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Standardized Protocol and Guidelines for Point-of-Care Lung Ultrasound in Diagnosing Neonatal Pulmonary Diseases:International Expert Consensus
--Manuscript Draft--

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3
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KEYWORDS:

Lung ultrasound (LUS), Point-of-care lung ultrasound (POC-LUS), Lung diseases, Infant, Newborn, Neonatal ward, Neonatal intensive care unit (NICU), Emergency, Critically ill

SUMMARY:

Lung ultrasound is a noninvasive and valuable tool for bedside evaluation of neonatal lung diseases. However, a relative lack of reference standards, protocols and guidelines may limit its

89 application. Here, we aim to develop a standardized neonatal lung ultrasound diagnostic protocol
90 to be used in clinical decision-making.

91

92 **ABSTRACT:**
93 Ultrasound is a safe bedside imaging tool that obviates the use of ionizing radiation diagnostic
94 procedures. Due to its convenience, the lung ultrasound has received increasing attention from
95 neonatal physicians. Nevertheless, clear reference standards and guideline limits are needed for
96 accurate application of this diagnostic modality. This document aims to summarize expert
97 opinions and to provide precise guidance to help facilitate the use of the lung ultrasound in the
98 diagnosis of neonatal lung diseases.

99

100 **INTRODUCTION:**
101 The chest x-ray (CXR) and/or chest computerized tomography (chest CT) are the main imaging
102 tools in the diagnosis of lung diseases. For a long time, the lung ultrasound (LUS) was considered
103 a "forbidden zone" in the diagnosis of lung diseases since ultrasonic waves are totally reflected
104 when encountering air. However, by utilizing ultrasonic artifacts formed by different pathological
105 changes in adults, children and newborn infants[1-5], this "forbidden zone" has been contested and
106 point-of-care lung ultrasound (POC-LUS) has been successfully used for the diagnosis of lung
107 diseases. Some authors have recommended POC-LUS as a preferred imaging modality in
108 evaluation of lung diseases due to its greater accuracy, reliability, ease of performance and lack
109 of potential adverse effects (i.e., radiation)[5-7]. In some neonatal intensive care units (NICUs), POC-
110 LUS has replaced CXR and become the first-line approach used for the diagnosis and differential
111 diagnosis of various neonatal lung diseases[5-9].

112

113 Nonetheless, the use of POC-LUS remains limited due to the lack of operating protocols,
114 diagnostic standards and guidelines. To promote proper utilization of POC-LUS in the neonatal
115 field, the Division of Perinatology of the Society of Chinese Pediatrics and the Division of Neonatal
116 Ultrasound Society of the Chinese Neonatologist Association in combination with the Chinese
117 College of Critical Ultrasound have organized an international expert panel to review the latest
118 publications on neonatal LUS. The panel summarized these expert-opinions and developed the
119 present LUS protocols and guidelines for its use. The main objective is to popularize the
120 application of POC-LUS in NICUs is reducing the number of CXR and thus avoiding the potential
121 radiation-induced harmful effects. As a real-time imaging technique, LUS is user-friendly, easy to
122 learn, and easy to replicate with appropriate training.

123

124 **Patients and timing of the LUS examination**
125 Indications for the initial POC-LUS exam include: (i) a neonate admitted for respiratory distress
126 (ii) prenatal suspicion of lung lesions, and (iii) a neonate with a sudden deterioration of
127 respiratory status.

128

129 Indications for a follow-up POC-LUS exam include: (i) helping to guide respiratory support (In
130 experienced hands, ultrasonography-assisted weaning of mechanical ventilation may
131 significantly shorten the duration of mechanical ventilation and reduce extubation failure.); (ii)
132 helping to guide changes in the level of respiratory support after surfactant delivery as well as to

133   determine the need for a repeat surfactant treatment; (iii) monitoring the progress of respiratory
134   illness when needed; (iv) following up on the changes in the lung volume or the degree of
135   atelectasis in the post-bronchoalveolar lavage period (i.e., for infants with meconium aspiration
136   syndrome, severe pneumonia, or atelectasis) as well as to improve visualization of the
137   therapeutic effects of thoracentesis (i.e. pleural effusion or pneumothorax)[10,11].

138

139   **Lung Ultrasonography Terminology**

140

141   **Pleural line and lung sliding**[12,13]: A pleural line is a hyperechoic reflection formed by the
142   difference in acoustic impedance between the pleural-lung surface interface. It appears as a
143   smooth, regular and relatively straight hyperechoic line (**Supplemental Figure 1**). Blurring,
144   irregularities, interruption of continuity or absence of the pleural line indicates abnormalities. In
145   a real-time ultrasound, the pleural line moves in a to- and fro- pattern, synchronized with
146   respiratory movement. This kind of movement is called lung sliding (**Video 1**). The absence of
147   lung sliding is always pathologic.

148

149   **A-line**[12,13]: An A-line is a type of reverberation artifact caused by multiple reflections of the pleura
150   when the probe is perpendicular to the ribs for scanning. A-lines are situated below the pleural
151   line and present as a series of smooth, clear, regular and equidistant hyperechoic parallel lines.
152   The echoes of the A-lines gradually diminish as they move deeper into the lung field where they
153   ultimately disappear (**Supplemental Figure 2**).

154

155   **B-line, confluent B-line and alveolar-interstitial syndrome**[13-15]: Based on current literature and
156   our clinical experiences in the field of neonatal lung diseases, we have defined these terms as
157   follows: A single B-line is a type of linear hyperechoic reflection of an artifact caused by an
158   ultrasound wave encountering the alveolar gas-liquid interface. B-lines arise from and are
159   roughly vertical to the pleural line. They spread downwards to the edge of the screen without
160   fading and move in synchrony with lung sliding. A confluent B-line is defined as the entire
161   intercostal space filled with B-lines (B-line fusion, reflecting B-lines that are difficult to distinguish
162   and count) between two acoustic shadows of the ribs. Alveolar-interstitial syndrome (AIS) is
163   defined as two or more sequential intercostal spaces with confluent B-lines in any scanning area
164   (**Supplemental Figure 3**).

165

166   **Compact B-lines and white lung**[15,16]: When the probe is used to scan perpendicular to the ribs,
167   the presence of concentrated B-lines may cause the acoustic shadow of the ribs to disappear
168   within the entire scanning zone. This type of B-line is called a compact B-line. A white lung is
169   present when each scanning zone on both sides of the lung presents as compact B-lines. Compact
170   B-lines and a white lung are manifestations of severe pulmonary edema (**Supplemental Figure
171   4**).

172

173   **Lung consolidation and shred sign**[17,18]: On LUS, lung fields may have a tissue-like density (lung
174   tissue 'hepatization'), which usually represents lung consolidation. Lung consolidation may be
175   accompanied by air bronchograms, fluid bronchograms, or even dynamic air bronchograms in
176   the most severe cases (**Video 2**). When the boundary between the consolidated lung tissue and

177 the aerated lung tissue is unclear, the hyperechoic ultrasonic signs formed between the two areas
178 are called shred signs (**Supplemental Figure 5**).
179
180 **Lung pulse**[19]: If lung consolidation is sufficiently large and near the edges of the heart, the
181 consolidated lung may appear to be pulsating synchronized with the heartbeat when observed
182 with real-time ultrasound. This sign is called the lung pulse (**Video 3**).
183
184 **Lung point**[13,18,20]: Under real-time ultrasound, the appearance of an alternate area where lung
185 sliding is present and then absent is called a lung point. The lung point is a specific sign of a
186 pneumothorax and can accurately locate the position of the gas boundary when a mild-moderate
187 pneumothorax is present (**Supplemental Figure 6**).
188
189 **Double lung point**[21]: Due to the differences in the severities and/or the natures of the lesions in
190 different area of the lungs, a clear difference between the upper and lower lung fields could be
191 found with perpendicular scans, which forms a sharp cut-off point between the upper and lower
192 lung field known as a double point (**Supplemental Figure 7**).
193
194 **Sandy beach sign and stratosphere sign**[20-22]: Under M-mode ultrasonography, a series of wavy-
195 line echo above the pleural line and the uniform granular dot echo (generated by the lung sliding)
196 below the pleural line can together form a beach-like sign known as a sandy beach sign or
197 seashore sign. When lung sliding disappears, the granular dot echoes are replaced by a series of
198 horizontal parallel lines. This kind of ultrasonic sign is known as a stratosphere sign or barcode
199 sign (**Supplemental Figure 8**).
200
201 **PROTOCOL:**
202
203 This work was approved by the research ethics committee of Beijing Chaoyang District
204 Committee of Science & Technology and the ethics committee of Beijing Chaoyang District
205 Maternal and child Healthcare Hospital, and that the protocol follows the guidelines of the
206 hospital's human research ethics committee.
207
208 **1. Ultrasound exam preparation**
209
210 1.1. Probe selection
211
212 1.1.1. Select a high-frequency linear probe (≥9.0 MHz) for POC-LUS to ensure high resolution.
213
214 NOTE: A higher frequency linear probe is used to assure higher resolution. For infants with a
215 lower gestational age or lower birth weight, a higher frequency probe is required. When the
216 penetration is not enough, decrease the frequency or change to a lower frequency liner probe.
217 If no suitable linear probe is available, consider using a high-frequency (≥8.0 MHz) convex array
218 probe.
219
220 1.2. Probe disinfection

1.2.1. Disinfect the probe before and after patient examination to avoid nosocomial infection and cross-contamination.

NOTE: The easiest, most convenient and effective disinfecting method is the use of special disinfection wipes. Alternatively, powderless gloves or probe covers can also be considered.

1.3. Preset selection

1.3.1. Choose a LUS preset.

1.3.2. Optimize the image for lung scanning if there is no LUS preset.

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

1.3.2.2. Modify the parameters to perform lung scanning. Adjust the **Depth** button to make it 4-5 cm.

1.3.2.3. Press the **Focus Zone** button to have 1-2 focuses and adjust it to change the focus position near the level of pleural line. Turn on the **SRI** (Speckle Reduction Imaging) button and select level 2-3 to reduce the speckle noise.

1.3.2.4. Turn on the **CRI** button (Crossbeam) and select level 2 to improve contrast resolution. Activate the **Harmonics** to improve the signal-noise ratio or use the fundamental frequency for sharper A-lines or B-lines.

1.4. Ultrasound gel application

1.4.1. Warm up the gel.

1.4.2. Apply a layer of gel on the transducer. Make sure to avoid air bubbles between the transducer and the skin surface.

2. Infant positioning

2.1. Keep the infant in a quiet state.

2.2. Swaddle the infant exposing only the area to be examined.

2.3. Place the infant in the supine, prone or side position before and during the process of examination.

NOTE: In general, we do not recommend using sedatives while pacifier use is encouraged. Supine positioning is convenient for scanning of the anterior and lateral chest. Prone or side positioning

265 is convenient for scanning of the back and lateral chest.

3. Lung partitioning

3.1. Six-region method

3.1.1. Divide each lung into three regions: anterior, lateral and posterior lung area. To do this, use the anterior axillary line and the posterior axillary line as boundaries. Divide both lungs into a total of six regions.

3.2. Twelve-region method

3.2.1. By using the line that connects the nipples, divide each lung into upper and lower lung fields, resulting in a total of 12 regions on both sides of the lungs.

NOTE: Carefully scan the entire lung fields. Each of the 6 or 12 areas should be scanned separately to ensure comprehensive coverage and to minimize the possibility of missing existing lung lesions.

4. Scan mode selection

4.1. B-mode ultrasound

4.1.1. Press the **2D** button on the user interface to start B-mode scanning.

NOTE: B-mode scanning is the most important and the most commonly used mode in obtaining LUS images. The majority of lung diseases can be found with B-mode scanning.

4.2. M-mode ultrasound

4.2.1. Press the **M** button on the user interface to start M mode scanning if needed.

NOTE: M-mode ultrasound is helpful for further confirmation of the possibility of pneumothorax.

4.3. Color or power Doppler ultrasound

4.3.1. Press the **C** button or **PD** button on the user interface to start the color or power Doppler examination if needed.

NOTE: Doppler ultrasound is used occasionally to assess the blood flow in the large area of lung consolidations or to distinguish the bronchi from blood vessels.

5. Scanning methods

309   5.1. Perpendicular scanning

310

311   5.1.1. Place the transducer perpendicular to the ribs and slide it from the midline to the lateral
312   side along the wide axis to perform the perpendicular scanning.

313

314   5.1.2. After initial area of the lung is scanned, move the transducer from up to down and scan
315   the remaining areas until all the lung fields are examined.

316

317   NOTE: Perpendicular scanning is the most important scanning method. Keeping the transducer
318   perpendicular to the ribs is the key to obtaining accurate and reliable results.

319

320   5.2. Parallel scanning

321

322   5.2.1. Rotate the transducer 90° after finishing the perpendicular scanning. Keep the transducer
323   parallel to the ribs and slide it along the narrow axis to realize the parallel scanning.

324

325   5.2.2. After the initial area of the lung is scanned, move the transducer from up to down to scan
326   the remaining areas until all the lung fields are examined.

327

328   5.3. Transdiaphragmatic scanning

329

330   5.3.1. Place the transducer below the xiphoid and angle the transducer from side to side to scan
331   the diaphragm and the bottom of lungs via the liver as the acoustic window.

332

333   NOTE: Increase the depth and turn on virtual convex scanning to expand the far field area if
334   needed.

335

336   **REPRESENTATIVE RESULTS:**
337   The main purpose of this protocol and guideline is to instruct users on how to use LUS to diagnose
338   and differentiate common neonatal lung diseases. These include respiratory distress syndrome
339   (RDS), transient tachypnea of the newborn (TTN), pneumonia, meconium aspiration syndrome
340   (MAS), pulmonary hemorrhage, pulmonary atelectasis and pneumothorax, etc. Thus, the normal
341   neonatal LUS characteristics and the LUS diagnostic criteria for different lung diseases are
342   described in detail.

343

344   **Normal Neonatal Lung Ultrasound**
345   The neonatal normal lung field appears hypoechoic on a B-mode ultrasound. Pleural lines and A-
346   lines are smooth, regular and straight. As mentioned previously, A-lines are hyperechoic,
347   arranged in parallel and equidistant from one each other. A-line echoes gradually diminish until
348   they disappear from the shallow to the deep part of the lung fields. There may not be any B-lines
349   (three to seven days after birth) or just a few B-lines (within three to seven days after birth) in
350   the lung fields. However, there is no AIS, pleural effusion, or lung consolidation. Lung sliding is
351   detectable by real-time ultrasound, whereas in M-mode imaging, a linear pattern appears in

352 tissues superficial to the pleural line, and a grainy or sandy pattern appears below the pleural
353 line, creating the seashore sign (**Figure 1**)[23, 24].
354
355 **LUS Characteristics and Diagnostic Criteria for Lung Diseases of the Newborn Infants**
356
357 **Respiratory distress syndrome (RDS) of the newborn**
358 RDS refers to a lung disease where main clinical manifestations are tachypnea, retractions,
359 grunting and cyanosis. It presents immediately after birth. RDS is caused by a primary or
360 secondary deficiency of pulmonary surfactant in preterm and term neonates respectively. Lack
361 of surfactant causes development of pulmonary atelectasis and low lung volumes[25-27]. Currently
362 the diagnosis of RDS is based on history, clinical manifestations and CXR findings. However, RDS
363 can also be diagnosed easily and accurately by LUS. A meta-analysis that included 673 newborn
364 infants with RDS showed that the sensitivity and specificity of LUS in diagnosing RDS was 99%
365 and 96%, respectively[28].
366
367 LUS diagnosis of RDS is based on the following findings[16,28-34]. (i) Lung consolidations
368 accompanied by air-bronchograms are the most important LUS manifestation of RDS, which is
369 characterized by the following: (a) Consolidations are most often observed in the posterior parts
370 of the lungs. The degree of consolidation is related to the severity of the disease. (b)
371 Consolidations are limited only to the region beneath the pleura in mild RDS patients. Conversely,
372 the areas of consolidation may extend to deeper parts of the lung fields in more severe RDS. (c)
373 Usually, consolidations are visible in different lung fields bilaterally. Nevertheless, they may be
374 limited to certain intercostal spaces on one side of the lung. Consolidated areas show an uneven
375 hypoechoic quality and the boundary with surrounding lung tissue is clear and easy to distinguish.
376 (d) Air-bronchograms show dense, speckled or snowflake-like shapes. (ii) The pleural line is
377 abnormal, and the A-lines disappear. (iii) The non-consolidated zones may appear as AIS. (iv) 15%
378 to 20% of patients may have different degrees of unilateral or bilateral pleural effusion.
379
380 In addition, changes in pulmonary status can be efficiently followed-up by LUS. Improvements in
381 LUS findings are often first observed in anterior lung areas because these areas are non-
382 dependent and better ventilated. Transition from consolidation to aggregation-induced emission
383 (AIE), AIE to interstitial edema (IE), and IE to a normal LUS pattern or vice-versa can be seen. This
384 LUS quality allows for estimation of the surfactant replacement therapy effect (**Figure 2**).
385
386 **Transient tachypnea of the newborn (TTN)**
387 TTN is also known as 'wet lung' of the newborn. It is one of the most common respiratory diseases
388 in newborn infants. TTN is self-limited with most patients recovering within 24-72 hours without
389 any special intervention. Rarely, it can lead to severe respiratory distress, hypoxemia,
390 pneumothorax or even death[35,36]. TTN is often underdiagnosed, especially among premature
391 infants. It has been reported that 62% to 77% of infants who were clinically diagnosed with RDS
392 actually had TTN according to the traditional diagnostic criteria[36,37]. LUS can eliminate such
393 misdiagnoses since TTN can be easily differentiated from RDS and other lung diseases by LUS.
394

395 The main characteristic of TTN is lung edema without lung consolidations, and it is diagnosed
396 based on the following findings[21,30,31,38,39]. (i) Mild TTN mainly manifests as AIS and a double lung
397 point. Severe TTN in the acute period mainly manifests as a compact B-line, white lung, or severe
398 AIS, while a double lung point may appear with disease recovery. (ii) Mild or severe TTN is
399 characterized by pleural line abnormalities, A-line disappearance, and different degrees of
400 pleural effusion in one or the bilateral side of chest. (iii) No consolidation is observed in the lung
401 fields (**Figure 3**).
402
403 **Pneumonia of the newborn**
404 Pneumonia refers to inflammation of the lung parenchyma, including the terminal airway,
405 alveolar space and pulmonary interstitial areas. It is caused by infectious microorganisms or
406 physical or chemical factors. Pathologically, alveolar inflammatory exudates, hyperemia and
407 edema are present. When bronchiolar epithelium cell necrosis occurs, mucous and cellular debris
408 in the lumen can cause regional air trapping and atelectasis. Pneumonia is responsible for more
409 than 1/3 of all newborn hospitalizations and infectious pneumonia accounts for more than 1/4
410 of all neonatal deaths especially in the developing world[40,41]. A meta-analysis showed a sensitivity
411 higher than 96% and specificity higher than 93% when LUS is used to diagnose pneumonia both
412 in adults and children[42,43].
413
414 LUS imaging characteristics of pneumonia include the following[43-48]. (i) Lung consolidations
415 accompanied by air-bronchograms or fluid-bronchograms; Lung consolidations are the main
416 ultrasound-imaging feature of pneumonia, which are characterized by the following: (a) The size
417 of the consolidation in severe pneumonia is usually large with irregular or jagged boundaries. The
418 shred sign is visible at the edges of the consolidated areas and the dynamic-bronchograms are
419 often visible in severe patients. (b) Consolidations may be located at one or more positions in the
420 lung fields, and consolidated areas may differ in size and shape in the different lung fields. (ii) The
421 pleural line is abnormal and A-lines disappear. (iii) B-lines or AIS are visible in the nonconsolidated
422 areas. (iv) Different degrees of unilateral or bilateral pleural effusion are visible in some infants.
423 (v) The main manifestations of mild or early pneumonia may be presented as small subpleural
424 focal consolidations and AIS (**Figure 4**).
425
426 **Meconium aspiration syndrome (MAS) of the newborn**
427 MAS is due to fetal hypoxia leading to defecation and inhalation of meconium-stained amniotic
428 fluid by the infant before or during the delivery process. Meconium particles cause mechanical
429 obstruction of the terminal bronchioles and alveoli together with chemical inflammation and
430 secondary surfactant deficiency. These changes further lead to air-trapping, atelectasis and
431 alveolar or interstitial pulmonary edema. Infants with severe MAS often present with signs of
432 severe respiratory distress including cyanosis, tachypnea, nasal flaring, and retractions and
433 grunting within hours of birth. MAS is a serious lung disease accounting for approximately 10%
434 of all cases of neonatal respiratory failure. Among these patients 10% to 20% will experience
435 pneumothorax and the reported mortality can be as high as 39% in developing and newly
436 industrialized countries[49,50].
437

438   The bases for the LUS diagnosis of MAS are as follows[51-53]: (i) Lung consolidations accompanied
439   by air-bronchograms are the most important sonogram characteristic of MAS. The scope of
440   consolidation is related to the degree of the disease. The edges of the consolidation area are
441   irregular or jagged and the shred sign is visible. The degrees of consolidation may differ between
442   the two sides of the lung. Similarly, different sizes of consolidation may be present on the same
443   side of the lung. (ii) The pleural line is abnormal, and the A-line disappears. (iii) The B-lines or AIS
444   are visible in the nonconsolidated zone. (iv) Some patients may have different degrees of
445   unilateral or bilateral pleural effusion. It is difficult to differentiate MAS and pneumonia solely
446   based on ultrasound manifestations. Therefore, to obtain a definitive diagnosis it is often
447   necessary to combine ultrasound findings with perinatal history, physical exam and laboratory
448   findings (**Figure 5**).
449
450   **Pulmonary hemorrhage of the newborn (PHN)**
451   PHN is not an independent lung disease. In general, it is a late complication of other diseases, its
452   onset is sudden and the infant deteriorates rapidly causing PHN to have a high mortality rate.
453   Pathologically, PHN can present as a focal, regional, or diffuse hemorrhage, usually with alveolar
454   structural damage. The interstitial area of the lung can also be affected. PHN often occurs within
455   the first several days after birth with nearly 90% of PHN occurring within the first week of life[54,55].
456
457   The main LUS characteristics in PHN are as follows[56,57]: (i) The shred sign is the most common
458   and the most important LUS sign of *PHN*. (ii) The degree of lung consolidations accompanied by
459   air-bronchograms are closely related to the severity of the primary diseases. (iii) More than 80%
460   of the patients have different degrees of unilateral or bilateral pleural effusion. Thoracentesis
461   usually confirms the effusion is bleeding. In severe cases, fibrous, cordlike, floating objects
462   formed by fibrin degeneration are visible within the effusion. These objects can be seen floating
463   in the effusion along with respiratory movement by real-time ultrasound. (iv) Miscellaneous signs
464   include pleural line abnormalities, A-line disappearance and AIS (**Figure 6**).
465
466   **Pulmonary atelectasis of the newborn**
467   Inadequate aeration resulting from collapse of previously expanded pulmonary tissue is defined
468   as atelectasis[49,50]. Atelectasis can be divided into obstructive and compressive atelectasis based
469   on the pathophysiology. It can also be divided into complete atelectasis and incomplete
470   atelectasis according to the degree of atelectasis. It is not also an independent disease but rather
471   a common complication of multiple diseases. Atelectasis is a common cause of neonatal
472   respiratory distress and often contributes to prolonged illness or difficulty weaning from
473   ventilator support. Correct diagnosis and appropriate treatment lead to improved outcomes[58,59].
474   LUS has a great diagnostic value in cases of pulmonary atelectasis.
475
476   Characteristic LUS findings include[60-62]: (i) Lung consolidation accompanied by air bronchograms,
477   or even dynamic bronchograms or parallel air bronchograms are visible in severe cases. (ii) The
478   edges of the consolidation area are relatively clear and regular in severe large-area pulmonary
479   atelectasis. If the atelectasis is limited to a small region, the edges of the consolidation area may
480   not be obvious. (iii) The pleural line in the consolidation area is abnormal and A-lines disappear.
481   (iv) In the early stages of severe or large-area atelectasis, the lung pulse may be visible while lung

482   sliding often disappears under real-time ultrasound. (v) The pulmonary blood flow may be visible
483   in the consolidated areas by color or power Doppler ultrasound. If atelectasis persists (the final
484   stages of atelectasis), both the dynamic bronchograms and the blood flow will disappear (**Figure**
485   **7, Figure 8, Supplemental Video 1, Supplemental Video 2**).
486

487 **Pneumothorax of the newborn**
488   Abnormal accumulation of air in the pleural space is defined as a pneumothorax. It is a relatively
489   common but critical neonatal illness associated with high morbidity and mortality especially in
490   preterm infants[63,64]. Ultrasound diagnosis of a pneumothorax is very sensitive and specific. Both
491   meta-analysis and prospective controlled studies have shown that LUS is more accurate than CXR
492   for the detection of pneumothorax[66,67].
493

494   Pneumothorax is diagnosed based on the following LUS signs[20,65-68]: (i) Disappearance of lung
495   sliding is the most important sign in the ultrasound diagnosis of pneumothorax. If lung sliding is
496   present, pneumothorax can essentially be excluded. (ii) There are no B-line or comet tail signs, if
497   present pneumothorax can also be excluded. (iii) The clear presence of the lung point is a specific
498   sign for ultrasound diagnosis of mild-to-moderate pneumothorax. However, there is no lung
499   point in severe pneumothorax. The specificity of the lung point in diagnosing pneumothorax is
500   100% while the sensitivity of approximately 70% or higher[21]. (iv) The pleural line and A-lines are
501   present. Pneumothorax can be excluded if these lines disappear. (v) On M-mode imaging the
502   sandy beach signs are replaced by the stratosphere signs (**Figure 9, Figure 10, Video, 5, Video 6**).
503

504   For beginners, the following steps may be taken if there are clinical doubts. (i) First, observe the
505   pleural line and the A-line: if they are absent, pneumothorax can be excluded. (ii) If the pleural
506   line and A-lines are present (that is normal lung appearance under B-mode ultrasound), observe
507   lung sliding under real-time ultrasound. If it is present, pneumothorax can be excluded. (iii) If lung
508   sliding disappears, observe the B-line or comet tail sign. If either is present, pneumothorax can
509   be excluded. (iv) If lung sliding disappears and there is no B-line, observe the lung point. If it is
510   present, then mild-to-moderate pneumothorax is essentially confirmed. If it is absent, then
511   severe pneumothorax may have occurred. (v) On M-mode imaging, if the beach sign is replaced
512   by a stratosphere sign, the existence of pneumothorax is further confirmed. The pneumothorax
513   diagnostic procedure is shown in the **Figure 11.**
514

515 **[Place Fig 11 here]**
516

517 **Pulmonary edema in cardiac insufficiency**
518   Causes of pulmonary edema in newborns are similar to the ones in the adult population. In
519   addition to the newborns with congenital heart diseases or cardiac insufficiency, many preterm
520   infants with bronchopulmonary dysplasia (BPD) may show signs that are consistent with
521   pulmonary edema[69,70]. Occasionally, LUS shows an increase in bilateral B-lines or interstitial fluid
522   even before CXR. This pattern may improve upon cardiac treatment or surgery.
523

524 **Examining correct ETT placement and position**

In pediatric and neonatal populations, studies have shown that POC-US is a feasible tool that has been used clinically to verify both correct endotracheal tube (ETT) placement and an acceptable ETT tip position[71-75]. Proper ETT placement includes both tracheal intubation and an acceptable ETT tip position. Visualization of the ETT tip at a distance ranging from 0.5 to 1.0 cm from the upper border of the aortic arch suggests that the ETT is not too deep. This method has been validated in several studies[73]. A recent study confirmed these findings and found that ultrasound provided images more rapidly than CXR (mean 19.3 vs. 47 minutes, respectively)[72]. The concordance of POC-US with CXR to recognize deep and shallow ETT tips was 95%. The sensitivity of LUS to detect deeply positioned ETT tips on X-ray was 86% (specificity of 96%)[73]. Other studies have evaluated the distance from the ETT tip to the superior aspect of the main pulmonary artery that anatomically corresponds to the level of the carina and found a good correlation between this technique and radiography[75,76].

FIGURE LEGENDS:

Figure 1: Neonatal normal LUS characteristics.
On B-mode imaging, the pleural line and A-line show smooth, regular and hyperechoic lines arranged in parallel and equidistant from each other. The A-line echoes gradually diminish until they disappear. In M-mode, a seashore sign is present.

Figure 2: LUS image characteristics of RDS patients.
(**A**) CXR of a patient with grade II-III RDS (A-1). LUS shows lung consolidation with air bronchograms in bilateral lung fields, disappearance of the pleural line and A-lines (A-2: left lung, A-3: right lung).
(**B**) CXR of a patient with grade III RDS (B-1). LUS shows a large area of consolidation and a small effusion in the left lung (B-2), significant consolidation in the upper field and a large amount of pleural effusion in the lower field of the right lung (B-3).

Figure 3: LUS image characteristics of TTN patients.
(**A**) Double lung point. Clear, sharp cut-off point between the upper and lower lung fields. It is formed when there are differences in the degrees of pathological changes. This sign is often observed in mild TTN.
(**B**) LUS shows a disappearance of the pleural line and A-lines, as well as AIS in the lung fields.
(**C**) An area of fluid in the right lung indicating a pleural effusion.
(**D**) The dense B-line causes the acoustic shadows of the ribs to disappear from the entire scanned area. This type of B-line is called a compact B-line. White lung is defined as the existence of compact B-lines within each lung field. Both compact B-lines and white lung are common ultrasound signs of severe TTN.

Figure 4: LUS image characteristics of pneumonia patients.
(**A**) Vertical scanning: The image demonstrates large areas of lung consolidation with air bronchograms in the lung field. The consolidation area has irregular boundaries.
(**B**) Horizontal scanning: The image shows large areas of lung consolidation with significant air bronchograms in the lung field.

569 (**C**) Extended view: A severe pneumonia patient. Extended view shows a whole aspect of the
570 consolidations involving the left lung.
571
572 **Figure 5: LUS image characteristics of MAS patients.**
573 (**A**) LUS shows large areas of pulmonary consolidation with irregular edges, especially in the right
574 lung. This finding is consistent with the CXR.
575 (**B**) LUS shows a large lung consolidation with the air bronchograms, irregular edges, abnormal
576 pleural line and the absence of A-lines. The CXR shows patchy opacities that highly suggest MAS.
577
578 **Figure 6: LUS image characteristics of PHN.**
579 (**A**) Ultrasound findings in a severe PHN patient. CXR shows bilateral hazy lung fields with low
580 lung volumes and pleural effusions. Middle and right: LUS shows a large area of lung
581 consolidation with an air bronchogram, shred sign at the edge of the consolidation and pleural
582 effusions in both sides of the lungs. The pleural effusion confirmed to be hemorrhagic by
583 thoracentesis. Pleural line and A-line are absent. Fibrous protein depositions are observed as
584 cordlike floating objects on real-time ultrasound.
585 (**B**) Pleural effusion as the main ultrasound finding in PHN patients. LUS shows significant pleural
586 effusion on both sides of the chest (more severe on the right). This finding is consistent with the
587 CXR. The fluid was confirmed to be bloody by thoracentesis. The other findings are AIS and mild
588 shred signs.
589
590 **Figure 7: LUS image characteristics of pulmonary atelectasis of the newborn.**
591 LUS shows a large consolidation area with regular edges in the right lung (**A, B, C**). The
592 echogenicity of the consolidated lung tissue is similar to that of the adjacent liver tissue (**B, C**).
593 Significant air bronchograms are observed (**C**).
594
595 **Figure 8: Blood flow within atelectasis**
596 (**A**) B-mode LUS shows a large area consolidation with a significant air-bronchograms as well as
597 regular margins, presented as atelectasis.
598 (**B**) Color Doppler ultrasound shows significant arterial blood supply within consolidated area of
599 the lung (**Video 4**).
600
601 **Figure 9: Lung point in mild-moderate pneumothorax**
602 (**A**) TTN patient with pneumothorax. The B-mode LUS shows an abnormal pleural line, AIS and
603 disappearing A-lines in the left lung. Right lung shows a lung point. Lung sliding occurs in the B-
604 line area but is absent in the A-line area on real-time ultrasound (Video 5).
605 (**B**) RDS patient with pneumothorax. B-mode LUS shows a large lung consolidation with air
606 bronchograms in the left lung and a small consolidation in the right lung. The pleural line and A-
607 lines are present on the right side of the right lung.
608 (**C**) Lung point under M-mode ultrasound. Left lung shows the beach sign. Right lung shows the
609 lung point (the point between beach sign and stratosphere sign), confirming mild pneumothorax.
610
611 **Figure 10: LUS in massive pneumothorax**

(**A**) CXR shows severe pneumothorax in the left lung. Pleural line and A-lines are present on the left lung but no lung point is found. LUS shows AIS in the right lung. Lung sliding disappears in the whole left lung field while present on the right on real-time ultrasound (**Video 6**).
(**B**) Under M-mode ultrasound the right lung shows a beach sign while the left lung presents a stratosphere sign (also known as a barcode sign). This confirms a severe pneumothorax in the left hemithorax.

Figure 11: Flowchart for pneumothorax diagnostic procedure

Video 1: Lung sliding
The pleural line moves in synchrony with respirations.

Video 2: Dynamic air bronchograms
When severe lung consolidation is present air-bronchograms move with the respirations. This kind of air-bronchogram is also known as dynamic air-bronchogram.

Video 3: Lung pulse
If the area of lung consolidation is large enough, the consolidated lung pulsates in synchrony with heartbeats, this kind of pulsation is called the lung pulse.

Video 4: Blood supply in atelectasis area
The rich blood supplying can be found under Color Doppler ultrasound.

Video 5: Lung point in a mild-moderate pneumothorax patient
Lung sliding occurs in the B-line area but is absent in the A-line area on real-time ultrasound.

Videos 6: Disappeared lung sliding in a severe pneumothorax patient
Lung sliding disappeared in the entire right lung field. It is presented in the left lung.

Supplemental Figure 1: Pleural line
Under B-mode ultrasound, the pleural line appears as a smooth, regular hyperechoic lines.

Supplemental Figure 2: A-lines
A-lines are situated below the pleural line. They present as a series of smooth, linear hyperechoic parallel lines.

Supplemental Figure 3: B-line, confluent B-line, and AIS
(**A**) B-lines. B-lines arise from and are roughly vertical to the pleural line.
(**B**) Confluent B-lines. Confluent B-lines occur when the entire intercostal space is full of intense B-lines, but the acoustic shadow of the ribs is still clearly displayed.
(**C**) Alveolar-interstitial syndrome. AIS is defined by the presence of two or more sequential intercostal spaces with confluent B-lines in any scanning area.

Supplemental Figure 4: Compact B-lines.

656 Compact B-lines refer to the concentration of B-lines that causes acoustic shadow of the ribs to
657 disappear within the scanning zone. White lung occurs when each scanning zone on both sides
658 of the lung presents as compact B-lines.
659
660 **Supplemental Figure 5: Lung consolidation and shred sign.**
661 (**A**) Lung consolidation. On LUS lung tissues gives appearance of tissue-like density, also called
662 'hepatization' of the lung.
663 (**B**) Shred sign. When the boundary between consolidated lung tissue and aerated lung tissue is
664 unclear the ultrasound sign formed between the two areas is called a shred sign.
665
666 **Supplemental Figure 6: Lung point**
667 The transition point from the B-line area to the parietal pleura and A-line existing area is the lung
668 point.
669
670 **Supplemental Figure 7: Double lung point.**
671 Differences in the degree or pathologic changes between upper and lower lung fields indicate a
672 double lung point.
673
674 **Supplemental Figure 8 Sandy beach sign and stratosphere sign**
675 Under M-mode ultrasound, the part A presents the sandy beach sign (generally excluded
676 pneumothorax) while the part B shows the stratosphere sign (generally seen in pneumothorax).
677
678 **Supplemental Video 1: Lung pulse in a patient with severe atelectasis**
679 Severe atelectasis in the left lung. Movement of the atelectatic lung can be observed with the
680 heart beat by real-time ultrasound; this movement is called the lung pulse.
681
682 **Supplemental Video 2: Dynamic air bronchograms in a patient with severe atelectasis**
683 Air bronchograms are observed with respiratory movement by real-time ultrasound. This kind of
684 movement is known as a dynamic air bronchogram and is a common ultrasound sign in severe
685 atelectasis patients.
686
687 **DISCUSSION:**
688 POC-LUS is a feasible and convenient diagnostic method that can be performed in the NICU at
689 the bedside. It is very sensitive and reliable in the diagnosis of all types of neonatal lung
690 diseases[77]. Furthermore, it has many advantages over the CXR and CT scan such as accuracy,
691 reliability, low cost, simplicity and no risk of adverse effects due to radiation. Therefore, we
692 encourage the use of LUS in the NICU. When learning this imaging modality, the following issues
693 need to be carefully considered: (1) Examiners require at least 6-8 weeks of training. They have
694 to evaluate 20-30 patients with each type of lung disease to master the technique. The diagnostic
695 sequence for pneumothorax is more challenging in neonates compared to older children or
696 adults. We suggest that in this case trainees receive extra training time. (2) Examiners operate in
697 strict accordance with the operating procedures of the ultrasound instrument. (3) Examiners
698 should reduce adverse stimulation of neonate as much as possible. The ultrasound exam is to be
699 performed at appropriate times, especially in high-risk infants. (4) The exam is to be ideally

700 performed with a quiet and calm neonate. No sedatives are needed to perform the examination.
701 (5) Care must be taken to keep the neonate warm. Ultrasound gel must be preheated. (6)
702 Sterilization and isolation procedures must be observed. The operators should wash their hands,
703 carefully clean and sterilize the probe and use a protective plastic probe cover to avoid cross-
704 contamination.

706 Perpendicular scanning is the most important and most commonly used scanning method. Since
707 sub-pleural lung tissue is located at the distal end of the bronchial and blood supply, it is more
708 likely to be affected by different lung diseases. Therefore, perpendicular scanning can delineate
709 nearly the entire lung anatomy in neonates. Certainly, parallel scanning is also very helpful in
710 detecting mild lung lesions (i.e., pathological changes involving only 1-2 intercostal spaces and
711 limited to the subpleural areas) or in identifying the "lung point" when a mild-moderate
712 pneumothorax is suspected[10]. When the lesions mainly involve the bottom of the bilateral lungs,
713 scanning may also be performed below the diaphragm via the liver as an acoustic window. This
714 type of scanning can also be used to examine the integrity of the diaphragm and the presence of
715 pleural effusions.

717 In clinical practice, however, LUS examination should not be limited to a fixed scanning sequence.
718 The scan can be performed from the most convenient place based on the infant's position during
719 the examination. Starting LUS scanning from the back is acceptable and easy to perform. It also
720 avoids interference from the heart and the large vessels. Further scanning in other areas of the
721 lungs must be performed in any infant with high suspicion of a pulmonary lesion in a situation
722 where scanning of the back reveals no abnormalities.

724 Occasionally, we may use the extended view (XTD-View) function. The XTD-View function can
725 construct an extended image from individual image frames as the operator slides the transducer
726 along the narrow axis of the probe. XTD-View allows the doctors to assess the interesting areas
727 and neighboring structures fully (**Figure 4C**). To do this, we should orient the transducer parallel
728 to the direction of transducer motion before activating the XTD-View button. It is necessary to
729 slide the transducer towards the notch and keep the transducer perpendicular to the ribs during
730 the whole scanning.

732 LUS has some limitations. (1) It is highly operator dependent. Therefore, it is necessary to gain
733 sufficient experience to fully understand the basic principles of LUS before performing
734 examinations. (2) Subcutaneous emphysema affects the image quality as well as accuracy of the
735 results, thus it may interfere with scanning. (3) The role of LUS in emphysema,
736 pneumomediastinum and the diagnosis of bronchopulmonary dysplasia remains uncertain. (4)
737 Some mild cases may be missed if the scanning is not performed carefully. (5) It was reported
738 that LUS has a limited value as a diagnostic tool for rare cystic lung diseases, such as
739 lymphangioleiomyomatosis, pulmonary Langerhans cell histiocytosis and Birt-Hogg-Dubé
740 syndrome[78].

742 Current literature offers well-designed, systematic and in depth research in the area of LUS.
743 Research findings have been validated and confirmed in clinical practice. Our protocol and

744 guidelines have been developed after a thorough evidence-based review of the currently
745 available data by a panel of international experts in this field.
746

761 **DISCLOSURES**
762 The authors have nothing to disclose.
763

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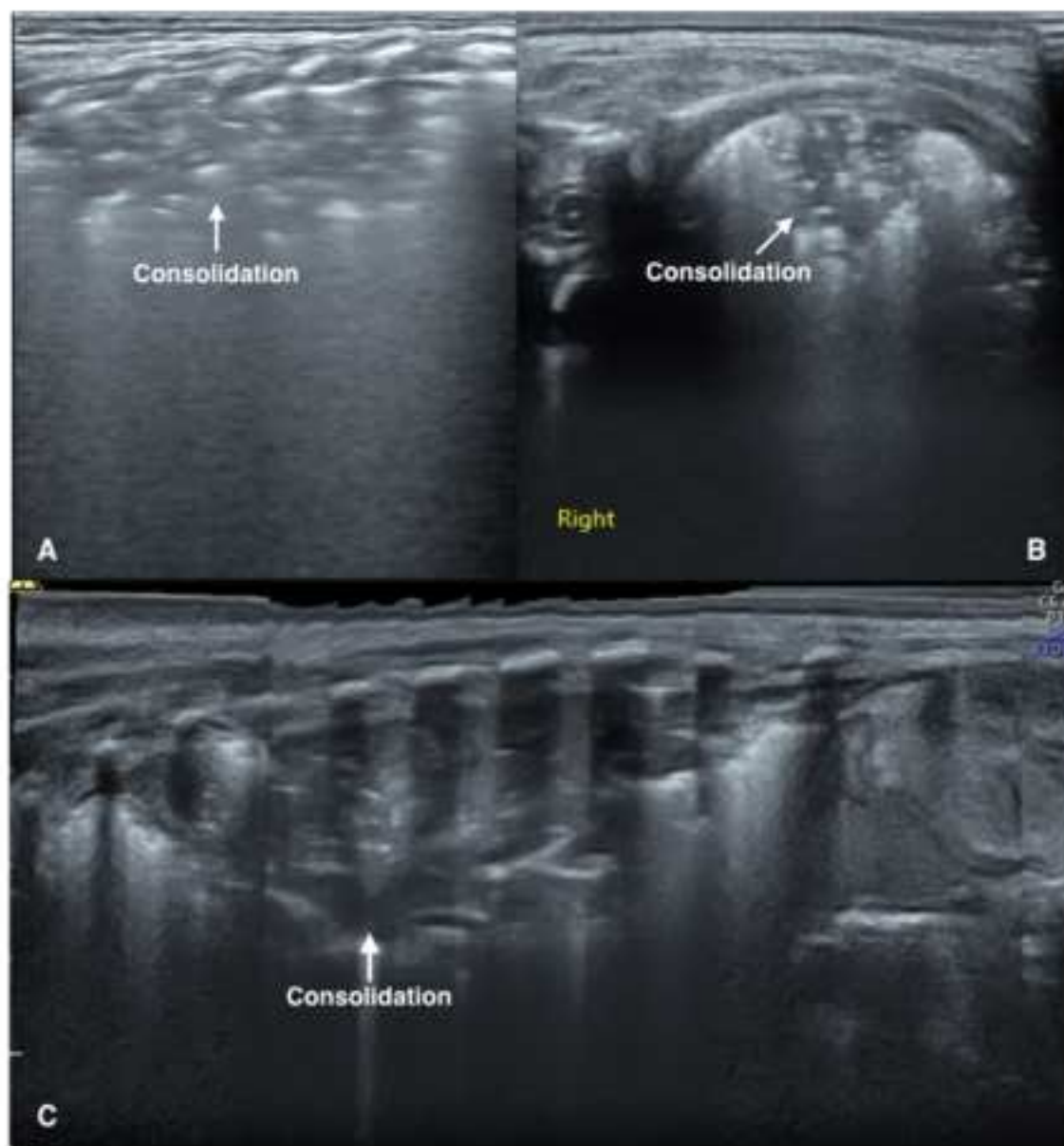938   tube position in neonates. *Neonatology.* **102** (3), 185-189(2012).

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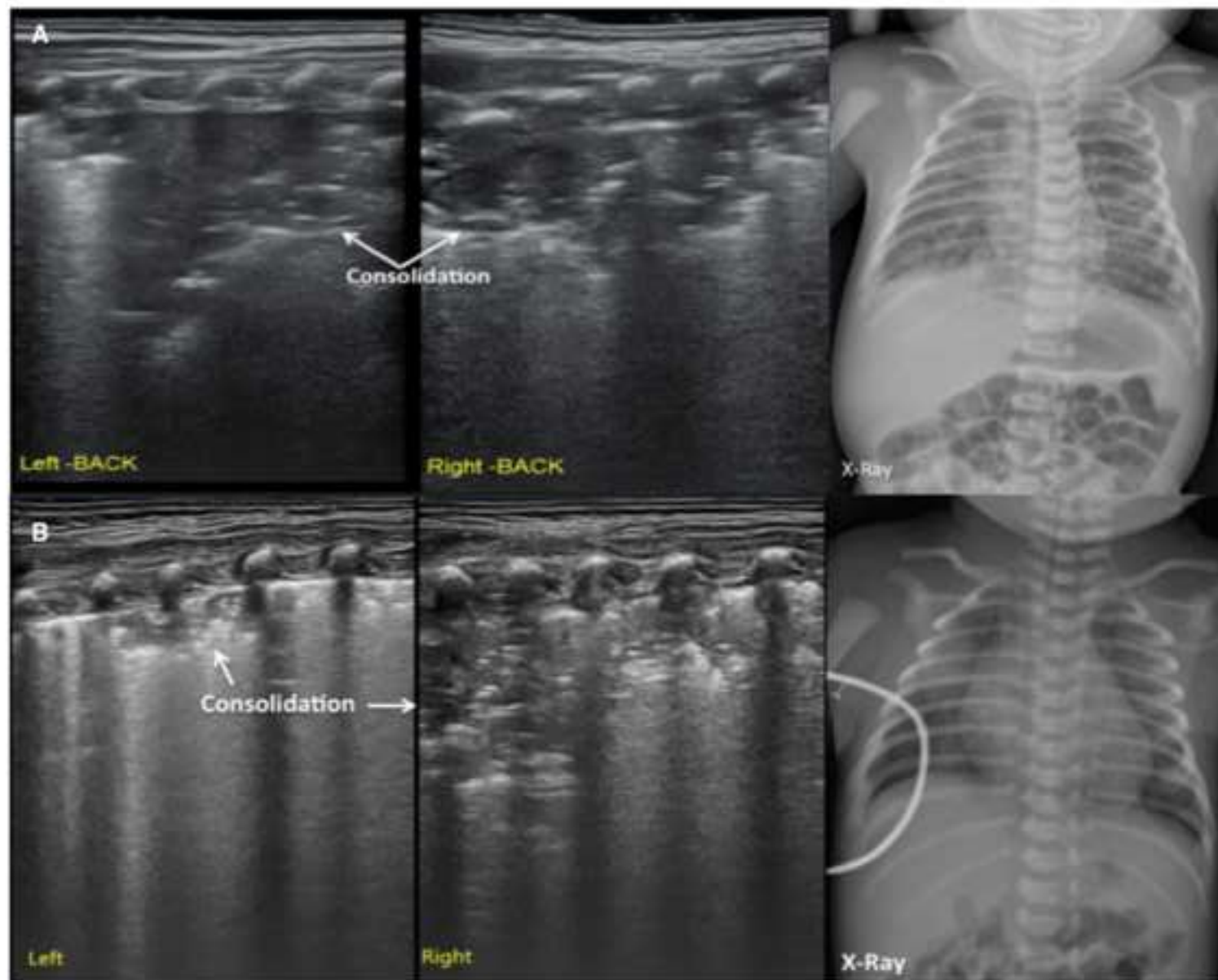
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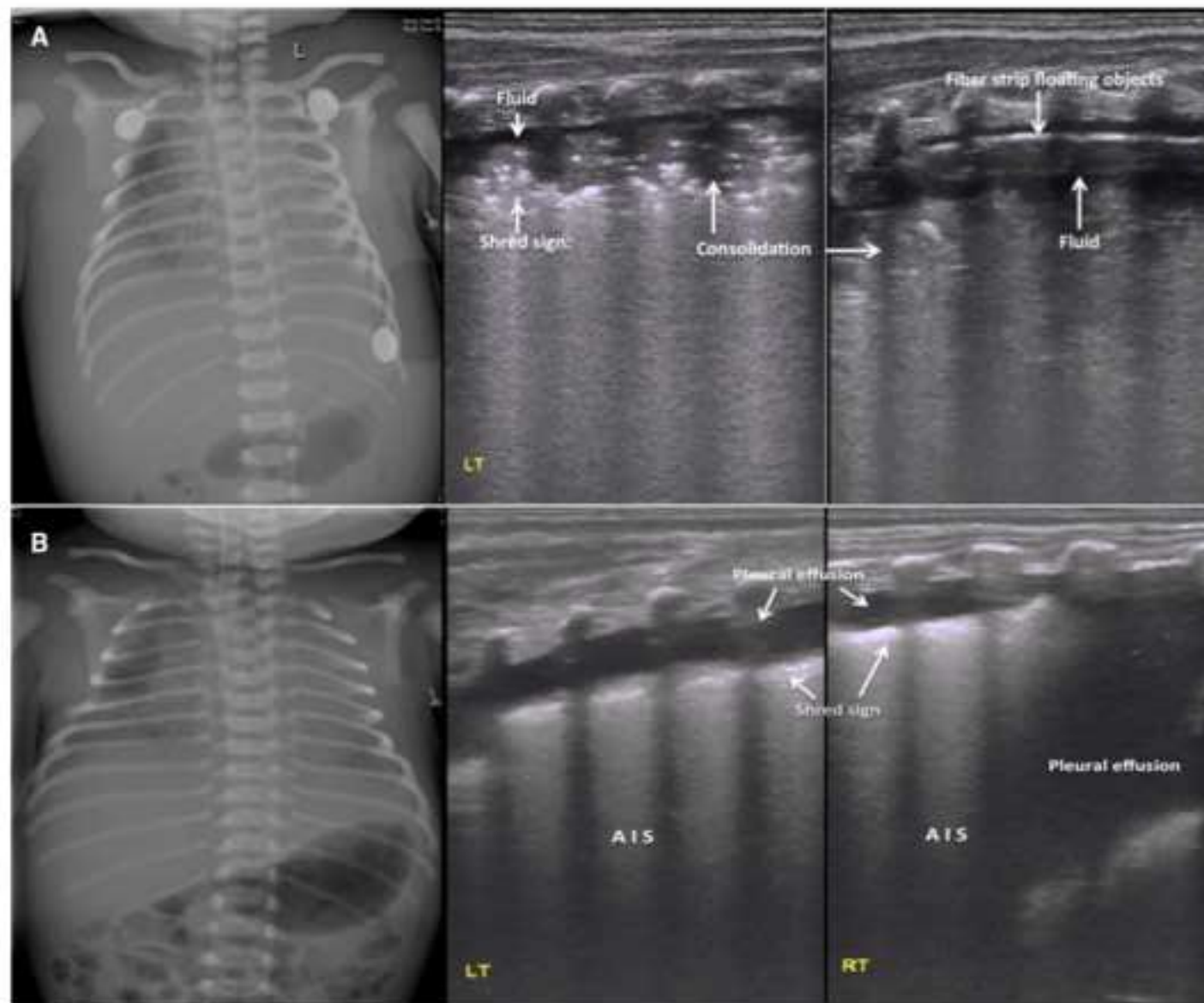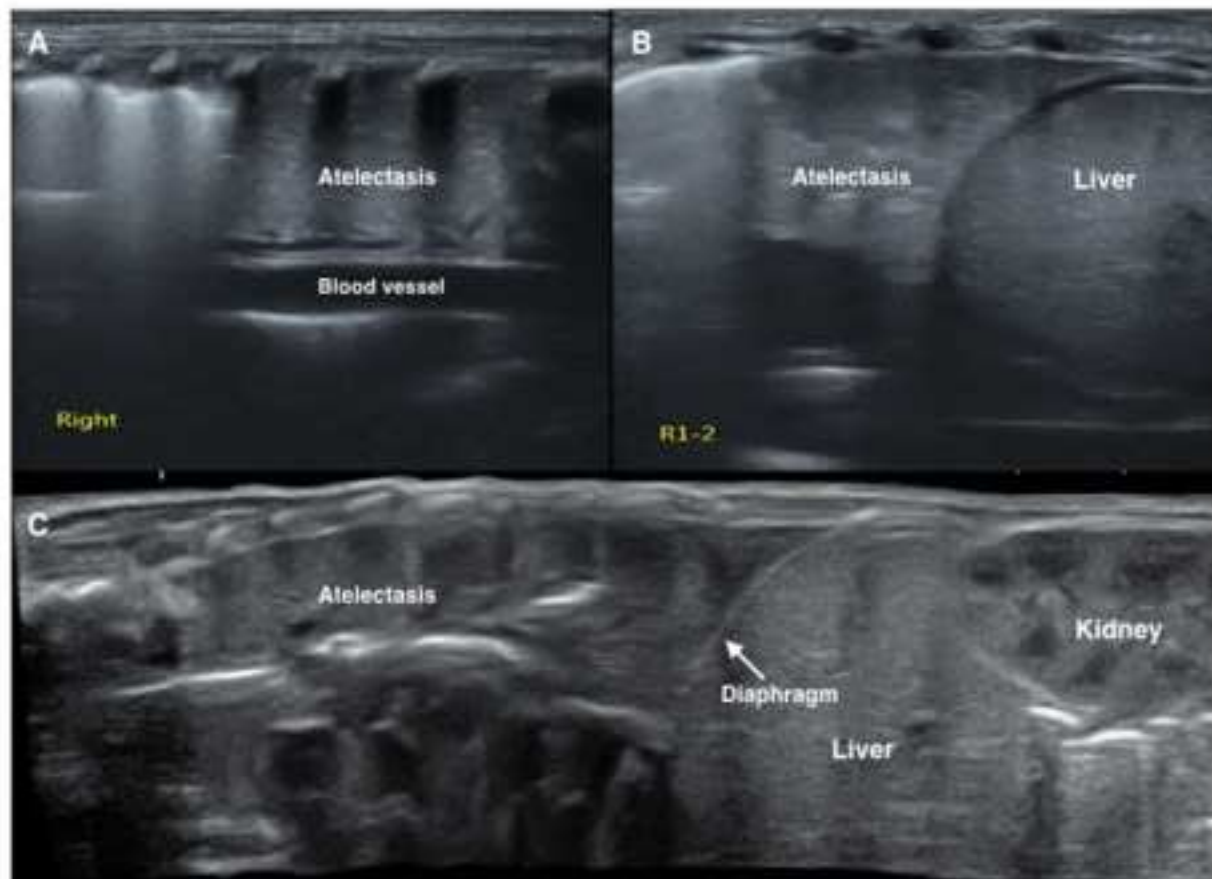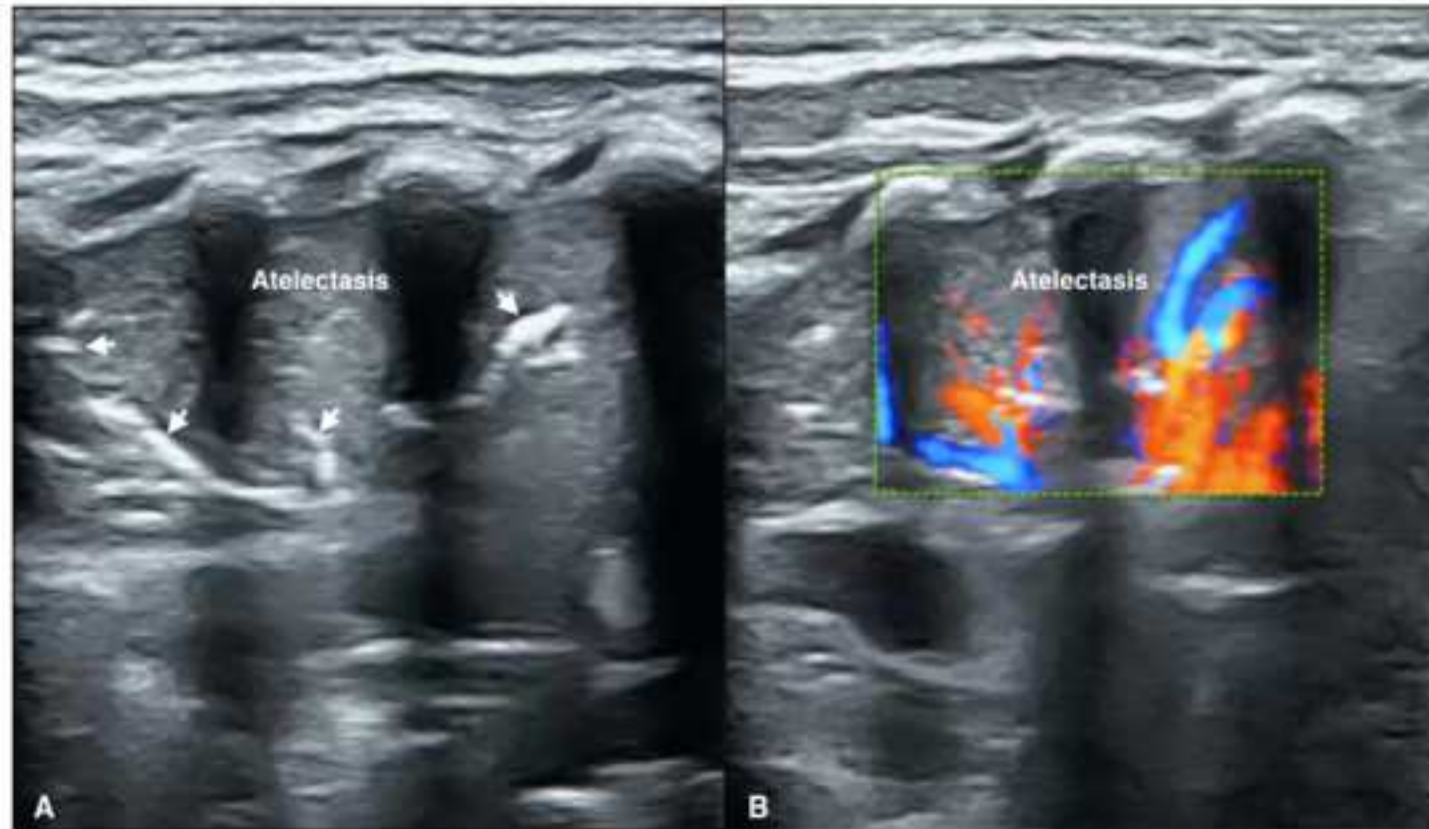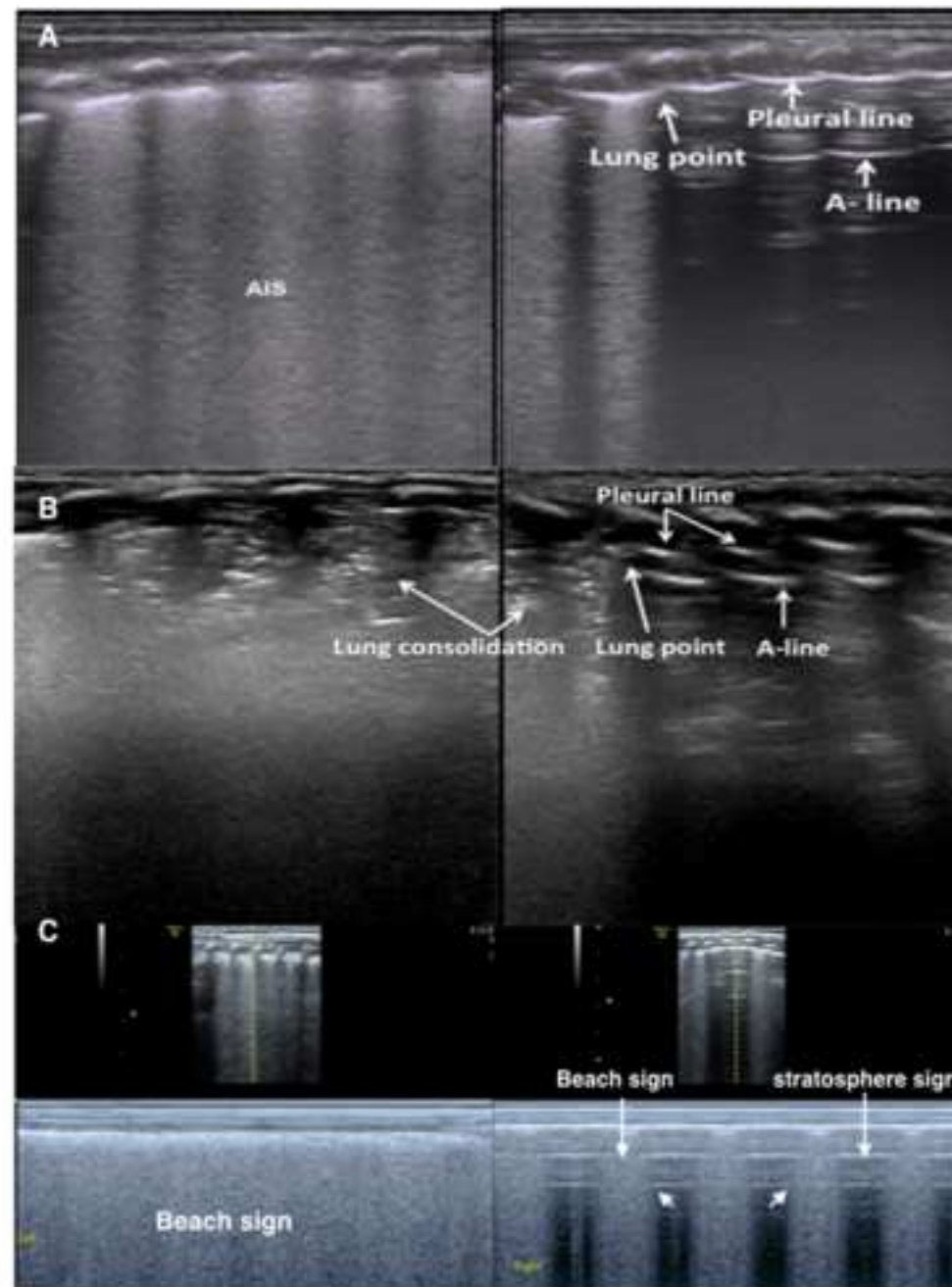943   *European Clinical Respiratory Journal*. **4** (1), 1330111 (2017).
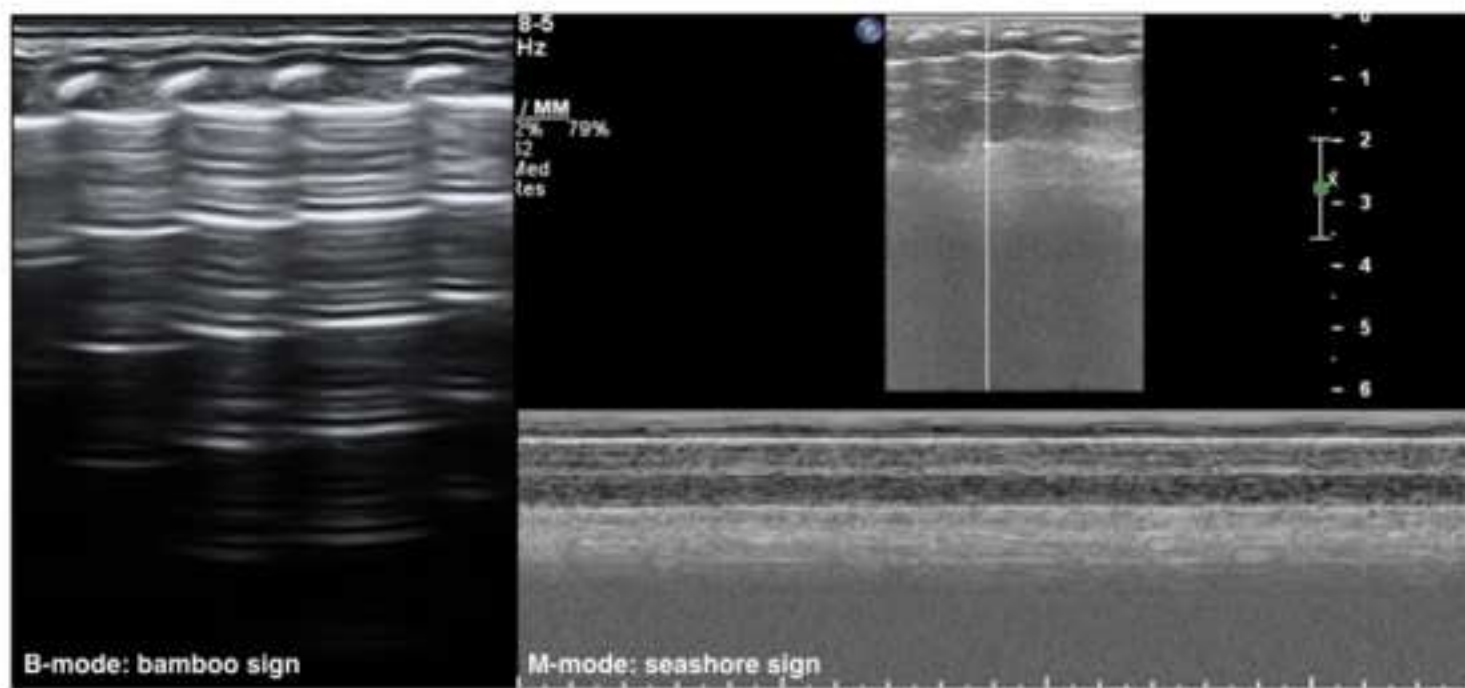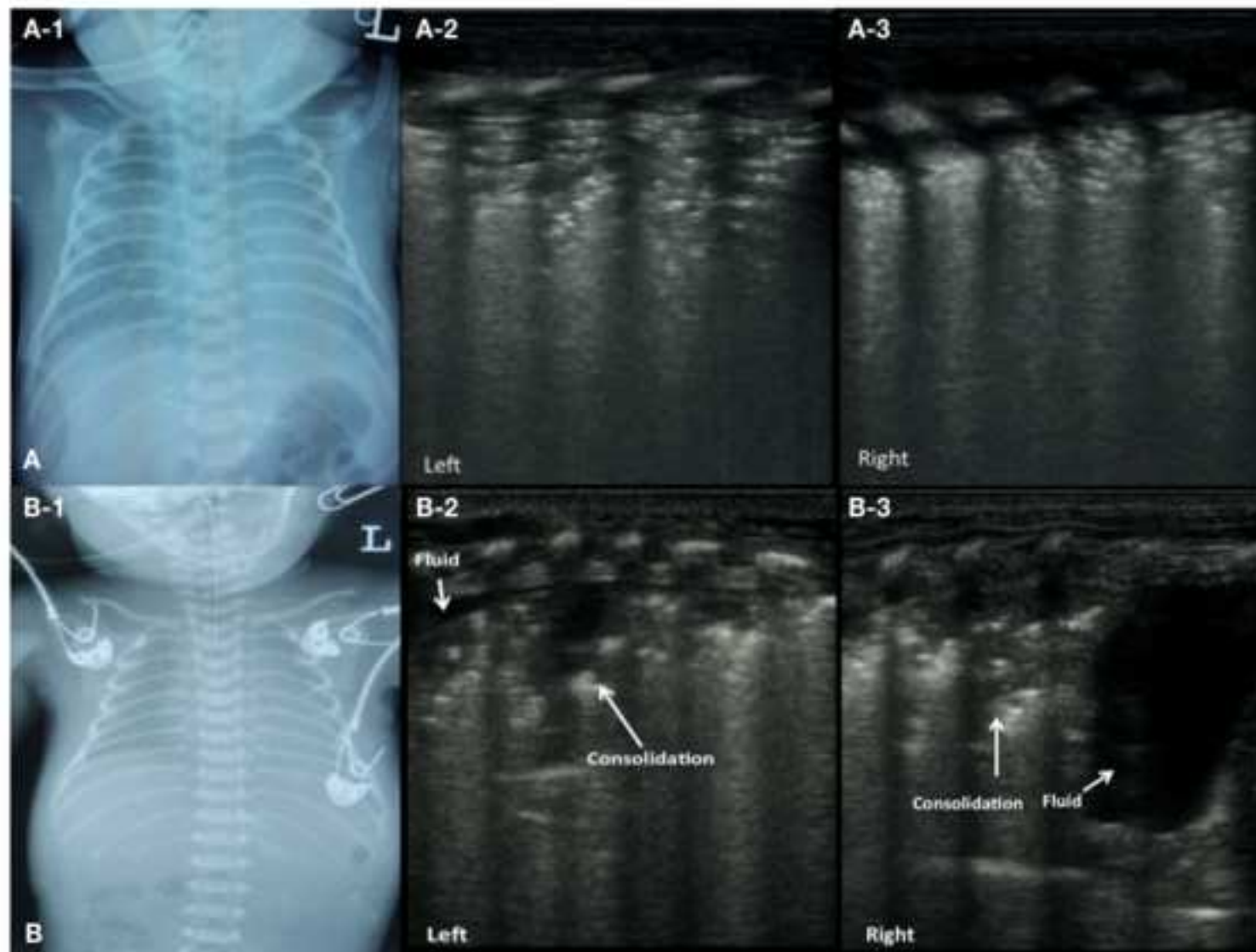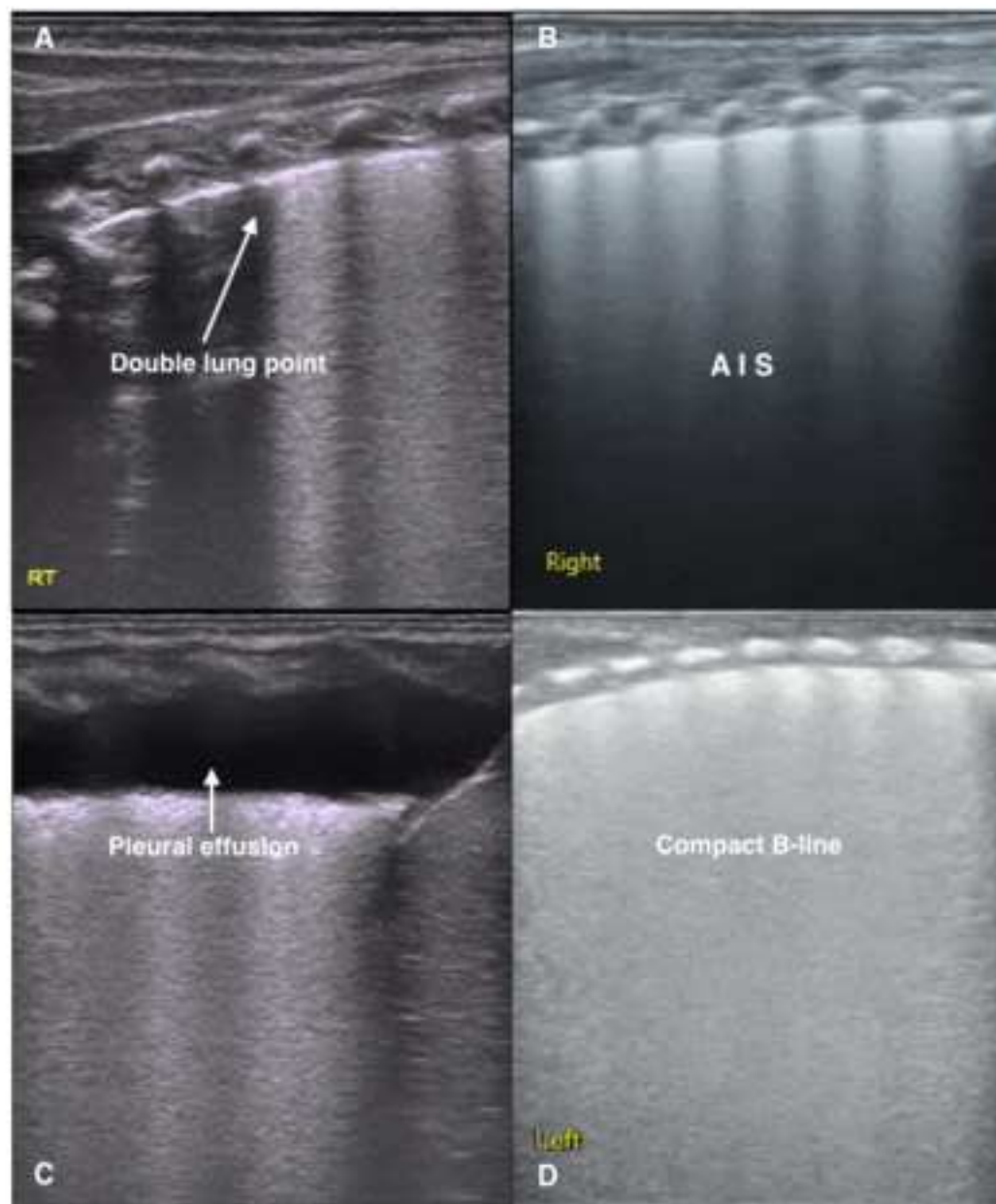
Figure 1

B-mode: bamboo sign

M-mode: seashore sign

Figure 8

Fig.11 Pneumothorax flowchart

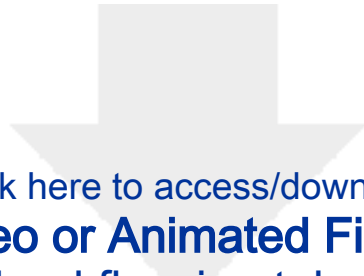**Pneumothorax Diagnostic Flowchart**

Video 1 Lung sliding
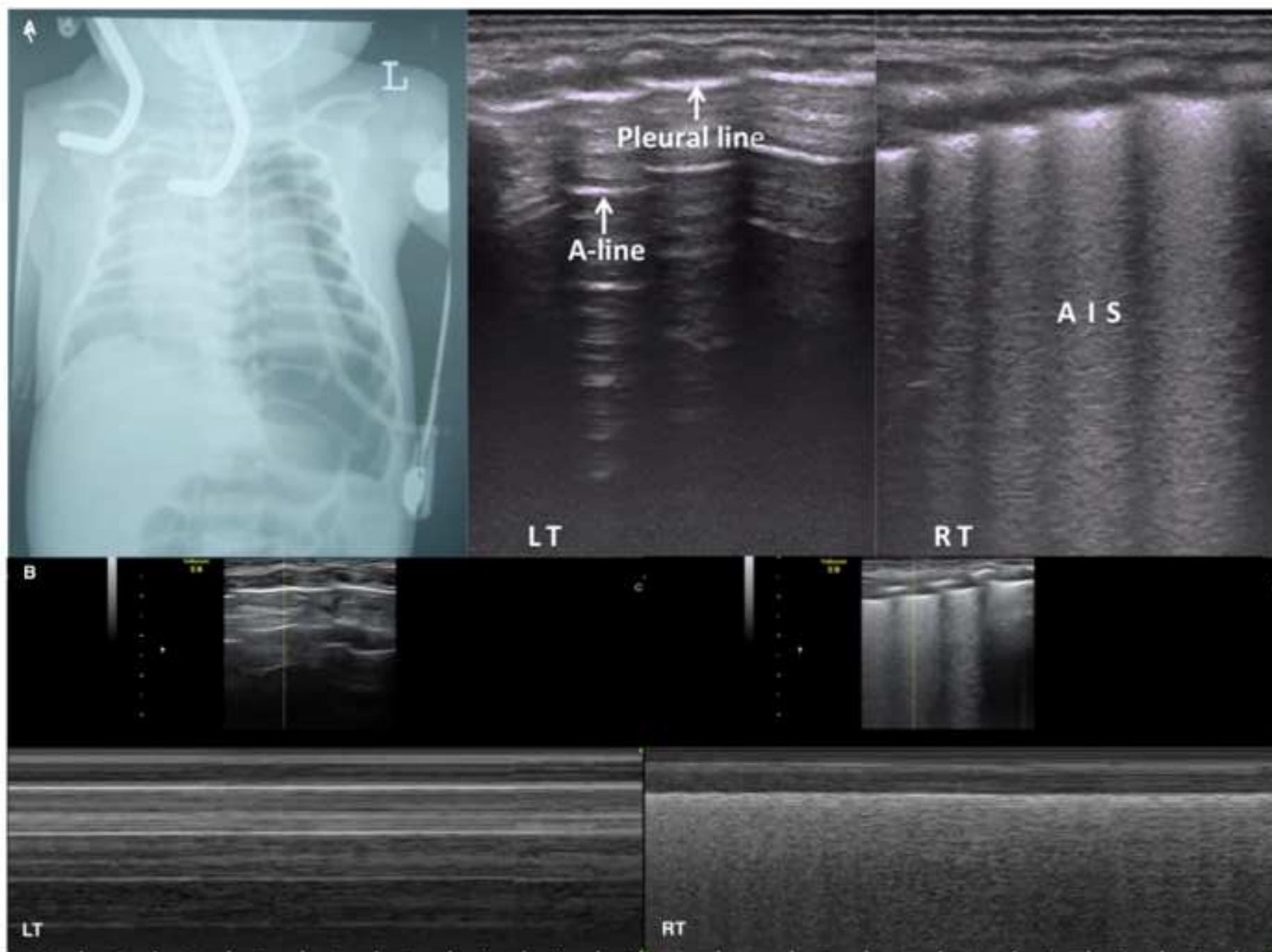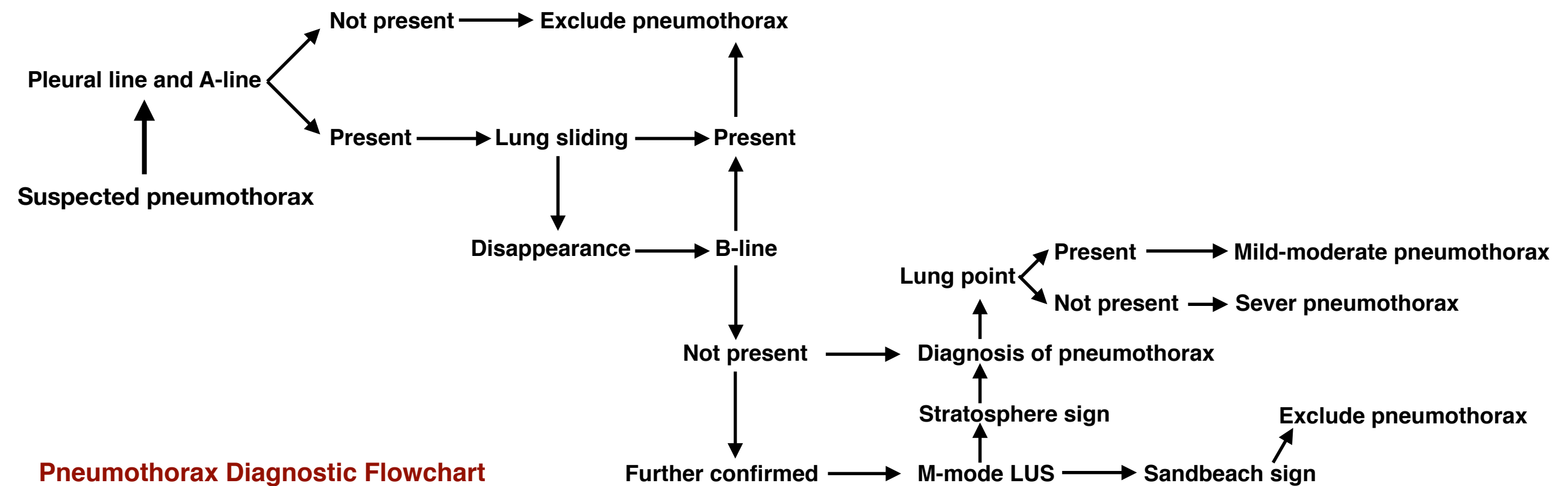
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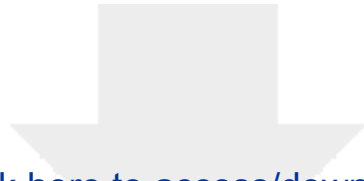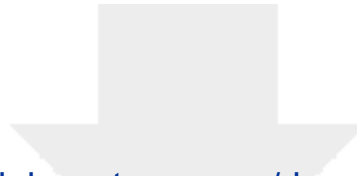Click here to access/download
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Video 4 Blood flow in atelactasis

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video.4 blood flow in atelectasis.mov

Video 6 Lung sliding disappearence

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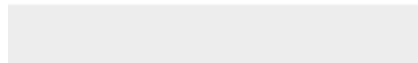Video.6-Lung sliding disappearence.avi

SUPPLE Video 1 lung pulse in atelactasis

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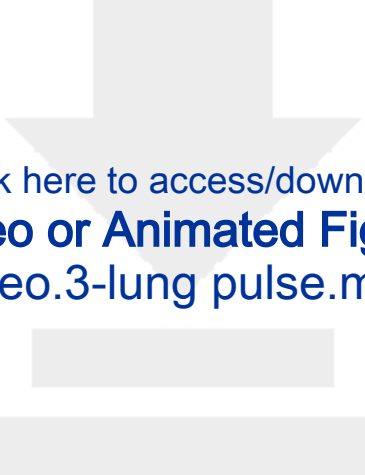supple video.1-lung pulse in atelactisis patient.avi

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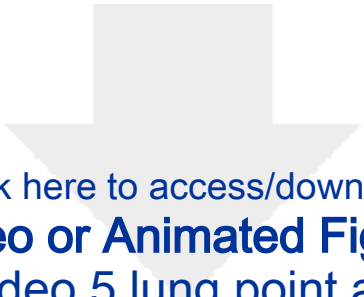**Video or Animated Figure**
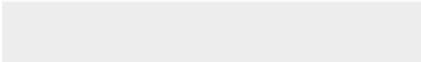supple video.2 DAB in atelactisis patient.mov

Video 3 Lung pulse

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Video 5 Lung point

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Video.5 lung point.avi

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Ultrasound machine	GE Healthcare	H48701UZ	Ultrasound machine,Voluson E10 BT18 OLED,Probe ML6-15 & 9L	
Ultrasound machine	Philips	US818C0258	Ultrasound machine,EpiQ5,Probe L18-5	
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Author(s):

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Editorial comments:

Changes to be made by the author(s) regarding the manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response:we have invited a native English speaker to revise the manuscript carefully.

2. Please revise lines 361-363, 543-545, 587-589 to avoid previously published text.

Response:we have rewritten the related parts.

3.Please revise the Introduction to include all of the following: a) A clear statement of the overall goal of this method b) The rationale behind the development and/or use of this technique c) The advantages over alternative techniques with applicable references to previous studies d) A description of the context of the technique in the wider body of literature e) Information to help readers to determine whether the method is appropriate for their application

Response:we have revised this section and hope it is now suitable for the requirements.

4. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.

Response:we have added this information.

5.Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety

procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

Response: we have incorporated some revisions according to the requirements.

6.Please add more details 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. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below.

Response: we have incorporated some revisions according to the requirements.

7. 3.2: Please specify the effective ingredients of the special disinfection wet wipe.

Response: we have revised this information.

8.3.4.2, 3.4.4 and 3.5: Please describe how to perform horizontal/parallel/diaphragm scanning.

This has been revised in detail.

9.Lines 155-457: The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use substeps as necessary. Please move the discussion about the protocol to the Discussion.

We have revised this section.

10. Please add a Representative Results section to describe how do results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. However for figures show-

ing the experimental set-up, please reference them in the Protocol. Data from both successful and sub-optimal experiments can be included.

Response: we have supplemented this section.

11. Please reduce the number of figures to less than 10 by combining some figures of small sizes.

We have incorporated these changes.

12.Figures 3, 9-15: Please combine all panels of one figure into a single image file. If the different panels are too big to combine, please number them as individual figures.

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Please note that the reviewers raised some significant concerns regarding your method and your manuscript. Please thoroughly address each concern by revising the manuscript or addressing the comment in your rebuttal letter. Reviewer #1: Manuscript Summary: This manuscript summarizes the main issues on the lung ultrasound in Neonatology. Major Concerns: I don't think that this manuscript can be published as guideline because the best methodology to do guideline is the GRADE methodology. This manuscript at list can be edited

as review but only after major revision. Actually the paper is too poor in contenet to be published as review

Response: Dear reviewer, thank you for your comment. However, we disagree with this suggestion for the following reasons. First, this is really a guideline because it tells readers how to perform the lung ultrasound and how to use ultrasound to diagnose lung diseases. Second, all the criteria are based on long-term research results from international experts in this field.Third , We have developed this guideline after a thorough evidence-based review of the currently available data and included the opinions and suggestions from a panel of international experts in this field.

Reviewer #2: Manuscript Summary:

1.Needs revision with step by step approach for Lung ultrasound

We have revised it.

2.Diagrammatic presentation - Vertical / Horizontal / Diaphragm scanning 3. Training component should be written in a proper way. Reviewer

Thank you very much.Please note we have incorporated the related revisions.

Reviewer #3: Guidelines are well written and done following an optimal criteria. based on a consensus of experts. This work can be accepted as it is.

Many thanks for this kindly and positive comment.