Lung ultrasound and diaphragmatic excursion assessment for evaluating perioperative atelectasis and aeration loss during video-assisted thoracic surgery: a feasibility study

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Background: Although lung-protective strategies are widely used in thoracic surgery, postoperative atelectasis can still occur. Both lung ultrasound (LUS) and diaphragmatic excursion assessments are accurate and noninvasive for bedside imaging and examination. This study aimed to test the feasibility of using LUS during the perioperative period of video-assisted thoracic surgery (VATS) and to continuously evaluate aeration changes through LUS examination and diaphragmatic excursion assessment.

Methods: Between January 2019 and May 2019, data were prospectively collected from patients that were scheduled to undergo a VATS with one-lung ventilation (OLV). LUS was performed at four specific timepoints: before the induction of general anesthesia (timepoint A), 5 minutes after intubation (timepoint B), at the end of surgery (timepoint C), and 15 minutes after extubation (timepoint D). Diaphragmatic excursion assessment was performed only at the first (timepoint A) and last timepoints (timepoint D) for the use of paralytics during surgery.

Results: This study included 80 consecutive patients (37 men, 43 women). Among them were patients undergoing lobectomy (14 patients; 17.5%), segmentectomy (35 patients, 43.8%), wedge resection (19 patients; 23.8%), or mediastinal tumor resection (12 patients, 15.0%). LUS was possible for all patients. As a result, LUS helped detect postoperative atelectasis in 12 patients (15.0%). Among them were 4 (33.3%) lung resection patients and 8 (66.7%) mediastinal tumor resection patients. Pneumothorax and small effusions were also diagnosed through LUS examination. There was significant aeration loss throughout the surgery from the start of induction (P<0.001). We discovered that changes in LUS scores were found to be associated with an increase of diaphragmatic excursions after assessment (Spearman’s r=−0.54, P<0.001).

Conclusions: LUS is feasible during all phases of the perioperative period in VATS and can facilitate the early investigation of perioperative atelectasis. Perioperative LUS and diaphragmatic excursion assessment are also feasible for the continuous assessment of aeration loss in patients undergoing VATS.

Keywords: Lung ultrasound (LUS); diaphragmatic excursion; video-assisted thoracic surgery; atelectasis; aeration loss

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Introduction

The use of one-lung ventilation (OLV) is widely applied in video-assisted thoracoscopic surgery (VATS) to achieve optimal surgical conditions (1). Lung-protective strategies such as lowering the tidal volume (VT), lowering the value of inspired oxygen (FiO₂), recruitment maneuvers, and the moderate positive end-expiratory pressure (PEEP) has reduced postoperative respiratory complications significantly (2,3). Unfortunately, atelectasis is a common phenomenon during general anesthesia and intrathoracic surgery (4). Meanwhile, thoracic surgery causes unusual weaknesses of the diaphragm’s muscle fibers, and one of the major components of total respiration is diaphragm movement (5). Thus, impairment of diaphragm muscle fibers significantly increases postoperative pulmonary complications (6,7).

The use of bedside lung ultrasound (LUS) has been growing rapidly as a method for the diagnosis of respiratory diseases in the intensive care unit (ICU), operating room (OR), and emergency department due to its lack of radiation, high accuracy, repeatability, portability, and noninvasiveness (8,9). LUS can quickly detect atelectasis (sensitivity 87.7%, specificity 92.1%, accuracy 90.8%) (10), pleural effusion (sensitivity 100%, specificity 100%, accuracy 100%) (11), pneumonia (sensitivity 88%, specificity 86%) (12), and pneumothorax (sensitivity 100%, specificity 82%) (13). Ultrasound assessments of diaphragmatic excursions are correlated with vital capacity (5). Furthermore, the use of LUS is straightforward and safe to use while simultaneously enhancing the specificity and sensitivity of phrenic nerve activity measurement (14). It has also been found to be useful for the serial assessment of diaphragmatic function and motion (sensitivity 91%, specificity 91%) (15).

Although it has been deemed necessary to use LUS daily in the postoperative period to enhance recovery after thoracic surgery (16), there have been no studies on LUS that continuously investigate atelectasis and aeration changes during the perioperative period of VATS. We hypothesized that LUS would allow for the diagnosis of pulmonary complications, especially atelectasis during VATS. Furthermore, it would be possible to use LUS to track and monitor changes in lung aeration. Additionally, we hypothesized that diaphragmatic excursion assessments by ultrasonography could also evaluate aeration loss after VATS. This study primarily aimed to investigate the feasibility of bedside LUS in the perioperative period of VATS and to diagnose perioperative atelectasis. The secondary aim was to investigate the use of continuous evaluation of perioperative lung aeration changes during VATS through LUS examination and diaphragmatic excursion assessment. We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/apm-19-595b).

Methods

Patients

The trial was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study protocol was approved by the institutional review board of the Second Affiliated Hospital of Zhejiang University (I2018001364, 2018/12/05) and registered at ClinicalTrials.gov (no. NCT03802868) and informed consent was taken from all the patients. Consecutive adult patients scheduled for elective video-assisted thoracoscopic lung surgery or mediastinal tumor resection were recruited. Exclusion criteria included the following: noncooperation or delirium after extubation; a body mass index (BMI) of ≥40 kg/m²; a history of respiratory infection; chronic obstructive pulmonary disease or chronic heart disease, history of brachial plexus nerve blockage or general anesthesia within 2 weeks of surgery; previous thoracic procedures (e.g., thoracotomy, thoracoscopy or thoracic drain). Patients were not included for further data analysis in cases of conversion to open thoracotomy, OLV failure (failure to maintain oxygenation and need to convert to two-lung ventilation, TLV), or postoperative admission to the ICU.

Anesthesia protocol

All enrolled patients were pre-oxygenated with 100% oxygen for 3 minutes and received intravenous administration of midazolam 0.03–0.05 mg/kg, sufentanil 0.4–0.6 µg/kg, and etomidate 0.2–0.3 mg/kg for anesthesia induction. Endotracheal intubation was facilitated with rocuronium 0.6–1.0 mg/kg. The proper double-lumen endotracheal tube was inserted to perform the OLV. Anesthesia was maintained with a continuous intravenous infusion of remifentanil and propofol. Supplemental cisatracurium was provided for adequate muscle relaxation (no more than 1 twitch on the train-of-four) when needed. The depth of anesthesia monitoring was completed by the bispectral index (BIS) with an appropriate value of 40–60. Mechanical
ventilation with the volume-controlled modes was used for each participant. The OLV parameters were set as a Vt of 5–6 mL/kg, a respiratory rate of 12–14 breaths/min adjusted to sustain an end-tidal carbon dioxide partial pressure (P$_{ET}$CO$_2$) of 35–45 mmHg, and an FiO$_2$ of 0.6–0.8 with a PEEP of 5 cmH$_2$O to maintain a peak airway pressure of less than 30 cmH$_2$O. Lung resection was performed in the lateral position, whereas mediastinal tumor resection was performed in the supine position. Before closing the chest, an alveolar recruitment maneuver set the peak inspiratory pressure limit to 45 cmH$_2$O. Next, a respiratory rate of 6 breaths/min and inspiratory-to-expiratory ratio (I:E) of 1:1 was induced by the anesthesiologist and then returned to pre-recruitment values. The complete expansion of the collapsed lung was directly visualized by the surgeon, and then the OLV was converted to TIV until extubation. The surgeon routinely placed a chest tube attached to a water-sealed bottle at −20 cmH$_2$O suction to drain any subsequent fluid and/or air leakage. Analgesic therapy at the incision site was accomplished with ropivacaine (0.375%, 10 mL) through an intercostal peripheral nerve blockade in each patient. Postoperative pain therapy was implemented by continuous patient-controlled intravenous analgesia for 48 hours. In the post-anesthesia care unit (PACU), mechanical ventilation with the same set of pre-recruitment parameters were used for all patients. Neostigmine was used for the reversal of neuromuscular blocking before extubation in all patients. Patients inhaled oxygen through a mask at 5–6 L/min for at least 15 minutes after extubation.

**LUS examination**

LUS imaging was performed by 2 trained anesthesiologists (Chen X and Na S, both with at least 1 year of ultrasound training) using a 2- to 5-MHz convex probe in an ultrasound device (Mindray, Guangdong, China). Images were acquired at 4 pre-established timepoints: before anesthesia induction (the timepoint A), 5 minutes after intubation (the timepoint B), at the end of the surgery (the timepoint C), and 15 minutes after extubation (the timepoint D). Each hemithorax was divided into 6 quadrants by anterior and posterior axillary lines, and axial lines (superoanterior, inferoanterior, superolateral, inferolateral, superoposterior, and inferoposterior quadrants) (Figure 1) (17). With the patient in the supine position, the anterior and lateral quadrants were examined.

Meanwhile, the examinations of the posterior quadrants were accomplished with the patient slightly turned to the lateral position. The ultrasound probe was moved horizontally along the intercostal spaces in each quadrant. The following data were recorded for each scan: the presence of A lines, the amount or coalescence of B lines, and the number of subpleural consolidations. Once atelectasis was detected at timepoint D, the patient was transported to the Radiology Department for a thoracic computed tomography (CT) scan within 1 hour of detection. Those who did not have anyatelectasis discovered on the LUS were transported to their respective wards without any additional investigations except for...
a routine chest X-ray (CXR) check on postoperative day 1 (Figure 2). A complete ultrasound examination needed a mean duration time of 5 to 10 minutes. For the characterization of the regions, a recorded video of the worst abnormality for each quadrant was analyzed offline by the two anesthesiologists mentioned above (Chen X and Na S). In the cases of disagreement, a third anesthesiologist (Kai S, with 3 years of ultrasound experience) reviewed the uncertain images and made the final diagnosis.

**Lung ultrasonography scores**

The LUS scores were used to assess aeration loss by retrieving the sum of the score values from 0 to 3 for each quadrant, with a higher grade indicating more severe aeration loss, and no scores being applicable for pneumothorax. The scoring was defined as follows: 0= normal lung with sliding pleura and equidistant A lines parallel to the smooth pleural line; 1= moderate aeration loss and no less than 3 scattered B lines deriving from the pleural line; 2= severe aeration loss and irregular pleural line with coalescent B lines; 3= complete aeration loss and a tissue-like pattern or subpleural consolidation (Figure 3) (10,18). Atelectasis was diagnosed with juxta-pleural hypoechoic consolidations using bright echogenic static air bronchograms or a tissue-like pattern on ultrasonography as a model (Figure 3D) (19). Pleural effusion was described as an anechoic space between the visceral and parietal pleura that also fluctuated with respiratory movement (20). A maximal intrapleural distance of less than 15 mm on ultrasonography was defined as a small effusion (21). Pneumothorax was diagnosed via a combination of the visualization of the “bar code” sign on the M-mode, the lung point, and the absence of lung sliding (22-24). Meanwhile, the presence of lung sliding could help to exclude the diagnosis of pneumothorax. The absence of pleural sliding in the second intercostal space on the LUS was defined as a small pneumothorax, whereas a large pneumothorax was recognized as the loss of sliding in both the second and third intercostal spaces (25).

**Diaphragmatic excursion assessment**

The anesthesiologists (Chen X and Na S) performed the
diaphragmatic excursion assessment at the timepoints A and D due to the use of paralytics during the surgery. The low-frequency curvilinear transducer was placed in bilateral costal margins between the anterior and posterior axillary lines for longitudinal scanning. Images of ultrasonographic diaphragmatic excursions were obtained using the liver and spleen as acoustic windows during full respiration (Figure 1). With normal breathing of patients in the 45° semi supination position, the maximum vertical axis between the adjacent peaks and valleys using the sinusoid in the M-mode was measured as the diaphragmatic excursion movement (14). Offline analysis was also completed by Chen X and Na S.

Data collection

Each participant's demographic data were collected. The data included sex, age, height, weight, BMI, American Society of Anesthesiologists (ASA) score, and lung function test data. Vital signs and hemodynamic index were recorded at all the study timepoints. At the timepoint B and C, mechanical ventilation parameters were noted, including Vt, respiratory rate, PEEP, FiO₂, and peak inspiratory pressure. Outcomes included the duration of mechanical ventilation, OLV time, and the procedure length, total infusion details (the sum of the crystalline and colloidal liquids), length of stay (defined as the period from operation to discharge), and short-term complications according to postoperative routine CXR or CT findings before discharge.

Statistical analysis

The sample size was calculated using the G-Power software (version 3.1). Using previous publication for inference (4), a total of 80 patients were needed with the following assumptions: α error of 0.05, a β value equal to 0.2, a nonsphericity correction of 0.5, an effect size of 0.25, and
a dropout rate set in 10% of patients. Continuous variables were described as the mean ± standard deviation or median and interquartile range after normality distribution testing and compared using a Student’s t-test or Mann-Whitney U-test as appropriate. Categorical variables were expressed as frequency and percentage, and comparison was made with Fisher’s exact test. The relationship between the changes in the diaphragmatic excursions and LUS scores was assessed by Spearman’s correlation. Repeated-measure one-way analysis of variance (ANOVA) was used to analyze the aeration changes in the ventilatory lungs. SPSS statistical software version 23.0 (IBM Corp, Armonk, NY, USA) was used for data analysis, and the level of statistical significance was set as a P value less than 0.05.

**Results**

**Patient participation and demographic information**

From January 2019 to May 2019, 98 adult patients were prospectively screened. After 18 patients met 1 or more exclusion criteria, a final total of 80 patients were enrolled (Figure 2). During the study, all of the ultrasound examinations of both the lungs and diaphragm were completed, and 4,800 cine-loops were stored. The demographic data of these patients are summarized in Table 1. Hemodynamic variables and ventilatory parameters are shown in Table 2.

### Table 1 Patient characteristics (N=80)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD)/median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>54.8 (12.8)</td>
</tr>
<tr>
<td>Sex, M/F (n)</td>
<td>37/43</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.5 (6.8)</td>
</tr>
<tr>
<td>Weight (cm)</td>
<td>63.2 (10.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5 (2.8)</td>
</tr>
<tr>
<td>ASA, 1/2/3 (n)</td>
<td>9/67/4</td>
</tr>
<tr>
<td>Length of mechanical ventilation (min)</td>
<td>145.0 (120.0, 178.8)</td>
</tr>
<tr>
<td>Length of OLV (min)</td>
<td>90.0 (71.3, 128.8)</td>
</tr>
<tr>
<td>Length of the procedure (min)</td>
<td>115.7 (42.3)</td>
</tr>
<tr>
<td>Length of stay (d)</td>
<td>7.0 (5.3, 7.0)</td>
</tr>
<tr>
<td>Infusion (mL)</td>
<td>1,000.0 (1,000.0, 1,500.0)</td>
</tr>
<tr>
<td>Type of surgery, n (%)</td>
<td></td>
</tr>
<tr>
<td>Wedge</td>
<td>19 (23.8)</td>
</tr>
<tr>
<td>Lobectomy</td>
<td>14 (17.5)</td>
</tr>
<tr>
<td>Segmentectomy</td>
<td>35 (43.8)</td>
</tr>
<tr>
<td>Mediastinal</td>
<td>12 (15.0)</td>
</tr>
</tbody>
</table>

Data are reported as the mean ± standard deviation or median and inter-quartile range as appropriate. SD, standard deviation; IQR, inter-quartile range; M, male; F, female; BMI, body mass index; American Society of Anesthesiologists; OLV, one-lung ventilation. Length of stay was defined as the hospital length after the date of operation.

### Table 2 Hemodynamic variables and ventilatory parameters (N=80)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Timepoints</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>96.0 (10.4)</td>
</tr>
<tr>
<td>Pulse (beats/min)</td>
<td>77.0 (72.0, 84.5)</td>
</tr>
<tr>
<td>SPO₂ (%)</td>
<td>98.0 (98.0, 99.0)</td>
</tr>
<tr>
<td>Tidal volume (mL/kg)</td>
<td>6.0 (5.0, 7.0)</td>
</tr>
<tr>
<td>Respiratory rate (breaths/min)</td>
<td>12.5 (12.0, 14.0)</td>
</tr>
<tr>
<td>FiO₂</td>
<td>0.21 (0.21, 0.21)</td>
</tr>
<tr>
<td>PEEP (cmH₂O)</td>
<td>5.0 (5.0, 5.0)</td>
</tr>
<tr>
<td>Peak airway pressure (cmH₂O)</td>
<td>23.3 (3.8)</td>
</tr>
</tbody>
</table>

Data are described as mean ± standard deviation or median and inter-quartile range as appropriate. Timepoint A, before induction of general anesthesia; timepoint B, 5 minutes after intubation; timepoint C, the end of surgery; timepoint D, 15 minutes after being extubated. *, P<0.05 compared to baseline or the timepoint B; **, P<0.01 compared to baseline or the timepoint B. SPO₂, peripheral oxygen saturation; FiO₂, fraction of inspiration oxygen; PEEP, positive end expiratory pressure.
All 12 cases of postoperative atelectasis were confirmed by CT scan and remained in their dependent quadrants when scanned 15 minutes after extubation. No new atelectasis was discovered in the postoperative routine CXR in the LUS atelectasis-free group. There was no evidence of prolonged pneumothorax or pleural effusion, except for atelectasis, which had not completely disappeared on routine CXR at discharge. No complications such as bleeding, pneumonia, or infection were experienced by any participant.

Continuous LUS scores in non-surgical lungs

Serial scoring was only performed on non-surgical lung since LUS scores were inapplicable for pneumothorax. The mean LUS scores of the lungs undergoing ventilation before induction was 0.15±0.58. After the induction of general anesthesia, the score significantly increased to 0.78±1.02 (P<0.001). At the end of the surgery, the LUS score markedly jumped to 6.73±2.67 (P<0.001). Aeration improved after extubation when compared to the end of surgery (5.64±2.72, P<0.001) but was still higher than baseline (P<0.001) (Figure 4).

Preoperative and postoperative diaphragmatic excursion assessment on both sides

The diaphragmatic excursion on the operative side and contralateral sides were 1.68±0.10 and 1.68±0.11 cm before induction, respectively (P=0.562). After extubation, the excursion declined on both sides (1.20±0.19 cm, P<0.001; 1.30±0.16 cm, P<0.001) but was worse on the operative side (P<0.001) (Figure 5).

Relationship between LUS scores and diaphragmatic excursion assessment

The changes in the LUS scores of the ventilated lungs were correlated with the changes in diaphragmatic excursion between the baseline and after extubation (r=−0.54; P<0.001). No correlation was found between the changes in LUS scores with age, BMI, duration of mechanical ventilation, or procedure. Perioperative temporal unilateral LUS signs of collapsed lung (Figure 6A), diaphragmatic excursion assessment of the homolateral side of the inferoposterior quadrant (Figure 6B), and signs of atelectasis in LUS and CT investigation (Figure 6C) of an atelectatic patient with mediastinal tumour resection are displayed in sequence in Figure 6.
Besides lung resection for which postoperative physiotherapy was not yet in place the earlier observational time (15 minutes after extubation) in the previous report (the thoracic CT scan in our study and was higher than that incidence rate of atelectasis (15%)). Postoperative atelectasis is often the result (not always possible to open the collapsed lung completely). Cytokine release during reperfusion injury causes decreased respiratory muscle capacity from lost maximal muscle forces due to mechanical ventilation and surgery (27 patients in the Subotic study). These factors may explain the increased frequency of atelectasis in the lung resection group compared to the mediastinal tumor resection group throughout which the supine position was used. The lateral decubitus position was shown to alleviate compression from the mediastinum and the heart and might also decrease atelectasis and atelectasis (32).

The LUS score has been proven to significantly correlate with CT-measured lung reaeration in patients with atelectasis and pneumonia (10). As expected, the induction of general anesthesia caused a decrease in aeration, which was primarily located in the dependent areas (4). A pure oxygen content being used for mechanical ventilation intubation may account for this phenomenon, with either gas absorption or airway closure resulting from the preoxygenation (33). Sustained high FiO2, sigh maneuver, breath disappearance, and prolonged dyskinesis are the main mechanisms for aeration loss during general anesthesia (34), with restoration of spontaneous breathing being shown to induce a marked improvement in aeration. Relief of the mediastinal compression and the influence of gravity allowed the ventilation and perfusion to slowly recover, but they did not return to baseline values 15 minutes after extubation.

The present study demonstrates, for the first time, a correlation between changes in LUS scores and diaphragmatic excursions. Diaphragmatic excursions assessment via ultrasound has been widely used to identify severe respiratory dysfunction and to predict success in weaning patients from mechanical ventilation (35). Preoperative bilateral diaphragmatic excursions showed no differences, and the findings were consistent with normal diaphragmatic functions (36). In contrast to Subotic et al., our study showed a decrease in amplitudes of the contralateral diaphragm after lung resection (37). A possible explanation for this result is our earlier observation time (15 minutes after extubation) and our larger sample size (27 patients in the Subotic study). Patients showed decreased respiratory muscle endurance and inspiratory capacity from lost maximal muscle forces due to mechanical ventilation and surgery (38). These factors may explain the significant postoperative reduction in bilateral diaphragmatic excursions.

As a result of the limited diagnostic tools available to anesthesiologists in the intraoperative period, the role of...
Figure 6 Perioperative temporal unilateral LUS signs of collapsed lung, diaphragmatic excursion assessment of the homolateral side of the inferoposterior quadrant, and signs of atelectasis in LUS and CT investigation of an atelectatic patient with mediastinal tumor resection. Lung ultrasonography was recorded in the inferoposterior quadrant. (A1) Preoperative normal aeration before induction of general anesthesia; (A2) a small degree of aeration loss with more than 3 B lines; (A3) the operation caused severe aeration loss with lung consolidation (atelectasis); (A4) lung consolidation was not improved after extubation. (B1) Normal diaphragmatic excursion before induction of general anesthesia; (B2) significantly decreased postoperative diaphragmatic excursion. (C1) Atelectasis in LUS examination; (C2) atelectasis in CT examination (yellow arrows). LUS, lung ultrasound, CT, computed tomography.
LUS in the OR should be examined. During all phases of VATS, atelectasis can be first effectively detected at the end of surgery. As each patient is still on mechanical ventilation at that time, the performance of recruitment maneuver again may effectively reduce postoperative atelectasis. Both LUS score and diaphragmatic excursion could reflect ventilation; the variation of the two indexes would be helpful for patients adapting to and being weaned from mechanical ventilation. Our present study is only a tentative exploration, and understanding the significance of these two methods will be our aim for a future study.

**Limitations**

Our study has several limitations. First, the small number of mediastinal tumor resections in our cohort makes it difficult to draw conclusions. Future studies with a larger sample size are needed to explore the optimal position to reduce postoperative atelectasis in mediastinal tumor resection patients. Also, as pneumothorax was inapplicable of the LUS score, continuous aeration changes were only assessed in the non-operative lungs, and further studies on LUS scores in non-thoracic surgery are encouraged. Last, only patients with ultrasound-detected atelectasis received a CT scan. Whether patients without ultrasound diagnosis of atelectasis have a similar atelectasis sign in the CT scan of atelectasis is unknown.

**Conclusions**

In conclusion, our study demonstrated the feasibility of using LUS during all phases of the perioperative period in VATS. LUS offers the ability to diagnose and track perioperative atelectasis. Both LUS and diaphragmatic excursion assessment can facilitate continuous evaluation of aeration loss during VATS.

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**Footnote**

*Reporting Checklist:* The authors have completed the STROBE reporting checklist. Available at [http://dx.doi.org/10.21037/apm-19-595b](http://dx.doi.org/10.21037/apm-19-595b)

*Data Sharing Statement:* Available at [http://dx.doi.org/10.21037/apm-19-595b](http://dx.doi.org/10.21037/apm-19-595b)

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at [http://dx.doi.org/10.21037/apm-19-595b](http://dx.doi.org/10.21037/apm-19-595b)). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The trial was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study protocol was approved by the institutional review board of the Second Affiliated Hospital of Zhejiang University (I2018001364, 2018/12/05) and registered at ClinicalTrials.gov (no. NCT03802868) and informed consent was taken from all the patients.

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