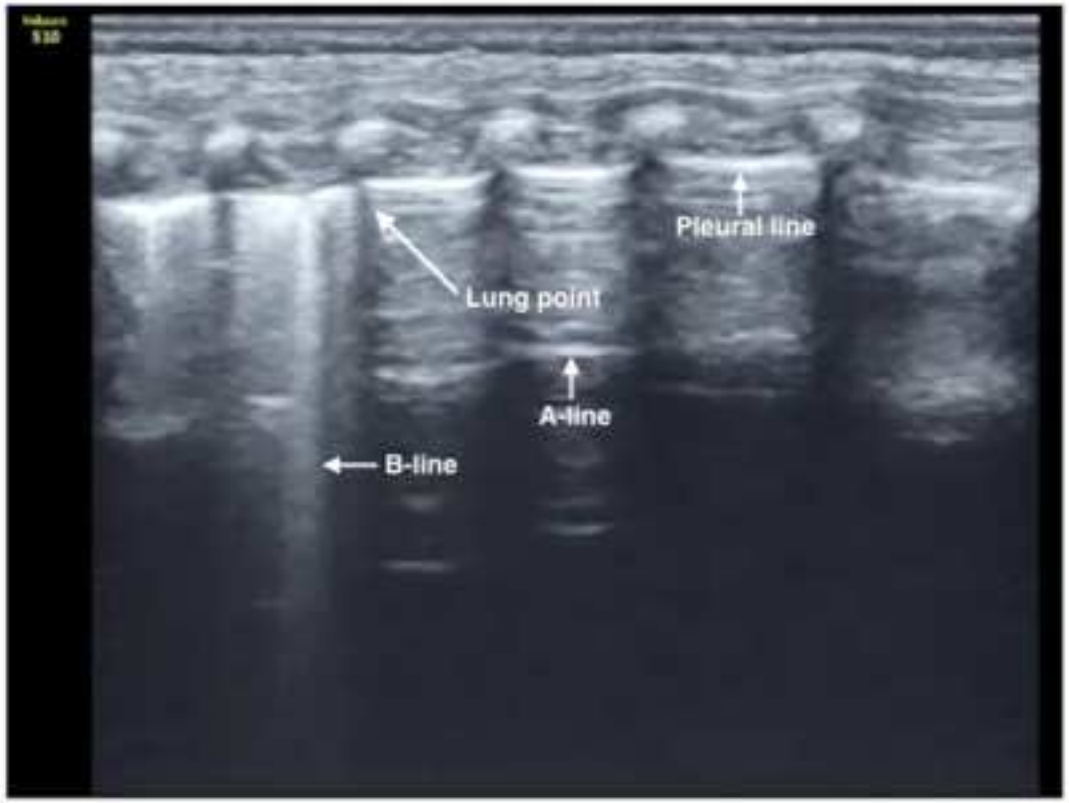

Figure-6

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Figure-7

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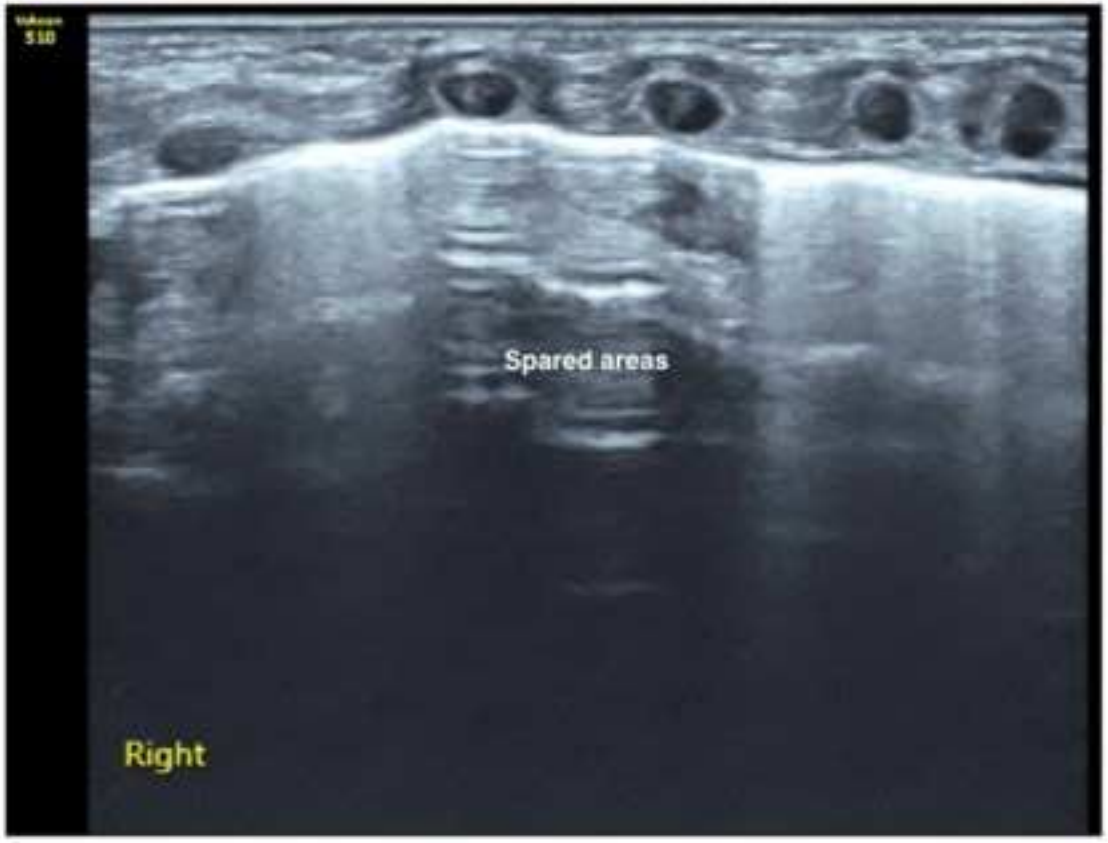
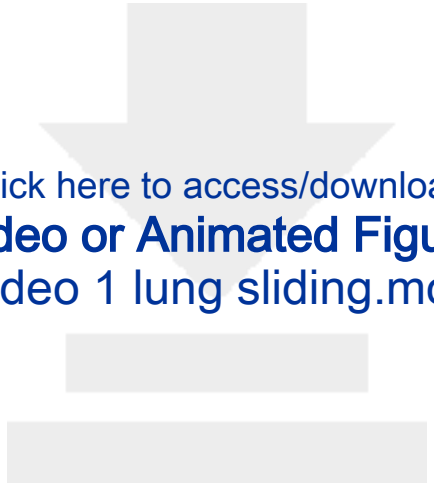


Figure-8

Click here to access/download;Figure;Figure 8 spared areas-mild PTX.001.tiff

Video-1



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Video or Animated Figure

Video 1 lung sliding.mov

Video-2

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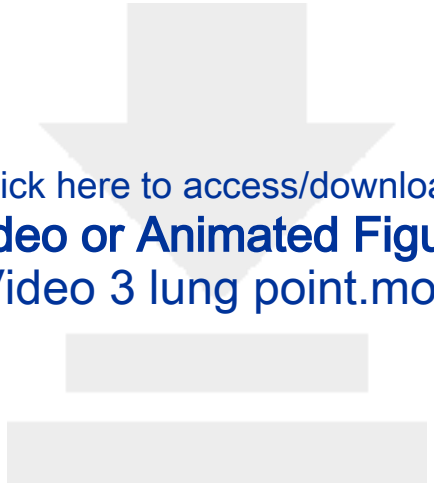
Video or Animated Figure

video 2 LS disappearing.mov

Video-3



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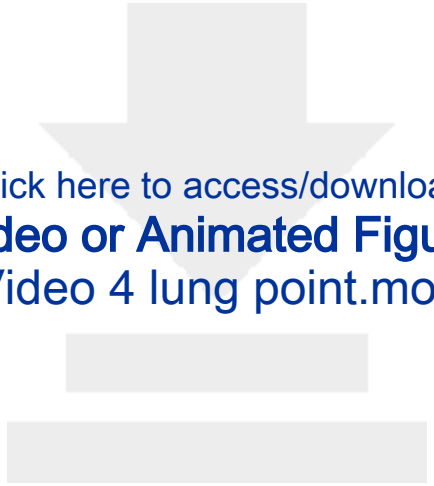
Video or Animated Figure

Video 3 lung point.mov

Video-4



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Video 4 lung point.mov

Video-5

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Video or Animated Figure

Video 5 lung point-mild PTX.mov

Video-6

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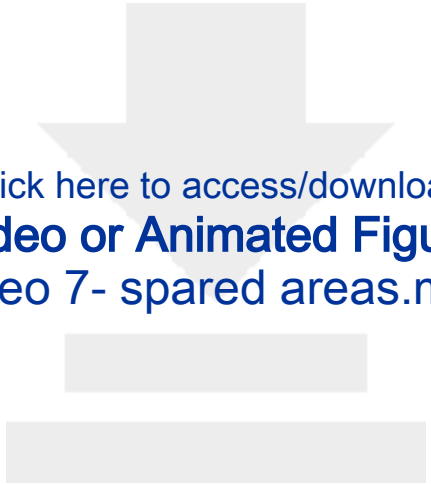
video 6 spared areas.mov

Video-7



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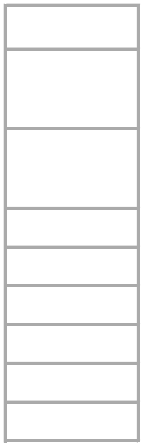
Video or Animated Figure

Video 7- spared areas.mov

Click here to access/download;Table of Materials;Materials Table-PTX.xlsx

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Disinfection wipe	Sirui Company	YZB0016-2013	Benzalkonium Bromide Patches
Ultrasound gel	Xiyuansi Company	TM20160195	Aquasonic 100 ultrasound transmission gel
Ultrasound machine	GE	H44792LW	Ultrasound machine,Voluson S10 BT16,Probe ML6-15 & 9L
Ultrasound machine	GE	H48701UZ	Ultrasound machine,Voluson E10 BT18 OLED,Probe ML6-15 & 9L



Rebuttal Letter
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Editorial Comments:

The manuscript will benefit from thorough language revision, as there are a number of grammatical errors throughout. Please thoroughly review the manuscript and edit any errors. Please avoid inconsistent formatting as well.
Response: Our authors from United States who are proficient in English language editing for scientific papers have carefully revised the manuscript. The formatting has been uniform.

Textual Overlap: Significant portions show significant overlap with previously published work. Please re-write paragraph 1 of discussion avoid this overlap.
Response: Paragraph 1 of the discussion has been rewritten according to the suggestions.

Introduction: Please avoid multiple subheadings under this section.
Response: We have removed multiple subheadings from the Introduction.

Protocol Language: Please ensure that all text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) Any text that cannot be written in the imperative tense may be added as a "Note", however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.
1) Examples NOT in the imperative: 3.a, 3c.a,b,c, 4.c.c1, 4.c., d
2) Avoid long discussions in the protocol. E.g., in section2
Response : We have ensured that all the verbs in the Protocol are written in the imperative voice.

• Protocol Detail: Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Some examples:
1) 2.a: Is the infant sedated? Mention acoustic gel application
2) 3.c: Under how the probe was place under the infant. Unclear how degree of PTX was evaluated.
3) 4.a: How is pain managed? Is the infant sedated? If so, how? Mention drugs and dosage.
4) 4.c, d, f: Several steps here are unclear. Please use complete sentences to describe the actions.
Response : We have included details that reflect infants sedation as needed, and as feasible considering that thoracentesis procedure may be done emergently.

Protocol Numbering: Please adjust the numbering of your protocol section to follow JoVE's instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. Please add a one-line space after each protocol step.

Response : We have changed the numbering as suggested.

• Protocol Highlight: After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.
1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
4) Notes cannot be filmed and should be excluded from highlighting.

Response : We have done the highlighting as per JoVE guidelines. With single spacing the Protocol is inside the required limits.

• Results:
1) Please avoid multiple subheadings under this section.
2) We require at least some results (figures/tables) that demonstrate the success of your technique, this can be an application of your method to a specific study or general results that validate the technique. These must be fully discussed in the Representative results. The current results do not sufficiently support and validate the technique you present.
Response: We have included several Figures and Videos that clearly reflect the text of the manuscript and the procedure itself.

• Discussion:
1) JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.
2) Remove all bulleted/numbered lists from this section.
3) After the Discussion, please add an "Acknowledgments" section. Please list acknowledgments and all funding sources for your work in this section.
Response: The Discussion has been reworded to include all the suggestions listed above.

Figure/Table Legends: Separate legends are required for each video.
Response: Legends have been rephrased as per JoVE guidelines.

• References:
1) Please make sure that your references comply with JoVE instructions for authors. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. Volume (Issue), FirstPage – LastPage, doi:DOI (YEAR).]
2) Please spell out journal names.
Response: All references have been redone.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."
Response: All our Figures and Videos are originals that do not require publishing permissions.

Comments from Peer-Reviewers:

Reviewers' comments:
Reviewer #1:
Manuscript Summary: good
Major Concerns: None
Minor Concerns:
Can this technique be used in pneumopericardium? If yes, maybe a brief mention in discussion will enhance the scope of the paper.
Response:Dear reviewer,pneumopericardium is not a common condition. Neonatal literature is quite scarce for this condition.Also,US-guided pneumocardium has been described in the literature and we wanted to stay focused on PTX.

Reviewer #2:
Manuscript Summary:
The manuscript describes a protocol to guide point of care ultrasound use to diagnose pneumothorax in neonates using specific ultrasonographic features. It also describes step by step the US guided thoracentesis for clinically significant Pneumothorax. The introduction is nicely written to describe the importance of US use in diagnosing and managing pneumothorax. The procedure description is well organized, Images and videos are very clear.
Response: Thank you for the positive comment.We appreciate it.

Major Concerns: No Major concerns

Minor Concerns:
1- This is a straight forward protocol, yet there are so many authors listed. Are those all authors or collaborators?
Response: Yes, all of the listed authors here are co-authors.We have made multiple cycles of e-mail and on-line discussions on this protocol. All authors actively participated with their suggestions and opinions. We used those to revise the manuscript and write the final version of the protocol.

2- There is a great article by Husain L et all (Sonographic diagnosis of pneumothorax 2012) describing all the feature of Pneumothorax on US. This can be a great reference.
Response: Good point. Thank you very much. We have studied this publication, included it in the paper and listed it as reference 30 in the revised manuscript.

3- There are few grammar mistakes and sentence structure that needs to be fixed in the text.
Response: We have included English-native colleagues to correct all the mistakes and the syntax. It was done to the best of our ability.

Reviewer #3:
Manuscript Summary:
Agree LUS is a useful tool in the rapid diagnosis of pneumothorax, value adding to clinical information in determining the extent of pneumothorax and facilitate safe procedural guidance of thoracocentesis. And also follow up post procedure. My concerns with this paper is a very 'black and white' depiction of the role of LUS, without healthy balance of the real limitations, potential pitfalls and caveats to use that only is appreciated with experience.
Response: There are several limitations and potential pitfalls within this recommendation. They have been mentioned and commented on in the Discussion.

Major Concerns:
I feel the need to extend the abstract to include further steps of diagnosis of air leak, beyond abolishing of pleural sliding from 2d and/or m-mode stratosphere. Authors must consider their role in clinical guidance and potential for operators to get it wrong.
"Watchful waiting is indicated in non-tension pneumothorax". An example of concrete thinking, could be better phrased…. Not all pneumothoraces warrant intervention. as some may necessitate drainage but not necessarily be under tension).
Response: Thank you for the comment. Abstract has a word limit that we tried to respect.The use of LUS in diagnosing PTX,assessing the size of PTX and correlating the findings with infant's clinical picture has been carefully addressed in the body of the manuscript as, in our opinion, needs more space than Abstract allows.

Procedure for scanning:
Make it clearer scanning in longitudinal and transverse planes and stepwise questions to ask as published Fig 1 in international recommendations 2012(Intensive Care Med.2012Apr;38(4):577-91.doi:10.1007/s00134-012-2513-4.Epub 2012 Mar 6.International evidence-based recommendations for point-of-care lung ultrasound).
Response: This is an important publication. We referenced it several times in different parts of our manuscript.

Examine for presence/ absence of pleural sliding, display of an A line artifact profile, Lack of B line artifacts, Identification of lung point- need to state here that this may not be found if large enough and no longer pleural contact.
Response: Revised version of the manuscript included all the above mentioned LUS findings.Thank you for the comment.

M-mode- can be helpful to determine presence of absence of pleural sliding- but authors must acknowledge caveats to its use- can be confused with parasitic artifact generated from intercostal muscle movement (sand above the pleural line) Lichtenstein provides expert guidance on scrutinising artifact generation above and below the pleural line with abolished lung sliding

(Lichtenstein DA. Lung Ultrasound in the Critically Ill: The BLUE Protocol: Springer International Publishing, 2015. Avicenne sign, chapter 14, page 93-94 figure 14.3).

Response : Thank you very much for this comment,very valid point. However, significant artifacts generated by the movement of intercostal muscle may not be present in infants since their chest wall excursions are not as apparent to M-mode as in in adults.

Not certain about defining mild/moderate cases of pneumothorax with definition of extent of signs across the chest. Authors must acknowledge what they are interpreting is lack of artifact generation due to separation of the visceral and parietal pleural and lung point is the defined physical limit. Must acknowledge cannot ascertain depth of air collection, only define the boarder. Some papers identify if meets the anterior axillary line more likely to be of significance and require intervention e.g. Cattarosi.

Response: Thank you for the constructive comments. We have now mentioned these limitations in the Discussion parts. Dr.Cattarosi's opinion was also listed in the revised manuscript in the Results part.

Clinical correlation is essential and in reality most would support correlation with conventional radiographs if used.

Response: Correct. Clinical correlation is essential. On the other hand, we encourage users to reduce the application of chest x-rays for several reasons explained at length in the manuscript.

I do not support the flowchart in figure 2.I understand the content however it's terminology is confusing e,g Pleural line and A line "not present"….exclude pneumothorax …Suggest use international recommendations figure or adapt but use consistent terminology as with Cattarossi paper (ref 17).

Response: Dear reviewer, The terminology description in this section has been modified.The Figure in the international recommendations (Ref.32) is excellent. According to extensive experience, we found that the sign of 'lung pulse' is not essential in the diagnosis neonatal PTX.

Referenced Doug Blank et al animal study, important work but more recent relevant work undertaken in human patients should be better referenced and discussed as supportive literature e.g.- ref 24 Fiorella Migliaro and ref 17 Cattarossi (CXR vs transillumination vs LUS). Authors seriously risk of offending an international audience by major referencing of dog studies.

Response : We have made the reference to the animal studies more concise. They are important because they included CT scanning comparison of PTX findings to US versus CXR.Understandably,this type of research cannot be done in infants.

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Author(s):	Jing Liu,Dalibor Kurepa, Roberto Copetti,Francesco Feletti, Erich Sorantin, Javier Rodriguez-Fanjul , Jovan Lovrenski, Xing Feng

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