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ORIGINAL ARTICLE

Lung ultrasound in children with pneumonia: interoperator agreement on specific thoracic regions

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Abstract

The objective of this study was to evaluate the interoperator agreement of lung ultrasonography (LUS) on specific thoracic regions in children diagnosed with pneumonia and to compare the findings of the LUS with the chest X-ray. Participants admitted to the ward or PICU underwent LUS examinations performed by an expert and a novice operator. A total of 261 thoracic regions in 23 patients were evaluated. Median age and weight of participants were 30 months and 11.6 kg, respectively. A substantial overall agreement between operators was found for normal lung tissue ($\kappa = 0.615$, 95% confidence interval (95% CI) = 0.516–0.715) and for consolidations ($\kappa = 0.635$, 95% CI = 0.532–0.738). For B-lines, a moderate agreement was observed ($\kappa = 0.573$, 95% CI = 0.475–0.671). An almost perfect agreement was found for pleural effusion ($\kappa = 0.868$, 95% CI = 0.754–0.982). The diagnosis of consolidations by LUS showed a high sensitivity (93% for both operators) but a low specificity (14% for expert and 25% for novice operator). While intubated patients presented significantly more consolidations, nonintubated patients presented more normal ultrasound patterns.

Conclusion: Even when performed by operators with very distinct degrees of experience, LUS had a good interoperator reliability for detecting sonographic patterns on specific thoracic regions.

What is Known:
- *Lung ultrasound is feasible, safe, and highly accurate for the diagnosis of pneumonia in children; however, it does not allow global visualization of the thorax in a single moment as in chest X-rays, and, similar to the stethoscope, partial thorax assessments must be performed sequentially.*

What is New:
- *This is the first study evaluating the agreement of LUS on specific thoracic regions between operators with distinct degrees of experience performing the sonograms.*
- *There is a good agreement between an expert operator and a novice operator who underwent a brief theoretical-practical training program on LUS.*

Keywords Ultrasound · Ultrasonography · Pneumonia · Pediatrics · Children

Abbreviation

LUS	Lung ultrasonography
CXR	Chest X-ray
PPV	Positive predictive value
NPV	Negative predictive value
IQR	Interquartile range
CI	Confidence interval

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Introduction

Community-acquired pneumonia is still the leading cause of illness and death in children worldwide remaining a condition which is challenging to accurately diagnose [35]. Although it usually does not affect the clinical outcome, chest X-rays (CXR) are frequently performed when managing pneumonia in children [11, 31]. However, the lack of findings on CXR does not rule out the diagnosis if there is a strong suspicion of pneumonia [13], and the great inter- and intraobserver

variability is a well-recognized problem [1, 36]. In addition, ionizing radiation in young children may have potential late adverse effects [15, 23]. Because of these circumstances, the British Thoracic Society and the Infectious Diseases Society of America do not recommend CXR in children with mild lower respiratory symptoms consistent with pneumonia who are candidates for outpatient treatment [4, 17]. Still, despite its limitations, treatment of childhood pneumonia on the basis of clinical parameters without CXR confirmation may lead to an overestimated incidence of pneumonia, causing among other problems an increase of inappropriate use of antibiotics [22, 40].

The point-of-care lung ultrasonography (LUS) emerged as an alternative to overcome the limitations of CXR in the diagnosis of pneumonia in adults and children [25, 34]. The literature has shown that LUS is feasible, safe, and highly accurate for the diagnosis of pneumonia in multiple settings, even when performed by nonradiologists [8]. Besides being a radiation-free method, the LUS might save costs for health systems and reduce length of stay in emergency departments when compared with CXR [16]. In this context, the good performance in addition to the various benefits of the LUS encouraged to recommend its use as a first-line examination for the diagnosis of pneumonia [3, 18]. To be identified by LUS, the consolidation needs to reach the pleura and be within acoustic window of the intercorstal space [34]. Fortunately, in adults, the lesion extends to the pleura in 98.5% of cases [20], and in children, this proportion is likely to be the same or even greater due to their smaller lungs. In patients with pneumonia, the consolidations on LUS are as often as the "B-line" artifacts (previously described as "comet tails") [3]. Although these artifacts are not specific for pneumonia, they are tightly related to interstitial involvement of lung diseases [34].

On the other hand, the LUS has some drawbacks. Despite being considered a repeatable exam, its use is highly operator dependent. Lack of sufficient operator skills could lead to diagnostic errors and damage to patient safety related to unnecessary tests or interventional procedures. The increasingly widespread accessibility to ultrasound equipment is leading medical students and untrained clinicians to start using LUS. As a matter of fact, there is no clear evidence-based guidelines or recommendations on the training needed to obtain adequate skills for performing an LUS examination [26]. A meta-analysis on the use of LUS for the diagnosis of pneumonia in children concluded that more studies are needed to validate the accuracy of LUS in novice users [25].

Therefore, our main objective was to evaluate the agreement between an experienced and a novice operator for identifying sonographic patterns on specific thoracic regions in children diagnosed with pneumonia. The secondary objective was to compare the findings of the LUS with the CXR.

Materials and methods

Study design, subjects, and setting

A prospective, observational pilot study was conducted at the Clinics Hospital of the State University of Campinas (UNICAMP) (a tertiary care academic teaching hospital), São Paulo, Brazil. Participants were enrolled from February 2017 to December 2018 as a convenience sample, with recruitment determined by operator's availability. The study was approved by UNICAMP's Research Ethics Committee (registration number 38170714.9.0000.5404) and a written informed consent was obtained from the participants' legal guardians.

All participants with clinically suspected community-acquired bacterial pneumonia admitted to the ward or pediatric intensive care unit were evaluated for eligibility. Participants younger than 14 years old were included if they presented the following clinical signs: persistent or repetitive axillary temperature of > 38.5 °C, chest retractions, and raised respiratory rate (in accordance with the British Thoracic Society guidelines) [17]. Exclusion criteria were (i) lack of parental consent, (ii) first episode of wheezing, (iii) previous diagnosis of chronic lung disease, (iv) presence of congenital or acquired heart diseases, (v) hemodynamic instability, and (vi) noncollaborative patients.

Lung ultrasound protocol

The chest sonograms were sequentially performed by two operators with different levels of experience. One of them (THdS) was a pediatric ultrasound instructor at the Brazilian Society of Intensive Care, with 5 years of experience in pediatric point-of-care ultrasound. The other operator (JAN) was a resident physician, enrolled in the pediatric intensive care program, who underwent a 14-h the theoretical-practical training in pediatric point-of-care ultrasonography. The specific training on LUS consisted of 1 h of theory and 1 h of practical hands on imaging session on healthy humans. The operators performing were made aware of the subject's clinical presentation but blinded to the CXR findings, as well as to the ultrasonographic findings obtained by each other. Both chest sonograms were performed within 24 h after chest X-ray examinations.

In accordance with international evidence-based recommendations for point-of-care LUS, four anterior thoracic regions were assessed in each hemithorax: anterior superior, anterior inferior, lateral superior, and lateral inferior [34]. These regions were delimited by three vertical lines (parasternal, anterior, and posterior axillary lines) and one horizontal line at mammillary level. The posterior thorax was assessed with the participant in lateral decubitus and sitting positions. Two posterior regions per side were defined:

posterior superior and posterior inferior. These regions were delimited by paravertebral lines, posterior axillary lines, and a horizontal line at the lower border of the scapula. Probes were placed perpendicularly, obliquely, and, when necessary, parallel to the ribs. This protocol is illustrated in Fig. 1.

As defined in literature, each thoracic region was classified as one or more among these four ultrasound diagnoses: normal lung, B-lines and/or confluent B-lines (interstitial syndrome), lung consolidation, and pleural effusion (Fig. 2) [34]. The normal lung was defined by the presence of sliding pleural line plus A-line artifacts. A-lines are horizontal artifacts separated by regular intervals that are equal to the distance between the skin and the pleural line. B-lines were considered in the presence of three or more B-line artifacts, which are discrete laser-like vertical hyperechoic reverberation artifacts arising from pleural line to the bottom of the ultrasonography screen without fading, moving synchronously with lung sliding. Lung consolidation was defined as a subpleural echopoor region or one tissue-like echotexture. Pleural effusion was defined as a generally hypoechoic space between parietal and visceral pleurae with or without consolidation. A structured data extraction form was piloted and then used to extract data during the reporting of the ultrasound examinations.

The ultrasound machine used in this study was a GE Healthcare Vivid Q (CA, USA) equipped with linear probe (5–13 MHz).

Radiological examinations

All participants received at admission a single anteroposterior CXR. Radiographs were analyzed by independent pediatric pneumologists who were unaware of the LUS results, but were aware of the clinical signs of pneumonia. Radiological findings were classified as pleural effusions, alveolar pneumonia, interstitial pneumonia, mixed interstitial and alveolar pneumonia, or no pneumonia in accordance with the World Health Organization criteria for the standardized interpretation of pediatric CXR for the diagnosis of pneumonia [38].

Statistical analysis

To take account of the agreement occurring by chance, unweighted Cohen's kappa (κ) coefficient was used to measure strength of agreement between the experienced and nonexperienced operators [29]. The Landis and Koch table was used to characterize κ values: <0 indicating no agreement, from 0 to 0.20 a slight agreement, from 0.21 to 0.40 a fair agreement, from 0.41 to 0.60 a moderate agreement, from 0.61 to 0.80 a substantial agreement, and from 0.81 to 1 an almost perfect agreement [19]. Assuming a κ coefficient of 0.65 between operators, a sample size of 260 thoracic regions was calculated to obtain a statistical power of 85% with $\alpha = 0.05$ [29].

Using the CXR as gold standard, the LUS sensibility, specificity, and positive/negative predictive values (PPV/NPV) were calculated for the following findings: normal lung, interstitial syndrome, lung consolidation, and pleural effusion.

Qualitative variables were expressed in number and associated percentages. Continuous variables were asymmetrically distributed and were thus described as medians and interquartile range (IQR). The Mann-Whitney test was used to compare continuous variables. Data were analyzed using IBM SPSS Statistics (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY: IBM Corp.).

Results

A total of 23 children were evaluated. Pulmonary tissue was not visualized in three left anterior inferior regions (cardiac interposition) and was not analyzed. The posterior thorax of two participants could not be assessed by the inexperienced operator because of respiratory instability. Four thoracic regions could not be assessed due to participant agitation. Thus, 261 thoracic regions were evaluated by the both operators. Table 1 summarizes the demographic and clinical characteristics of the participants enrolled.

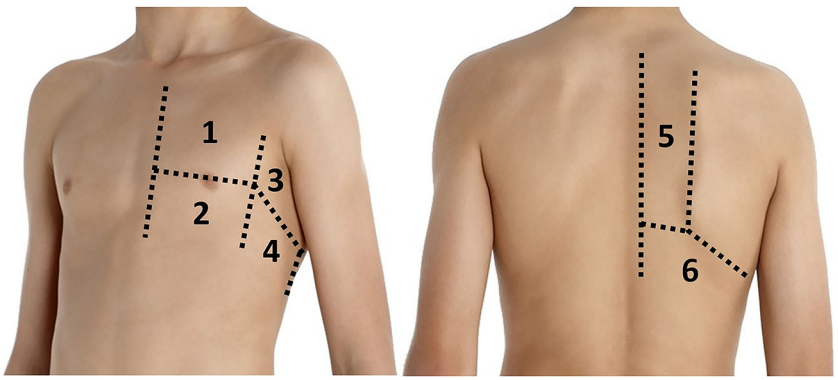

Fig. 1 Thoracic regions evaluated by lung ultrasound. 1—Anterior superior. 2—Anterior inferior. 3—Lateral superior. 4—Lateral inferior. 5—Posterior superior. 6—Posterior inferior

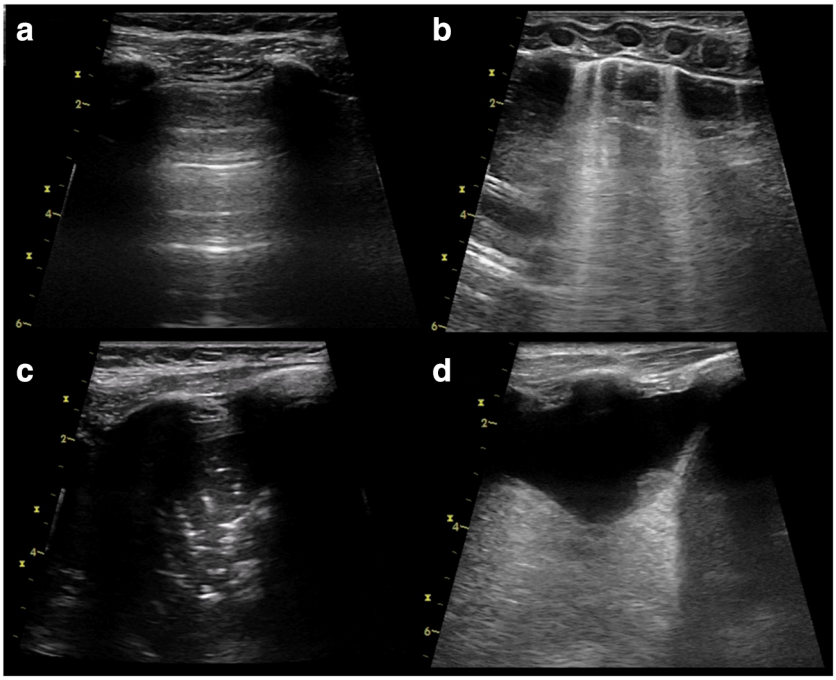

Fig. 2 Characteristics of the LUS patterns. **a** Normal appearance of the lung. **b** B-lines. **c** Lung consolidation. **d** Pleural effusion

The number of observed agreements for normal lung was 215 (82.38% of the observations), while the number of agreements expected by chance was 141.4 (54.16% of the observations). Thus, when assessing for binary outcome of normal versus abnormal lung tissue, we found substantial overall agreement between the operators ($\kappa = 0.615$, 95% confidence interval (95% CI) = 0.516–0.715).

B-lines presented agreement on 205 thoracic regions (78.54% of the observations), while the number of agreements expected by chance was 129.8 (49.73% of the observations). Cohen's κ value was 0.573 (95% CI = 0.475–0.671), meaning a moderate agreement between operators for interstitial syndrome.

Regarding the lung consolidations, there was an agreement in 222 thoracic regions (85.06% of the observations), whereas 151.4 agreements were expected by chance (59.04% of the observations). Therefore, when assessing the presence of lung consolidations, a substantial agreement was observed between the operators ($\kappa = 0.635$, 95% CI = 0.532–0.738).

Finally, the agreement for pleural effusion occurred in 256 thoracic regions (98.08% of the observations), while the agreement expected by chance was 223.2 (85.52% of the observations). Cohen's κ value was 0.868 (95% CI = 0.754–0.982), meaning an almost perfect agreement between operators.

The results of agreement analysis are summarized in Table 2. Concordance rates and Cohen's κ value for each thoracic region are presented in Table 3.

The CXR was used as gold standard to compare with LUS diagnoses. In general, abnormal patterns were more often visualized by LUS than by CXR. Consolidations were visualized by the expert operator but not by the CXR in six patients. The LUS examinations of these patients identify consolidations in 20 thoracic regions, 14 of which were less than 1-cm deep (eight localized on the posterior thorax). Of the six consolidations with more than 1-cm deep, five were visualized in the left hemithorax (three in the lateral inferior, one in lateral superior, and one in posterior inferior regions). Table 4 describes by CXR and LUS (expert and novice operators) the number of participants in whom abnormality patterns were visualized.

The results of sensitivity, specificity, PPV, and NPV obtained by both operators were similar and are presented in Table 5.

While intubated patients presented significantly more thoracic regions with lung consolidations than those nonintubated (47% vs 18%, $p = 0.002$), nonintubated patients presented more normal ultrasound patterns (20% vs 44%, $p = 0.011$)

Table 1 Distribution of demographic and clinical characteristics of the participants

Category	Data distribution
Total participants	23
Total thoracic zones evaluated	261
Age (months)*	30 (5–41)
Weight (kg)*	11.6 (7.2–17)
Sex (males)	12/23 (52%)
Sex (females)	11/23 (48%)
PICU admissions	13/23 (57%)
Invasive mechanical ventilation	12/23 (52%)
Hospital length of stay*	12 (4–18)

*Values described as median and interquartile range

Table 2 Interoperator agreement for lung ultrasound patterns. Concordance tests for expert and novice operators by using the Landis and Koch scale [19]

Lung ultrasound diagnosis	Cohen's κ coefficient	95% confidence interval	Strength of agreement
Normal lung	0.615	0.516–0.715	Substantial
B-lines and/or confluent B-lines	0.573	0.475–0.671	Moderate
Lung consolidation	0.635	0.532–0.738	Substantial
Pleural effusion	0.868	0.754–0.982	Almost perfect

(Online Resources 1 and 2). Distribution of the LUS abnormalities visualized by the expert operator is available in the Online Resource 3.

Discussion

The present study showed good agreements between an expert operator and a novice operator who underwent a brief theoretical-practical training program on LUS. These results may help establish the feasibility of point-of-care LUS in pediatric clinical practice, since being an operator-dependent examination, the low interrater reliability could inhibit its use. Despite its many advantages, LUS does not allow global visualization of the thorax in a single moment as in CXR, and, similar to the stethoscope, partial thorax assessments must be performed sequentially. In addition, ultrasound images are not frequently printed or recorded on video files, impairing prospective evaluation at the bedside. Therefore, it is critical to understand the capacity of different operators to detect pulmonary lesions in specific thoracic regions. To the best of our knowledge, this is the first study evaluating the agreement of LUS on specific thoracic regions between operators with distinct degrees of experience performing the sonograms.

Few studies involving children evaluated the agreement in LUS diagnoses [2, 10, 28, 32, 33]. When assessing for binary outcome of normal versus abnormal, Tripathi et al. [32] found only a fair agreement ($\kappa = 0.27$, 95% CI = 0.04–0.58) between the novice-expert pair, in contrast to our results ($\kappa = 0.615$, 95% CI = 0.516–0.715). However, in their study, only the

Table 3 Rates of agreements between operators and Cohen's κ coefficient (95% CI) stratified by specific thoracic regions and lung ultrasound patterns

Thoracic region		Normal lung	B-lines	Lung consolidations	Pleural effusion
Right hemithorax	Anterior superior	83%	78%	96%	100%
		0.65 (0.33–0.96)	0.56 (0.22–0.90)	0.65 (0.01–1.00)	1.00 (1.00–1.00)
	Anterior inferior	87%	87%	96%	96%
		0.74 (0.46–1.00)	0.73 (0.45–1.00)	0.83 (0.51–1.00)	0.65 (0.01–1.00)
	Lateral superior	83%	83%	91%	91%
		0.65 (0.35–0.96)	0.65 (0.34–0.96)	0.70 (0.30–1.00)	0.46 (0.13–1.00)
	Lateral inferior	70%	74%	87%	100%
		0.37 (− 0.01–0.76)	0.47 (0.12–0.83)	0.64 (0.27–1.00)	1.00 (1.00–1.00)
	Posterior superior	81%	67%	86%	100%
		0.48 (0.06–0.90)	0.35 (− 0.03–0.73)	0.72 (0.43–1.00)	1.00 (1.00–1.00)
	Posterior inferior	86%	76%	76%	100%
		0.67 (0.35–1.00)	0.52 (0.16–0.88)	0.54 (0.24–0.85)	1.00 (1.00–1.00)
Left hemithorax	Anterior superior	83%	83%	83%	100%
		0.65 (0.33–0.96)	0.65 (0.33–0.96)	0.39 (− 0.09–0.88)	1.00 (1.00–1.00)
	Anterior inferior	90%	85%	80%	100%
		0.73 (0.39–1.00)	0.69 (0.38–1.00)	0.38 (− 0.09–0.86)	1.00 (1.00–1.00)
	Lateral superior	73%	68%	86%	95%
		0.43 (0.07–0.79)	0.39 (0.06–0.73)	0.68 (0.36–1.00)	0
	Lateral inferior	78%	73%	68%	95%
		0.41 (0.00–0.82)	0.45 (0.11–0.80)	0.35 (− 0.04–0.74)	0.77 (0.36–1.00)
	Posterior superior	90%	90%	85%	100%
		0.69 (0.28–1.00)	0.78 (0.49–1.00)	0.66 (0.31–1.00)	1.00 (1.00–1.00)
	Posterior inferior	90%	80%	85%	100%
		0.76 (0.46–1.00)	0.58 (0.22–0.95)	0.71 (0.41–1.00)	1.00 (1.00–1.00)

Table 4 Distribution of abnormalities visualized in each participant by the chest X-ray and lung ultrasonography examinations (expert and novice operator)

Variable	Consolidation	Interstitial syndrome	Pleural effusion	Any abnormality
Chest X-ray	16	8	5	23
Expert operator	21	22	7	22
Novice operator	20	23	7	23

novice operator performed the LUS while the expert was just a reader of the recorded video clips. In addition, all thoracic regions were assessed together for normal or abnormal categorization. These differences in the study designs may explain the discrepancies between the results. Identifying the normality pattern on LUS is essential in order to rule out pulmonary lesions or to verify their improvement during the prospective evaluation [5, 6, 27].

Due to its relation with the diagnosis of pneumonia, the consolidations are the pulmonary injury most evaluated in pediatric LUS studies [2, 6, 8, 10, 12, 21, 25, 27, 28]. Despite the substantial strength of agreement for consolidation presented in our study ($\kappa = 0.635$, 95% CI = 0.532–0.738), it was lower than that found by other authors [2, 10, 28]. The studies conducted by Shah et al. and Biagi et al. found an almost perfect interrater agreement ($\kappa = 0.93$, 95% CI = 0.87–0.99) [2, 28]. However, in both studies, the agreement was not assessed in the exact thoracic region; moreover, Tripathi et al. observed that kappa decreased from 1 to 0.62 when the exact location of the consolidation was analyzed instead of the whole lung [32]. It is important to emphasize that most studies evaluate the agreement between an operator and an external reviewer, and not by two operators performing LUS examinations [10, 14, 28, 32, 33].

As in other studies, strength of agreement for B-lines presented here was the lowest among the LUS patterns evaluated in our data ($\kappa = 0.573$, 95% CI = 0.475–0.671) [10, 32]. However, our agreement was greater than others published. Ellington et al. reviewed 1062 LUS and found a kappa of only 0.38 (95% CI = 0.27–0.41) for interstitial syndrome and Tripathi et al. a kappa of 0.30 for exact "B-pattern" localization [10, 32]. Although B-lines themselves might be easy to identify, characterizing interstitial syndrome requires interpretation of these lines for significance which may be more challenging and subjective [32]. The same difficulty seems to occur in the interpretation of interstitial infiltrates in chest X-rays. Moncada et al. found a poor agreement between a pneumologist and a radiologist for identifying interstitial infiltrates on chest X-rays of adults with pneumonia [24]. LUS is considered superior to CXR for diagnosing interstitial syndrome [34].

Ultrasonography has been shown to be as effective or superior to computed tomography for the diagnosis and definition of pleural effusions [7, 30, 34]. As expected, the kappa for pleural effusion was the highest presented in our study ($\kappa = 0.868$, 95% CI = 0.754–0.982). This fact can be explained by the preferential localization of the pleural effusions in the lower regions of the thorax, making easier the agreement between operators. Even so, this almost perfect agreement found is important, since ultrasonography has proven to be superior to CXR for diagnosis of pleural effusion [39]. Moreover, lower strength of agreement on CXR for pleural effusion has been reported ($\kappa = 0.57$) [9].

In our study, two patients could not have their posterior thoraces assessed by ultrasound because of respiratory

Table 5 Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of lung ultrasound by the operators to detect pulmonary abnormality as compared with chest X-rays read by a pediatric pulmonologist

Variable	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Lung consolidations				
Expert operator	93	14	71	50
Novice operator	93	25	70	67
Interstitial syndrome				
Expert operator	87.5	NA*	32	NA*
Novice operator	100	NA*	35	NA*
Pleural effusion				
Expert operator	100	89	71	100
Novice operator	100	89	71	100
Any abnormality				
Expert operator	96	NA*	100	NA*
Novice operator	100	NA*	100	NA*

*The values could not be calculated due to lack of true negative

instability. These children were among the twelve intubated patients. Children under mechanical ventilation may present respiratory and/or hemodynamic instability during change of the decubitus position, which impairs the ultrasonography assessment. We believe that further studies are needed evaluating the security and clinical relevance of information obtained from posterior thorax in this specific population.

CXR interpretation carries a great intra- and interobserver variability and is not routinely recommended for the diagnosis of pneumonia, except when hospitalization is required [1, 10, 17, 36]. Even using a scheme for standardizing the interpretation of CXR, the concordance rate between two trained reviewers was only 48% (250/521) [37]. The risks of computed tomography make CXR the most commonly used reference standard for assessing LUS accuracy. However, its limitations must be taken into account when interpreting these results. A meta-analysis evaluating LUS observed that using CXR alone may be inadequate for diagnosis of childhood pneumonia [25]. Of the included studies, 3 used CXR alone as the reference standard for diagnosis of pneumonia, while 5 used both CXR and clinical criteria. The overall pooled sensitivity and specificity of LUS were 96% and 93%, respectively. However, when the reference standard was limited to findings based on CXR alone, the sensitivity remains the same, but the specificity decreased to 84%. Furthermore, small consolidations visualized on LUS may not be detectable by the CXR [28]. Using CXR as a reference standard, Shah et al. observed the specificity of LUS increasing from 89 to 97% when only > 1-cm consolidations were considered [28]. The sensitivity, specificity, and predictive values presented in our data are very limited because of the small sample size, but the low specificity found was interesting. The distribution of consolidations visualized by LUS but not by the CXR suggests that the cardiac area has impaired the visualization by radiography. Therefore, CXR does not appear to be an appropriate reference standard for assessing LUS accuracy.

Our study has some limitations, mainly regarding to secondary objective. Firstly, only one pair of operators performed the LUS, so the results presented here need to be confirmed by further studies. Secondly, the CXR was used as gold standard and, as previously commented, this may be inappropriate. This is a common limitation in all studies assessing the diagnostic performance of LUS compared with CXR for the diagnosis of pneumonia in children. Thirdly, because of researchers' disponibility, LUS and CXR were not always performed consecutively, and changes in the process of lung disease may have occurred due to time passed between the two imaging studies. Finally, the results of sensitivity, specificity, and predictive values presented were calculated on a very small sample size; thus, it must be interpreted carefully.

Conclusions

The LUS is a clinically useful diagnostic tool in children with pneumonia [34]. Even when performed by operators with very distinct degrees of experience, LUS had a good interoperator reliability for detecting sonographic patterns on specific thoracic regions. The results presented suggest that practitioners undergoing a brief theoretical-practical training may be able to identify the main sonographic signs of pneumonia in hospitalized children. Despite its safety and efficiency, more studies evaluating the agreement between operator are needed in order to encourage the use of LUS routinely in clinical practice.

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Authors' contributions T.H.dS. conceptualized and designed the study, collected data, drafted the initial manuscript, carried out the statisticalanalysis and reviewed and revised the manuscript. A.O.P. and J.A.H.N. designed the data collection instruments, collected data, and reviewed and revisedthe manuscript. M.P.G., A.C.S.S. and R.M.P. collected data. M.B.B. coordinated and supervised data collection, and critically reviewed the manuscript forimportant intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Compliance with ethical standards

The study was approved by UNICAMP's Research Ethics Committee (registration number 38170714.9.0000.5404), and a written informed consent was obtained from the participants' legal guardians.

Conflict of interest The authors declare that they have no conflict of interest.

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