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## Review article

Ultrasonography for confirmation of endotracheal tube placement: A systematic review and meta-analysis<sup>☆</sup>Eric H. Chou<sup>a,1</sup>, Eitan Dickman<sup>a</sup>, Po-Yang Tsou<sup>b</sup>, Mark Tessaro<sup>a</sup>, Yang-Ming Tsai<sup>c</sup>,  
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## ABSTRACT

**Objective:** This study aimed to undertake a systematic review and meta-analysis to summarize evidence on the diagnostic value of ultrasonography for the assessment of endotracheal tube placement in adult patients.

**Methods:** The major databases, PubMed, EMBASE, and the Cochrane Library, were searched for studies published from inception to June 2014. We selected studies that used ultrasonography to confirm endotracheal tube placement. The search was limited to human studies, and had no publication date or country restrictions. Exclusion criteria included case reports, comments, reviews, guidelines and animal studies. Two reviewers extracted and verified the data independently. We summarized test performance characteristics with the use of forest plots, hierarchical summary receiver operating characteristic (HSROC) curves, and bivariate random effect models. Meta-regression analysis was performed to explore the source of heterogeneity. The methodological quality of individual studies was evaluated using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool.

**Results:** A total of 12 eligible studies involving adult patients and cadaveric models were identified from 1488 references. For detection of esophageal intubation, the pooled sensitivity was 0.93 (95%CI: 0.86–0.96) and the specificity was 0.97 (95%CI: 0.95–0.98). The area under the summary ROC curve was 0.97 (95%CI: 0.95–0.98). The positive and negative likelihood ratios were 26.98 (95%CI: 19.32–37.66) and 0.08 (95%CI: 0.04–0.15), respectively.

**Conclusions:** Current evidence supports that ultrasonography has high diagnostic value for identifying esophageal intubation. With optimal sensitivity and specificity, ultrasonography can be a valuable adjunct in this aspect of airway assessment, especially in situations where capnography may be unreliable.

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## 1. Introduction

Tracheal intubation serves as definite airway control when resuscitating critically ill patients. Confirmation of proper tube placement should be completed in all patients at the time of initial intubation. Unrecognized misplacement of the endotracheal tube

may lead to avoidable morbidity including neurological damage, and death, with a reported incidence of 6–16%.<sup>1,2</sup> Thus, immediate post-intubation airway assessment is an essential clinical skill for every physician in emergency medicine (EM), anesthesia and critical care medicine. There are multiple options for confirming tracheal intubation and all methods have unique limitations.<sup>3–5</sup> According to the 2010 American Heart Association (AHA) guideline, endotracheal tube position should be verified by both clinical assessments and confirmation devices after intubation.<sup>6</sup> No single confirmatory method is entirely reliable in emergency situations.<sup>3–5</sup>

According to the 2009 American College of Emergency Physicians (ACEP) policy statement on the verification of endotracheal intubation, physical examination methods, such as auscultation of the chest and epigastrium, visualization of chest movement,

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and fogging in the tube, are not sufficiently reliable to confirm endotracheal tube placement.<sup>7</sup> Similarly, pulse oximetry and chest radiography are not reliable as sole techniques to determine endotracheal tube location.<sup>7</sup> In addition, chest radiography takes time and is not available during cardiopulmonary resuscitation (CPR). Quantitative waveform capnography is recommended as the criterion standard for confirming correct endotracheal tube placement in the 2010 AHA Guideline.<sup>6</sup> However, it has well-known limitations in cardiac arrest patients, and can be affected by low cardiac output, low pulmonary blood flow or epinephrine use.<sup>8</sup>

Focused ultrasonography is a novel tool for airway assessment. In the last decade, there have been many studies providing promising results for endotracheal tube confirmation using ultrasonography.<sup>9-21</sup> The 2009 ACEP policy statement on the verification of endotracheal intubation described ultrasonography as a possible future adjunct.<sup>7</sup> Many studies with updated data were published after the policy was written.<sup>9-17</sup> However, most of these studies had a small sample size, had heterogeneous design and demonstrated mixed results. In this study, we systematically reviewed the literature and performed a meta-analysis to determine the diagnostic value of ultrasonography for the assessment of endotracheal tube placement in adult patients.

## 2. Methods

### 2.1. Data sources and searches

The meta-analysis was performed in accordance with the Preferred Reporting Items for Systemic reviews and Meta-Analysis (PRISMA) guidelines and the Assessment of Multiple Systematic Reviews (AMSTAR) tool.<sup>22,23</sup> General bibliographic databases (PubMed and EMBASE) were searched from inception to June 2014. The medical subject heading (MeSH) and text words for the term "intubation" were combined with the MeSH term "ultrasonography". The search terms for the primary intervention included "intubation", "endotracheal intubation" or "esophageal intubation". The search results were then cross-checked for the population of interest and searched using the terms, "ultrasonography," "ultrasound," "sonogram," "sonography", "sono" and "echo". The search was limited to human studies, and there were no publication date or country restrictions. In addition to the electronic search, reference lists in all known reviews and primary studies were checked manually.

### 2.2. Selection criteria

This meta-analysis focused on studies in which the bedside ultrasound was used to detect esophageal intubation in adult patients. We included studies using a cohort design or case-control design with appropriate controls. Selection of pertinent studies was performed independently by two reviewers. Discrepancies between the reviewers were resolved by a consensus meeting initially and using arbitration by a third reviewer if consensus could not be reached. Data collected included study design and setting, participants, sonographer experience, ultrasound transducer type, sonographic method, and criterion standard. One reviewer extracted the data and a second reviewer independently verified the correctness of the extracted data. Exclusion criteria included case reports, comments, reviews, guidelines and animal studies. Studies enrolling pediatric patients were excluded as well.

### 2.3. Data abstraction and quality assessment

Data were extracted for overall study characteristics (including the first author, country, language, and date of publication), settings, patient characteristics, sonographic scanning methods

and time of measurement, sonographers' experience, criterion standard, and quantitative data required for construction of a 2 × 2 table (including number of participants, sensitivity, and specificity). One reviewer extracted the data and a second reviewer verified the data independently. In studies that reported multiple pairs of sensitivity and specificity data, we consistently used the data with the highest Youden index (sensitivity + specificity - 1) and performed a sensitivity analysis at a later stage. We used the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool to assess the methodological quality of the select studies before meta-analyses.<sup>24</sup> This tool evaluated characteristics of study designs, population, index tests, and reference standards that may be associated with risk of bias.

### 2.4. Quantitative data synthesis

We performed a meta-analysis of diagnostic accuracy of ultrasonography for the detection of esophageal intubation. We calculated the pooled sensitivity and specificity, positive and negative likelihood ratios, and the diagnostic odds ratio of ultrasonography, along with the respective 95% confidence intervals (CIs), using a bivariate meta-analysis model.<sup>25</sup> When 2 × 2 tables contained 0 cells, we performed continuity correction by adding 0.5 to each cell. We constructed a hierarchical summary receiver operating characteristic (HSROC) curve that plots sensitivity versus specificity and calculated the area under the curve (AUROC).<sup>26</sup> We evaluated the degree of between-study heterogeneity by using the  $I^2$  test.<sup>27</sup> To explore the clinical sources of heterogeneity, we defined the potential explanatory variables a priori and performed subgroup analysis to see if the accuracy estimates changed significantly across various subgroups. The presence and the effect of publication bias were examined using a combination of the Begg and Egger tests.<sup>28</sup> Statistical analyses were conducted using the statistical package STATA (Version 11.0, Stata Corp, College Station, TX), notably with the user-written "midas" and "metandi" programs. All statistical tests were two-sided and statistical significance was defined as a *P* value less than .05.

## 3. Results

### 3.1. Search results and study characteristics

The flow of inclusion and exclusion is summarized in Fig. 1. Using our search criteria, we identified 1488 studies, of which 659 were from PubMed and 829 were from EMBASE. A total of 1413 citations were excluded based on pre-defined criteria. No additional citations were identified from the reference lists. A total of 75 articles were retrieved for full-text review, and 63 were excluded due to various reasons detailed in Fig. 1. A total of 12 studies that evaluated the accuracy of ultrasonography in detecting esophageal intubation were included in the meta-analysis. The 12 studies included a total of 1656 intubation attempts, and 550 (33.2%) were identified as esophageal intubation.

### 3.2. Characteristics of included studies

Table 1 lists the study and population characteristics. Three studies used different ultrasound techniques in their subgroups independently, so that they can be regarded as different estimates.<sup>9,17,20</sup> Three studies included human cadaver models<sup>16,20,21</sup>, while three recruited patients in an operating room (OR) setting<sup>14,18,19</sup>, and six recruited emergency department (ED) patients.<sup>9-12,15,17</sup> Tracheal ultrasonography was used in ten studies<sup>9,11,12,14-20</sup>, and lung ultrasonography was used in four studies<sup>9,10,17,21</sup>. Two studies used both tracheal and lung ultrasonography to confirm endotracheal tube placement<sup>9,17</sup>. Seven

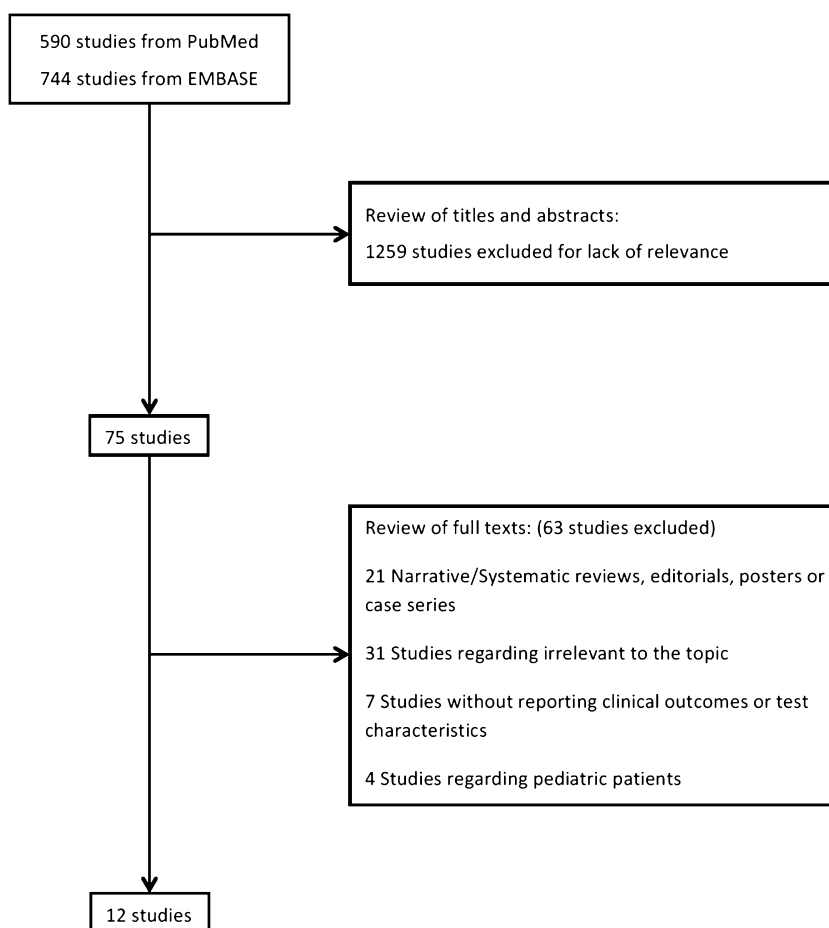


Fig. 1. Literature search flow diagram.

studies used capnography or EtCO<sub>2</sub> detectors as criterion standard of tracheal intubation<sup>9,11,12,14,15,17,19</sup>, whereas the remaining studies used other traditional methods, such as direct laryngoscopy, chest auscultation, or chest radiography.<sup>10,16,18,20,21</sup> The sensitivities and specificities of the various studies are summarized in Table 1.

### 3.3. Quality assessment

Results of the quality assessment of studies of diagnostic accuracy are summarized in Table 2. Study types included prospective cohort design and case-control studies. Enrolled subjects included human cadaver models, patients with impending respiratory failure, patients in cardiac arrest, and patients undergoing elective surgeries. There were three studies using human cadaveric models.<sup>16,20,21</sup> The anatomical structure of fresh, unfrozen cadavers can have a similar sonographic appearance when compared to patients in cardiac arrest. Although some studies did not specify the reference standard or blinding process, the determination of esophageal intubation by ultrasonography was not affected and the risk of incorporation bias was minimal.<sup>9,10,14,20</sup> None of the studies reported indeterminate results or subjects that were withdrawn from the final analysis.

### 3.4. Diagnostic accuracy indices

Table 3 shows the results of individual sensitivity and specificity estimates for the tests. The estimated sensitivity and specificity were relatively variable across studies [ $I^2=61.7\%$  (95%CI: 34.0–77.7)]. The estimated pooled sensitivity and specificity for

ultrasonography was 0.93 (95%CI: 0.86–0.96) and 0.97 (95%CI: 0.95–0.98), respectively. The pooled positive likelihood ratio was 26.98 (95%CI: 19.32–37.66) and the negative likelihood ratio was 0.08 (95%CI: 0.04–0.15). The area under the ROC curve showed an acceptable overall accuracy (0.97, Table 3 and Fig. 2). Fig. 3 shows the forest plot of the overall odd ratios.

We performed subgroup analyses by evaluating studies with a similar methodology. We found the specificities of the sonographic examination were generally comparable, while there was a small variation in sensitivities across different subgroups. Tracheal ultrasonography was used in ten studies<sup>9,11,12,14–20</sup>, and lung ultrasonography was used in four studies.<sup>9,10,17,21</sup> Studies using the direct tracheal scan approach have a comparable sensitivity (0.92, 95%CI: 0.84–0.97) to the overall estimate (sensitivity: 0.93, 95%CI: 0.86–0.96).

In tracheal ultrasonography, attending physicians demonstrated superior sensitivity (0.98, 95%CI: 0.96–0.99) when compared to resident physicians (0.92, 95%CI: 0.78–0.96). Different settings also affected the accuracy. Tracheal ultrasonography performed in the ED had a lower sensitivity (0.88, 95%CI: 0.76–0.94) in comparison to other settings. Tracheal ultrasonography performed in real-time was associated with superior sensitivity (0.94, 95%CI: 0.86–0.98) than that performed in a static (post-intubation) fashion (0.91, 95%CI: 0.70–0.98). Despite the variation of sensitivities noted in different subgroups, none of these comparisons was significant.

## 4. Discussion

In this systematic review and meta-analysis, the performance of ultrasonography for the detection of esophageal intubation was

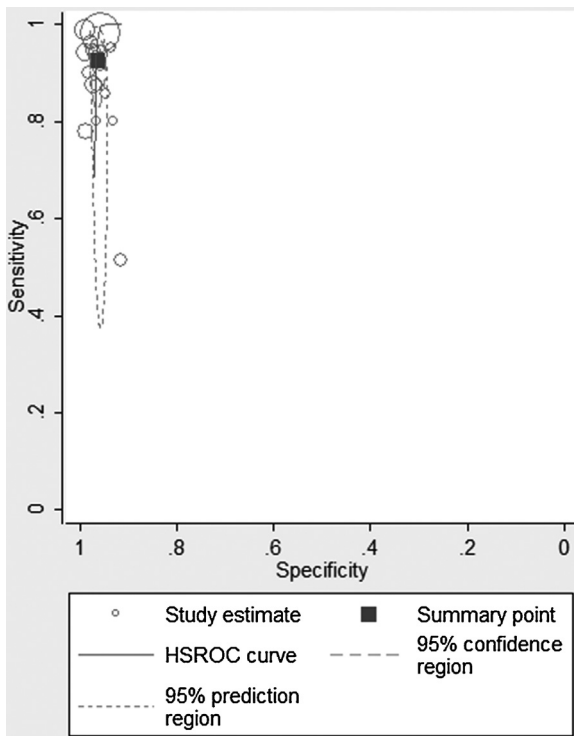


Fig. 2. Hierarchical summary receiver operating characteristic model for diagnosis of esophageal intubation using ultrasonography.

excellent, especially in its specificity value. Different settings, operator experience, and timing of the ultrasound can cause a small but non-significant influence on the diagnostic accuracy. Ultrasonography can be a useful tool for confirmation of tracheal intubation. However, the use of ultrasonography or any method as the sole

test for detection of esophageal intubation is not recommended, given the grave consequences of endotracheal tube malposition.

Point-of-care ultrasonography was considered a promising method for airway confirmation in previous narrative reviews.<sup>29-31</sup> The confirmation of endotracheal tube placement can be made either directly by scanning the anterior neck during the intubation, indirectly by looking for ventilation at the pleural or the diaphragmatic level, or by combining these techniques. The reported diagnostic accuracy varied by different study setting, patient population, and ultrasound scanning methods. To the best of our knowledge, this is the first meta-analysis of studies examining the test characteristics of ultrasonography for the detection of esophageal intubation in adult patients.

Chest auscultation and observation of chest wall expansion are the most common methods to confirm tracheal intubation. Based on the most recent AHA guidelines, capnography is the criterion standard for intubation assessment. For detection of esophageal intubation, chest auscultation and capnography both have high sensitivity but suboptimal specificity values, especially in patients with cardiac arrest or low pulmonary blood flow.<sup>3,32</sup> Tracheal ultrasonography has three major advantages for airway assessment. First, tracheal ultrasonography can be performed in real time as the tube is passed through the trachea or esophagus. Mistakes can be identified before any ventilation commences. Second, ultrasonography has excellent specificity for esophageal intubation detection. It can be used when the results of capnography are equivocal, and may therefore reduce unnecessary intubation attempts in critically ill patients. Finally, tracheal ultrasonography can be performed during CPR without interruption of chest compressions.<sup>11</sup>

In this analysis, tracheal ultrasonography was the most common ultrasound technique to detect esophageal intubation and was used in ten studies.<sup>9,11,12,14-20</sup> The sensitivity and specificity are both high in cadaveric models, ORs, and EDs. In general, real-time sonographic imaging during intubation has higher sensitivity for detection of esophageal intubation than post-intubation

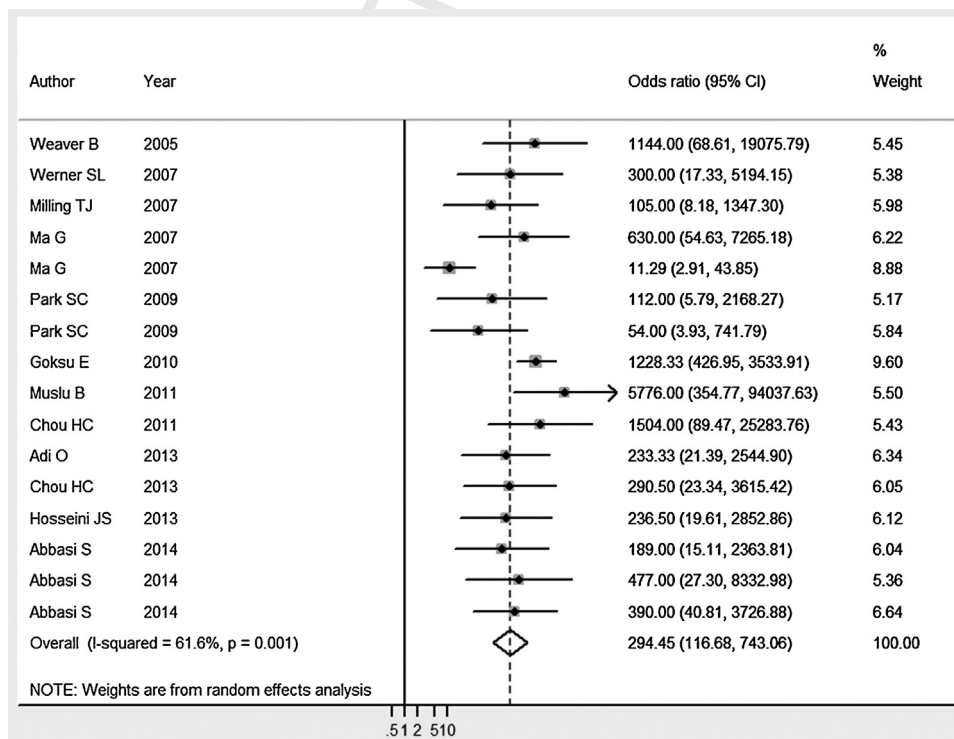


Fig. 3. Forest plot presents the odds ratio of ultrasonography for detection of esophageal intubation. The dot refers to the point estimate for the odds ratio, and the line represents the 95%CI. The vertical line is the summary odds ratio, and the diamond represents the associated 95%CI. Note that weights are from random effects analysis.

**Table 1**  
Summary of included studies.

Author	Design	Population characteristics	Time of measurement	Settings	Operator training levels	Scan type	Intubation attempts	Esophageal intubation	Sensitivity	Specificity
Weaver (2006)	Case control study	Cadavers	Post-intubation	Laboratory	EM physicians	Lung US	68	37%	100.0%	100.0%
Werner (2007)	Case control study	Adults	Real-time	OR	EM physicians	Tracheal US	33	58%	100.0%	100.0%
Ma (2007)	Case control study	Cadavers	Real-time	Laboratory	EM residents	Tracheal US	70	50%	97.1%	100.0%
			Post-intubation	Laboratory	EM residents	Tracheal US	70	50%	51.4%	91.4%
Milling (2007)	Prospective cohort study	Adults	Real-time	OR	EM physicians/residents	Tracheal US	40	13%	100.0%	97.0%
Park (2009)	Prospective cohort study	Adults	Real-time	ED	EM residents	Tracheal US	30	10%	100.0%	96.3%
			Post-intubation	ED	EM residents	Lung US	30	10%	100.0%	100.0%
Göksu (2010)	Case control study	Cadavers	Real-time	Laboratory	EM physicians/residents	Tracheal US	560	50%	98.2%	95.7%
Muslu (2011)	Case control study	Adults	Post-intubation	OR	Anesthesiologists	Tracheal US	150	50%	100.0%	100.0%
Chou (2011)	Prospective cohort study	Adults	Post-intubation	ED	EM residents	Tracheal US	112	15%	94.1%	98.9%
Adi (2013)	Prospective cohort study	Adults	Post-intubation	ED	EM residents	Tracheal US	107	6%	100.0%	98.0%
Chou (2013)	Prospective cohort study	Adults (cardiac arrest)	Real-time	ED	EM residents	Tracheal US	89	8%	85.7%	100.0%
Hosseini (2013)	Prospective cohort study	Adults	Post-intubation	ED	EM residents	Diaphragm movement	57	21%	91.7%	95.6%
Abbasia (2014)	Prospective cohort study	Adults	Real-time	ED	EM residents	Tracheal US	60	10%	100.0%	98.1%
			Post-intubation	ED	EM residents	Tracheal US	60	13%	100.0%	100.0%
			Post-intubation	ED	EM residents	Lung US	120	12%	100.0%	97.2%

EM, emergency medicine; US, ultrasonography; OR, operation room; ED, emergency department.

**Table 2**  
Quality assessment by QUADAS criteria.

Author	Representative spectrum of patients	Clear description of selection criteria	Adequate RS	Short time period between RS and index test	All patient verified by RS	Same RS used	RS independent of index test	Adequate index test description	Adequate RS	Blinding for index test	Blinding for RS	Clinical data available	Report of uninterpretable test result	Description of withdrawals
Weaver (2006)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Werner (2007)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ma (2007)	No	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	No	Yes	Unclear	No	Yes	Yes
Milling (2007)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Park (2009)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Göksu (2010)	No	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes
Muslu (2011)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Chou (2011)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Adi (2013)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Chou (2013)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hosseini (2013)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Abbasia (2014)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes

RS, reference standard.

**Table 3**  
Summary of pooled test characteristics.

Variables	Sensitivity (95%CI)	Specificity (95%CI)	LR+	LR-	AUROC (95%CI)	Diagnostic OR (95%CI)	I <sup>2</sup> (95%CI)	Publication bias (Egger's test p)
Ultrasonography <sup>a</sup>	0.93 (0.86-0.96)	0.97 (0.95-0.98)	26.98 (19.32-37.66)	0.08 (0.04-0.15)	0.97 (0.95-0.98)	354.78 (158.74-792.91)	61.65 (33.99-77.72)	0.83
Tracheal ultrasonography	0.92 (0.84-0.97)	0.97 (0.95-0.98)	27.11 (18.46-39.81)	0.08 (0.04-0.17)	0.97 (0.95-0.98)	344.01 (131.00-903.37)	70.80 (47.38-83.79)	0.88
Resident-performed	0.92 (0.78-0.96)	0.97 (0.95-0.98)	25.87 (17.18-38.97)	0.10 (0.05-0.23)	0.97 (0.95-0.98)	247.22 (93.37-654.56)	70.80 (47.38-83.79)	0.87
Physician-performed	0.98 (0.96-0.99)	0.96 (0.94-0.98)	25.10 (15.50-40.60)	0.02 (0.01-0.04)	0.99 (0.98-1.00)	1160 (491-2743)	43.61 (0.00-81.13)	0.66
ED setting	0.88 (0.76-0.94)	0.98 (0.96-0.99)	36.90 (19.90-68.70)	0.13 (0.06-0.25)	0.98 (0.97-0.99)	294 (107-806)	0.00 (0.00-74.62)	0.31
Real-time	0.94 (0.86-0.98)	0.96 (0.94-0.98)	25.99 (15.67-43.13)	0.06 (0.03-0.15)	0.98 (0.96-0.99)	416.36 (150.39-1170.58)	23.95 (0.00-66.45)	0.01
Static	0.91 (0.70-0.98)	0.98 (0.94-0.99)	39.05 (14.30-106.67)	0.09 (0.02-0.36)	0.99 (0.98-1.00)	435.01 (50.16-3772.35)	82.77 (60.62-92.46)	0.01

ED, emergency department; LR, likelihood ratio; OR, odds ratio.  
<sup>a</sup> Either tracheal or lung ultrasonography.

scanning.<sup>9,11,16-20</sup> Ma et al.<sup>20</sup> reported high sensitivity and specificity in real-time, dynamic assessment, but poor sensitivity in static, post-intubation assessment in cadaveric models. However, static assessment can still achieve excellent sensitivity and specificity on live patients in the OR and ED.<sup>9,12,14,15</sup> The sensitivity, but not specificity, of ultrasonography varied in different settings and level of operators. Ultrasonography is highly operator-dependent, and ultrasounds performed by residents had a lower sensitivity than those performed by attending physicians. Ultrasonography also demonstrated decreased sensitivity in the ED setting, which is likely due to the emergent nature of many ED intubations.

Lung ultrasonography can provide indirect dynamic evidence of correct endotracheal intubation. Pleural and diaphragmatic movements, which are the indicators of lung expansion, can be identified with ultrasonography. Bilateral lung sliding and equal diaphragm movement synchronized with ventilation can be seen if the tube is in the trachea. Compared with tracheal ultrasonography, lung ultrasonography can be used to confirm endotracheal intubation and detection of main stem intubation.<sup>13,21</sup> Four studies using lung or diaphragm ultrasonography were enrolled in our final analysis.<sup>9,10,17,21</sup> These indirect ultrasound methods also achieved high sensitivity and specificity for detection of esophageal intubation. Lung ultrasonography can also be used to identify main stem intubation.<sup>13,21,33</sup> In this study, we did not pool the results of diagnostic accuracy of lung ultrasonography for detection of main stem intubation, because only a few studies were found in the adult population.<sup>13,21,33</sup> Future studies are still needed to clarify the value of this application.

There are several limitations in this study. First, three studies were performed in cadaveric models, and these results may not be reproduced when imaging actual patients. Two studies utilized only one cadaveric model for intubation attempts that may induce a potentially significant source of bias.<sup>16,20</sup> Second, we found moderate heterogeneity among the included studies, which was a result of differences in study setting, patient characteristics, and ultrasound scanning techniques between studies. This heterogeneity may affect the reliability and precision of the final test characteristics results. In addition, there was incomplete adherence of the included studies to the QUADAS tools. Fourth, the accuracy of ultrasonography depends on the operator's experience. However, the nature of training requirements for tracheal or lung ultrasonography was not investigated. Finally, we did not pool the results of diagnostic accuracy specific to cardiac arrest patients or mainstem intubation because only a few studies were found in adult populations related to these specific areas. To reduce the heterogeneity of this meta-analysis, pediatric studies were not included because the ultrasound techniques for detection of esophageal intubation are different between adult and pediatric studies. In pediatric patients, the researchers usually used diaphragmatic ultrasonography<sup>34,35</sup>, but not tracheal or lung ultrasonography, to confirm endotracheal intubation. Several studies used tracheal ultrasonography to confirm tracheal intubation in pediatric patients.<sup>36,37</sup> However, the researchers looked for direct visualization of the endotracheal tube in the trachea, but not the sonographic sign of esophageal intubation that was used in adult patients. Future systematic reviews and meta-analyses are still needed for this application in pediatric patients.

### 5. Conclusion

The pooled results of published studies suggest that ultrasonography can be used to detect esophageal intubation with a high degree of diagnostic accuracy. With optimal sensitivity and near-perfect specificity, ultrasonography can be a valuable adjunct in

322 this aspect of airway assessment, especially in situations where  
323 capnography may be unreliable.

### 324 Author contributions

325 EHC, ED, and MT conceived the study. EHC and CCL designed the  
326 study. PYT, MT, and YMT were responsible for acquisition of data.  
327 ED, MHMM, and CCL provided statistical advice on study design.  
328 EHC and PYT were responsible for analysis and interpretation of  
329 data. EHC and ED drafted the article. ED, CCL and JM contributed  
330 to article review and revision. CCL were responsible for statistical  
331 analysis. JM and CCL supervised the conduct of the study and pro-  
332 vided quality control. EHC takes responsibility for the paper as a  
333 whole.

### 334 Conflict of interest statement

335 The authors did not receive any financial support and have no  
336 conflict of interest.

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