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The predictive value of serial changes in diaphragm function during the spontaneous breathing trial for weaning outcome: a study protocol

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Keywords: Diaphragm; Ultrasonography; Weaning

Word count: 2319

Abstract

Introduction: There are many tools being used in clinical practice for the prediction of weaning success from mechanical ventilation. However, most of them are less than satisfaction. The purpose of this study was to investigate the value of diaphragm function measured serially by ultrasound during the spontaneous breathing trial(SBT) as a weaning predictor.

Methods and analysis: This is a prospective observational study conducted in a 10-bed medical emergency intensive care unit(EICU) in a university-affiliated hospital. The study will be performed from November 2016 to December 2017. All patients in the EICU who are expected to have mechanical ventilation for more than 48 hours through endotracheal tube are potentially eligible for this study. Patients will be included if they meet all the following criteria: 1) absence of fever; 2) alert and cooperative; 3) hemodynamically stabile without vasopressors; and 4) improved respiratory function with $FiO_2 < 0.5$, PEEP ≤ 5 cmH_2O, $PaO_2/FiO_2 > 200$ and respiratory rate <30 breaths per minute. We will use the tube compensation mode (TC) to perform the spontaneous breathing trial(SBT) with the compensation ratio at 85%. The patients will undergo SBT for 2 hours in a semi-recumbent position. If the patient fails to tolerant the SBT, we will terminate the trial immediately and turn back to the control mode. Patients who passed the 2-hour SBT were extubated. During the SBT, right diaphragm excursion and thickening fraction will be evaluated by ultrasonography. Images will be obtained at 5 min, 30min, one hour and two hours after the initiation of SBT. Rapid shallow breathing index (RSBI) was simultaneously calculated at the bedside.

Ethics and dissemination: The study protocol was approved by the ethics committee of Sir Runrun Shaw hospital, an affiliate of Zhejiang University, Medical College. The results will be published in a peer-reviewed journal and shared with the worldwide medical community.

Trial registration: The study was registered at International Standard Registered Clinical/Social Study number (ISRCTN) registry (ISRCTN42917473).

Strengths and limitations of this study

1. This is the first study to investigate the serial changes in diaphragm function during the SBT. It is possible to find a better weaning predictor through this study.
2. We use bedside ultrasound to assess the function of the diaphragm in this study. It is simple, rapid and noninvasive.
3. We only measured the right diaphragm. Because the measurement of the right diaphragm is technically simpler than the left, and we want to provide a simple and feasible method in clinical practice.
4. We did not compare the ultrasound result with trans-diaphragmatic pressure, and the latter is considered as the gold standard of diaphragm function.

Introduction

Approximately 20% of mechanically ventilated patients have difficulties in weaning from mechanical ventilation[1], and about 40% of ventilation time is spent to discontinue ventilatory support[2]. Measures such as breathing frequency, minute ventilation, maximum inspiratory pressure, tracheal airway occlusion pressure 0.1 s, and a combined index named CROP (compliance, rate, O_2, pressure index) have been employed to determine the optimal timing of weaning from mechanical ventilation [3]. Yang and Tobin[4] found that the most accurate predictor of weaning outcome was RSBI (respiratory frequency/tidal volume). However, the threshold of

RSBI varied in different studies[5-7], owing to the differences in ventilatory support settings, age, gender, body position, underlying illness and endotracheal size[8-10]. It is reported that RSBI measured early during an SBT is inaccurate in predicting weaning success in patients with chronic obstructive pulmoanry diseases[11]. Also RSBI is not a good predictor for patients whose primary problem is related to poor cough, increased secretion and compromised airway protection. The latter conditions are commonly seen in neurosurgical patients[12,13].

The diaphragm is the main respiratory muscle. Diaphragm dysfunction is the primary contributor to weaning failure and prolonged duration of mechanical ventilation [14]. Excursion and thickening fraction of the diaphragm has been measured by bedside ultrasonography and were found to be able to quantitatively reflect diaphragm function, and thus was useful in predicting weaning outcome[15-19]. However, these indices are usually measured at the start of an SBT. To the best of our knowledge, there is no report on the impact of serial changes in diaphragm excursion and thickening fraction during the SBT on weaning outcomes. The purpose of this study is to investigate whether the diaphragm function measured serially by ultrasonography during the SBT could be used to predict the weaning outcome.

Study design

This is a prospective observational study conducted in a 10-bed medical intensive care unit in a university-affiliated hospital. The hospital is a teaching hospital of Zhejiang University with about 2400 beds. Our EICU enrolled approximately 400 critically ill patients every year, and tracheal intubation patients accounted for 40% of the total admissions. The study starts from November 2016, and is planned to last for one year. All patients will be consecutively enrolled and followed up for the entire hospital stay.

Study population

All patients in the EICU who are mechanically ventilated for more than 48 hours through endotracheal tube are potentially eligible for this study. Patients will be included if they meet all the following criteria: 1) absence of fever; 2) alert and cooperative; 3) hemodynamically stabile without vasopressors; and 4) improved respiratory function with $FiO_2 < 0.5$, PEEP ≤ 5 cmH_2O, $PaO_2/FiO_2 >$ 200 and respiratory rate <30 breaths per minute. After inclusion, they will undergo an SBT. Subjects were excluded if they had a history of diaphragm paralysis, cervical spine injury, neuromuscular diseases, a current thoracostomy, pneumothorax, or pneumomediastinum.

Study protocol and some definitions

Patients who meet the above mentioned criteria were followed up. Demographics such as sex, age on admission, body mass index(BMI), underlying diseases, reasons for tracheal intubation, Acute Physiology and Chronic Health Evaluation II (APACHE II), duration of mechanical ventilation, were recorded. When the subjects are suitable for SBT, they will proceed to undergo an SBT for 2 hours. We will use the tube compensation mode (TC) to do the SBT with the compensation ratio at 85% [20]. During these two hours, if the SBT failed, we terminated the trial immediately and turned back to the control mode. Patients who passed the 2-hour SBT were extubated. Right diaphragm excursion and thickening fraction was evaluated by ultrasonography at 5 min, 30min, one hour and two hours after the initiation of SBT. RSBI was simultaneously calculated at the bedside. Laboratory measurements including blood gas, proBNP, chemistry profile, blood count and C reactive protein were obtained and recorded. Cardiac function were assessed by pro-BNP and echocardiography findings such as left ventricular ejection fraction (LVEF), E-point to septal separation(EPSS) and diastolic function. Attending physicians also assessed the amount of endotracheal secretion and the patient's ability to cough. And they were blinded to ultrasound measurements. According to the weaning outcome, subjects were divided into two groups, the successful group and the failure group.

A successful weaning was defined as spontaneous breathing(SB) for >48 h following extubation without any level of ventilator support. A failed weaning was defined as either SBT failure or the inability to maintain SB for at least 48 h after extubation. Criteria for failure to the SBT was the following: breathing frequency >35 breaths/min, change in heart rate > 20%, increase in systolic blood pressure > 25%, change in mental status, onset of discomfort, diaphoresis, or signs of increased work of breathing [21]. The time from the beginning to the end of mechanical ventilation is defined as total ventilation time. Weaning time was the time spent in partial support mode.

Diaphragm ultrasound

All patients will be measured in a semi-recumbent position with the head of bed elevated between 20° and 40°. The right diaphragmatic excursion is measured with a 3.5-5MHz ultrasound probe (Mindray M9, China). The probe was placed below the right costal margin along the mid-clavicular line. The liver is used as an acoustic window. Firstly, B-mode is used to get the best approach and to select the exploration line. Then M-mode is used to display the motion of the diaphragm along the selected line. The diaphragmatic excursion could be measured in figure 1.

The right diaphragm thickness was measured by a 7-10MHz linear ultrasound probe set to B mode placed perpendicularly to the chest wall, in the 8th or 9th intercostal spaces, between the anterior axillary and the midaxillary lines[22]. The diaphragm was imaged at the zone of apposition as three layer structure, including two parallel echoic lines (the diaphragmatic pleura and the peritoneal membrane) and a hypoechoic structure between them (the muscle itself) [22,23]. On frozen B-mode image, the distance from the middle of the pleural line to the middle of the peritoneal line was the diaphragm thickness (figure 2). Diaphragm thickening fraction (DTF)=(Thickness at end inspiration − Thickness at end expiration)/ Thickness at end expiration.

Follow-up study

Diaphragm dysfunction is common in critically ill patients. It can be caused by multiple factors including prolonged mechanical ventilation. We first examine the serial changes in diaphragm function during an SBT with ultrasound in order to assess its value for predicting weaning outcome. Then a follow-up diaphragm ultrasound will be performed in patients after extubation in order to find whether the damage of diaphragm is reversible.

Statistical analysis

Sample size calculation was performed based on the Cox proportional hazard regression model[24]. Date will be presented as the mean and standard error, or median and interquartile range for continuous variables as appropriate [25]. Means were compared between the groups by independent Student's *t* test. Differences between the parameters with each group were assessed by the paired Student's *t* test. The χ^2 tests were used to compare the categorical variables. Linear regression was utilized to examine the correlation between variables. Multivariate logistic regression was used to identify indexes that can independently predict the weaning outcome [26]. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for diaphragm excursion, DTF and RSBI in predicting weaning outcome. Receiver operating characteristic (ROC) curves are utilized to assess the diagnostic performance of diaphragm excursion, DTF and RSBI in predicting weaning outcome. Differences between ROC curves are identified using nonparametric comparisons of area under the curve. A two-tailed p value <0.05 is considered statistically significant. All statistical analyses will be performed using R software.

Discussion

There are approximately 20% of patients have difficulties in weaning from mechanical ventilation[1]. Weaning outcome can be affected by a variety of factors such as cardiac dysfunction, muscle weakness, pulmonary insufficiency and electrolyte disturbances[27]. As a result, single parameter is not enough to make an accurate prediction. RSBI is one of the most accurate weaning

indices currently being used. This indicator reflects the contribution of all inspiratory muscles, rather than the function of the diaphragm. Therefore, diaphragm fatigue can be masked by compensatory action of the other inspiratory muscle during SBT. However, the compensatory effect of non-diaphragm inspiratory muscles cannot be maintained for a long time [28,29]. Thus, a substantial proportion of patients who pass RSBI test may still fail to wean from mechanical ventilation.

A patient's breathing may be stable at the start of an SBT, but deteriorate a few hours later. Chatila *et al*. reported that RSBI measured at 30 min of an SBT was better than RSBI measured at the start of the trial as a weaning predictor [30]. Another study concluded that RSBI measured at the end of SBT had better diagnostic accuracy [31]. Also there was a study found that RSBI measured serially was more useful[32]. Segal *et al*. concluded that the percent change of RSBI during SBT was better in predicting the weaning outcome[33]. However, most reported weaning parameters were measured at the start of SBT. Our review of the literature failed to identify studies investigating the serial changes in diaphragm function during the SBT. We hypothesized that the changes of diaphragm function prior to RSBI was a better predictor of weaning outcome. The aim of this study is to explore weather the diaphragm function changed significantly during an SBT, as well as its impact on weaning outcome. Furthermore, we attempted to investigate the right time to measure the diaphragm function that was able to best predict the extubation outcome. Accordingly, we designed this experiment to observe the serial changes in diaphragm function during the SBT with ultrasound, and access its value of predicting weaning outcome.

Currently the gold standard for evaluating the function of the diaphragm is trans-diaphragmatic pressure, other methods such as fluoroscopy and magnetic resonance imaging are also used widely [34,35]. However, these methods are invasive, costly and uncomfortable, and have to transport patients and expose patients to radiations. Bedside ultrasound is an alternative to evaluate the diaphragm function. It is simple, rapid and noninvasive, therefore it is increasingly used in ICU patients. A number of studies have indicated that diaphragm excursion and thickening fraction

could reflect the function of the diaphragm and predict the weaning outcome [18,19,36]. So in this study, we used diaphragm excursion and thickening fraction measured by ultrasound to evaluate the function of diaphragm.

However, there are some limitations in our study. We only measured the right diaphragm. Because the measurement of the right diaphragm is technically simpler than the left, and we want to provide a simple and feasible method in clinical practice. Furthermore, we did not compare the ultrasound result with trans-diaphragmatic pressure, and the latter is considered as the gold standard of diaphragm function. Therefore, more experiments are needed to confirm this study's result.

Contributorship statement

All the listed authors have participated actively in the study, and have seen and approved the submitted manuscript. Pengmin Zhou, Yucai Hong and Peifeng Xu were responsible for the design of this study. Pengmin Zhou, Jiawei Guo, Yun Pan and Shengping Lin was responsible for the drafting of the article. Zhongheng Zhang, Huabo Cai, Hui Zhao and Junru Dai was responsible for the revision of the article.

Competing interests

The authors do not have any possible competing interest.

Funding

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Data sharing statement

No additional unpublished data are available.

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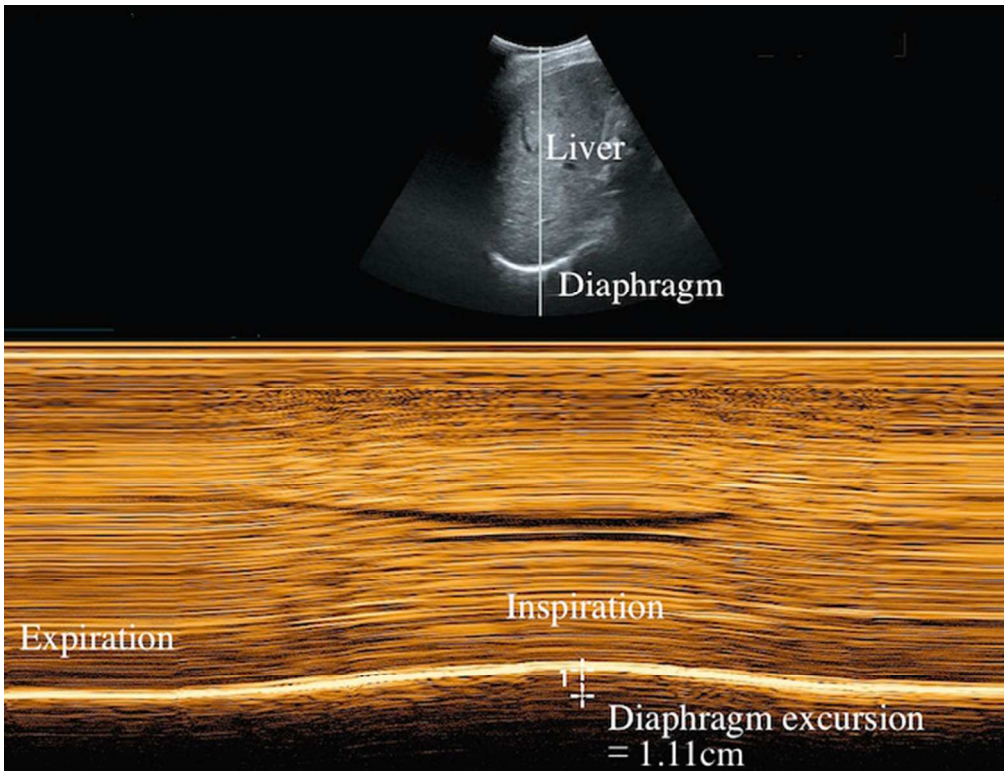

Figure 1 The liver was used as a window for the right diaphragm. The image showed an inspiratory peak above the baseline. The diaphragm excursion was measured as the vertical distance from the baseline to the peak.

240x183mm (72 x 72 DPI)

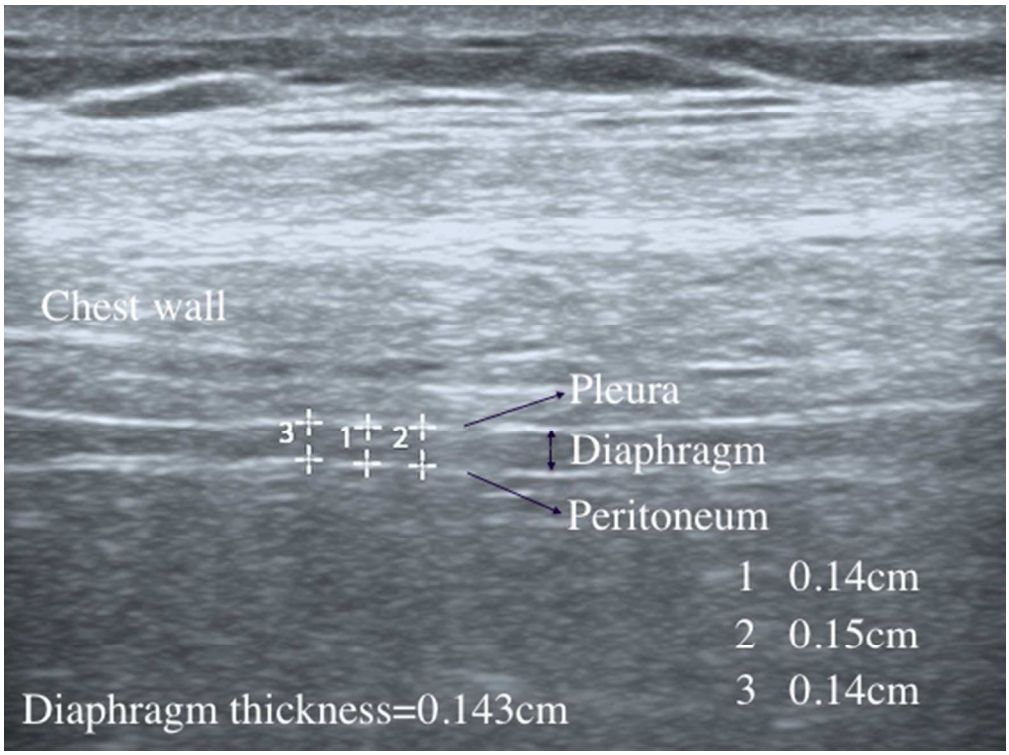

Figure 2 The diaphragm was imaged as three layer structure, including two parallel echoic lines (the pleura and the peritoneum) and a hypoechoic structure between them (the muscle itself). The distance from the middle of the pleural line to the middle of the peritoneal line was the diaphragm thickness. We measured the thickness three times and took the mean value.

201x150mm (72 x 72 DPI)

SPIRIT 2013 Checklist: Recommended Items to Address in Clinical Trial Protocol and Related Documents

Section/Item	Item Number	Description	Page number in the main document
Administrative information			
Title	1	The predictive value of serial changes in diaphragm function during the spontaneous breathing trial for weaning outcome: a study protocol	1
Trial registration	2a	Can serial changes in diaphragm function during the spontaneous breathing trial predict the weaning outcome?(ISRCTN42917473)	3
	2b	ISRCTN42917473	3
Protocol version	3	Date:1/11/2016 Version: 1.0	N/A
Funding	4	This study received the financial support from the Department of Health of Zhejiang Province.	9
Roles and responsibilities	5a	Pengmin Zhou, Yucai Hong and Peifeng Xu were responsible for the design of this study. Pengmin Zhou, Jiawei Guo, Yun Pan and Shengping Lin was responsible for the drafting of the article. Zhongheng Zhang, Huabo Cai, Hui Zhao and Junru Dai was responsible for the revision of the article.	9
	5b	Sponsor name: Department of Health of Zhejiang Province, Telephone number: (0571)87087342, E-mail: webmaster@zjwst.gov.cn, website: www.zjwst.gov.cn.	9
	5c	Department of Health of Zhejiang Province provide funding for this study. They will not have ultimate authority over any of these activities.	9
	5d	N/A	N/A
Introduction			
Background and rationale	6a	Excursion and thickening fraction of the diaphragm has been measured by bedside ultrasonography and were found to be able to quantitatively reflect diaphragm function, and thus was useful in predicting	4

		weaning outcome. However, these indices are usually measured at the start of an SBT. To the best of our knowledge, there is no report on the impact of serial changes in diaphragm excursion and thickening fraction during the SBT on weaning outcomes.	
	6b	There are no comparators in this study.	N/A
Objectives	7	The purpose of this study is to investigate whether the diaphragm function measured serially by ultrasonography during the SBT could be used to predict the weaning outcome.	2
Trial design	8	This is a prospective observational study.	2
Methods			
Participants,interventions, and outcomes			
Study setting	9	This study will be performed in an academic hospital in China.	2
Eligibility criteria	10	Inclusion criteria for participants: 1) endotracheal intubation for more than 48 hours; 2) absence of fever; 3) alert and cooperative; 4) hemodynamically stabile without vasopressors; 5) improved respiratory function with FiO2< 0.5, PEEP ≤5 cmH2O, PaO2/FiO2> 200 and respiratory rate <30 breaths per minute. Exclusion criteria for participants: 1) diaphragm paralysis; 2) cervical spine injury; 3) neuromuscular diseases; 4) a current thoracostomy; 5) pneumothorax; 6) pneumomediastinum.	5
Interventions	11a	When the objects meet the above inclusion criteria, they will undergo SBT for 2 hours. If the patient fails to tolerant the SBT, we will terminate the trial immediately and turn back to the control mode. Patients who passed the 2-hour SBT were extubated. During the SBT, right	5

		diaphragm excursion and thickening fraction will be evaluated by ultrasonography. Images will be obtained at 5 min, 30min, one hour and two hours after the initiation of SBT. Rapid shallow breathing index was simultaneously calculated at the bedside.	
	11b	If the patient fails to tolerant the SBT, we will terminate the trial immediately. Criteria for failure to the SBT was the following: 1) breathing frequency >35 breaths/min; 2)change in heart rate > 20%; 3)increase in systolic blood pressure > 25%; 4)change in mental status; 5)onset of discomfort, diaphoresis, or signs of increased work of breathing.	6
	11c	N/A	N/A
	11d	Relevant concomitant care and interventions are as usual.	N/A
Outcomes	12	Patients were divided into two groups according to their weaning outcomes. A successful weaning was defined as spontaneous breathing(SB) for >48 h following extubation without any level of ventilator support. A failed weaning was defined as either SBT failure or the inability to maintain SB for at least 48 h after extubation.	6
Participant timeline	13	When the objects meet the above inclusion criteria, they will undergo SBT for 2 hours. If the patient fails to tolerant the SBT, we will terminate the trial immediately and turn back to the control mode. Patients who passed the 2-hour SBT were extubated. During the SBT, right diaphragm excursion and thickening fraction will be evaluated by ultrasonography. Images will be obtained at 5 min, 30min, one hour and two hours after the initiation of SBT.	5
Sample size	14	This study conducted in a 10-bed medical intensive care unit in a university-affiliated hospital. The hospital is a teaching hospital	4,7

		of Zhejiang University with about 2400 beds. Our EICU enrolled approximately 400 critically ill patients every year, and tracheal intubation patients accounted for 40% of the total admissions. The study starts from November 2016, and is planned to last for one year. Sample size calculation was performed based on the Cox proportional hazard regression model. We intended to include more than 50 patients.	
Recruitment	15	Every patient in our EICU who met the inclusion criteria was enrolled.	5
Assignment of Interventions(for controlled trials)			
Allocation Sequence generation	16a	N/A	N/A
Allocation concealment mechanism	16b	N/A	N/A
Implementation	16c	N/A	N/A
Blinding(masking)	17a	N/A	N/A
	17b	N/A	N/A
Data collection, Management, and analysis			
Data collection methods	18a	Right diaphragm excursion and thickening fraction was evaluated by ultrasonography at 5 min, 30min, one hour and two hours after the initiation of SBT. RSBI was simultaneously calculated at the bedside. Laboratory measurements including blood gas, proBNP, chemistry profile, blood count and C reactive protein were obtained and recorded. Cardiac function were assessed by pro-BNP and echocardiography findings such as left ventricular ejection fraction (LVEF), E-point to septal separation(EPSS) and diastolic function.	5
	18b	All patients included into our cohort are followed up.	N/A
Data management	19	Double data entry	N/A
Statistical methods	20a	Date will be presented as the mean and standard error, or median and interquartile	7

		range for continuous variables as appropriate. Means were compared between the groups by independent Student's *t* test. Differences between the parameters with each group were assessed by the paired Student's *t* test. The χ^2 tests were used to compare the categorical variables. Linear regression was utilized to examine the correlation between variables. Multivariate logistic regression was used to identify indexes that can independently predict the weaning outcome. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for diaphragm excursion, DTF and RSBI in predicting weaning outcome. Receiver operating characteristic (ROC) curves are utilized to assess the diagnostic performance of diaphragm excursion, DTF and RSBI in predicting weaning outcome. Differences between ROC curves are identified using nonparametric comparisons of area under the curve. A two-tailed p value <0.05 is considered statistically significant. All statistical analyses will be performed using R software.	
	20b	N/A	N/A
	20c	N/A	N/A
Monitoring			
Data monitoring	21a	This is a prospective observational study. This study has no harm to the patient's physical, psychological and social relations. And this study accepted the supervision of Ethics Committee of Sir Run-Run Shaw Hospital, Zhejiang University School of Medicine. So a DMC is not needed.	3
	21b	N/A	N/A
Harms	22	This is a prospective observational study. This study has no harm to the patient's physical, psychological and social relations.	3
Auditing	23	This study was audited by Ethics Committee of Sir Run-Run Shaw Hospital, Zhejiang University School of Medicine	3

		every 12 months. And this process will be independent from investigators and the sponsor.	
Ethics and dissemination			
Research ethics approval	24	The study protocol was approved by the ethics committee of Sir Runrun Shaw hospital, an affiliate of Zhejiang University, Medical College.	3
Protocol amendments	25	If our protocol has modifications, we will communicate to relevant parties(including investigators, trial participants, trial registries, journals and regulators) as soon as possible.	N/A
Consent or assent	26a	Investigator will obtain informed consent from potential trial participants or authorized surrogates.	N/A
	26b	N/A	N/A
Confidentiality	27	The personal information of the participants is confidential, and the participant's medical records will be kept in hospital.	N/A
Declaration of interests	28	The principal investigators have no financial and other competing interests for the overall trial and each study site.	9
Access to data	29	Corresponding author will have access to the final trial data set.	9
Ancillary and post-trial care	30	Ancillary and post-trial care is as usual.	N/A
Dissemination policy	31a	The results will be published in a peer-reviewed journal and shared with the worldwide medical community.	3
	31b	N/A	N/A
	31c	N/A	N/A
Appendices			
Informed consent materials	32	Our informed consent materials are approved by ethics committee of Sir Runrun Shaw hospital, an affiliate of Zhejiang University, Medical College. All participants gave informed written consent.	N/A
Biological specimens	33	N/A	N/A

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The predictive value of serial changes in diaphragm function during the spontaneous breathing trial for weaning outcome: a study protocol

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The predictive value of serial changes in diaphragm function during the spontaneous breathing trial for weaning outcome: a study protocol

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Keywords: Diaphragm; Ultrasonography; Weaning

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Abstract

Introduction: There are many tools being used in clinical practice for the prediction of weaning success from mechanical ventilation. However, most of them are less than satisfaction. The purpose of this study is to investigate the value of diaphragm function measured serially by ultrasound during the spontaneous breathing trial (SBT) as a weaning predictor.

Methods and analysis: This is a prospective observational study conducted in a 10-bed medical emergency intensive care unit (EICU) in a university-affiliated hospital. The study will be performed from November 2016 to December 2017. All patients in the ICU who are expected to have mechanical ventilation for more than 48 hours through endotracheal tube are potentially eligible for this study. Patients will be included if they meet all the following criteria:1) adequate cough, absence of excessive tracheobronchial secretion, resolution of disease acute phase for which the patient was intubated; 2) alert and cooperative, no sedation; 3) hemodynamically stable (*i.e. f*C ⩽140 beats · min^{-1} , systolic BP 90-160mmHg, no or minimal vasopressors); 4) stable metabolic status and 5) improved respiratory function: SaO_2 >90% on ⩽FiO_2 0.4(or PaO_2/FiO_2⩾150mmHg), PEEP⩽8 cmH_2O, *f*R⩽35 beats · min^{-1}, no significant respiratory acidosis[1]. We will use the tube compensation mode (TC) to perform the spontaneous breathing trial (SBT) with the compensation ratio at 85%. The patients will undergo SBT for 2 hours in a semi-recumbent position. If the patient fails to tolerant the SBT, we will terminate the trial immediately and turn back to the control mode. Patients who pass the 2-hour SBT will be extubated. Right diaphragm excursion and bilateral

diaphragm thickening fraction will be evaluated by ultrasonography during spontaneous breathing. Images will be obtained before to start the SBT and at 5 min, 30min, one hour and two hours after the initiation of SBT. Rapid shallow breathing index (RSBI) will be simultaneously calculated at the bedside by a respiratory nurse.

Ethics and dissemination: The study protocol is approved by the ethics committee of Sir Runrun Shaw hospital, an affiliate of Zhejiang University, Medical College. The results will be published in a peer-reviewed journal and shared with the worldwide medical community.

Trial registration: The study was registered at International Standard Registered Clinical/Social Study number (ISRCTN) registry (ISRCTN42917473).

Strengths and limitations of this study:

1. This is the first study to investigate the serial changes in diaphragm function during the SBT. It is possible to find a better weaning predictor through this study.
2. We use bedside ultrasound to assess the function of the diaphragm in this study. It is simple, rapid and noninvasive.
3. We do not compare the ultrasound result with trans-diaphragmatic pressure, and the latter is considered as the gold standard of diaphragm function.

Introduction

Approximately 20% of mechanically ventilated patients have difficulties in weaning from mechanical ventilation [2], and about 40% of ventilation time is spent to discontinue ventilatory support [3]. Measures such as breathing frequency, minute ventilation, maximum inspiratory pressure, tracheal airway occlusion pressure 0.1 s, and a combined index named CROP (compliance, rate, O_2, pressure index) have been employed to determine the optimal timing of weaning from

mechanical ventilation [4]. Yang and Tobin [5] found that the most accurate predictor of weaning outcome was RSBI. However, the threshold of RSBI varied in different studies[6-8], owing to the differences in ventilatory support settings, age, gender, body position, underlying illness and endotracheal size[9-11]. It was reported that RSBI measured early during an SBT is inaccurate in predicting weaning success in patients with chronic obstructive pulmoanry diseases[12]. Also RSBI is not a good predictor for patients whose primary problem is related to poor cough, increased secretion and compromised airway protection. The latter conditions are commonly seen in neurosurgical patients [13,14].

Recently, some scholars put forward the idea that sonography may be useful during weaning from mechanical ventilation[15]. The diaphragm is the main respiratory muscle. Diaphragm dysfunction is the primary contributor to weaning failure and prolonged duration of mechanical ventilation [16, 17, 18, 19]. Excursion and thickening fraction of the diaphragm has been measured by bedside ultrasonography and is found to be able to quantitatively reflect diaphragm function, and thus is useful in predicting weaning outcome [20-24]. However, these indices are usually measured at the start of an SBT. To the best of our knowledge, there is no report on the impact of serial changes in diaphragm excursion and thickening fraction during the SBT on weaning outcomes. The purpose of this study is to investigate whether the diaphragm function measured serially by ultrasonography during the SBT could be used to predict the weaning outcome.

Study design

This is a prospective observational study conducted in a 10-bed medical intensive care unit in a university-affiliated hospital. The hospital is a teaching hospital of Zhejiang University with about 2400 beds. Our EICU enrolled approximately 600 critically ill patients every year, and tracheal intubation patients accounted for 40% of the total admissions. The study starts from November

2016, and is planned to last for one year. All patients will be consecutively enrolled and followed up for the entire hospital stay.

Study population

All patients in the ICU who are mechanically ventilated for more than 48 hours through endotracheal tube are potentially eligible for this study. Patients will be included if they meet all the following criteria: 1) adequate cough, absence of excessive tracheobronchial secretion, resolution of disease acute phase for which the patient was intubated; 2) alert and cooperative, no sedation; 3) hemodynamically stable (*i.e.* $fC \leq 140$ beats·min^{-1} , systolic BP 90-160mmHg, no or minimal vasopressors); 4) stable metabolic status and 5) improved respiratory function: SaO_2 >90% on ≤ FiO_2 0.4(or $PaO_2/FiO_2 \geq 150$mmHg), PEEP≤8 cmH_2O, $fR \leq 35$ beats·min^{-1}, no significant respiratory acidosis[1]. After inclusion, they will undergo an SBT. Subjects will be excluded if they have a history of diaphragm paralysis, cervical spine injury, neuromuscular diseases, a current thoracostomy, pneumothorax, or pneumomediastinum.

Study protocol and some definitions

Patients who meet the above mentioned criteria will be followed up. Demographics such as sex, age on admission, body mass index(BMI), underlying diseases, reasons for tracheal intubation, Acute Physiology and Chronic Health Evaluation II (APACHE II), duration of mechanical ventilation, will be recorded. When the subjects are suitable for SBT, they will proceed to undergo an SBT for 2 hours. We will use the tube compensation mode (TC) to do the SBT with the compensation ratio at 85% [25]. During these two hours, if the SBT fails, we will terminate the trial immediately and turn back to the control mode. Patients who pass the 2-hour SBT will be extubated and followed up for the presence of postextubation respiratory distress for 48 hours. Right diaphragm excursion and

bilateral diaphragm thickening fraction will be evaluated by ultrasonography during spontaneous breathing before to start the SBT and at 5 min, 30min, one hour and two hours after the initiation of SBT. RSBI will be simultaneously calculated at the bedside by a respiratory nurse. RSBI= respiratory frequency/tidal volume. The values of respiratory frequency and tidal volume can be obtained from the ventilator screen. Laboratory measurements including blood gas, pro-brain natriuretic peptide (pro-BNP), plasma protein, hemoglobin, chemistry profile, blood count and C reactive protein will be obtained and recorded before starting the SBT and at the end of the SBT. Cardiac function will be assessed by pro-BNP and echocardiography findings such as left ventricular ejection fraction (LVEF), E-point to septal separation(EPSS) and e'/a' at the end of the SBT. Attending physicians are blinded to ultrasound measurements. According to the weaning outcome, subjects will be divided into two groups, the successful group and the failure group.

Criteria for passing SBT are the following: good ability of patients to cooperate, oxygen saturation greater than 90%, $fR \leqslant 35$ breaths·min^{-1}, $fC \leqslant 140$ breaths·min^{-1} and no discomfort, diaphoresis, or signs of increased breathing work. Criteria for failure to the SBT are the following: agitation and anxiety, depressed mental status, diaphoresis, cyanosis, increased accessory muscle activity, facial signs of distress, dyspnea, $PaO_2 \leqslant$50-60mmHg on $FiO_2 \geqslant 0.5$ or SaO_2<90%, $PaCO_2$ >50mmHg or an increase in $PaCO_2$ >8mmHg, pH<7.32 or a decrease in pH$\geqslant$0.07 pH units, fR>35 breaths·min^{-1} or increased by $\geqslant$50%, fC>140 breaths·min^{-1} or increased by $\geqslant$20%, systolic BP>180mmHg or increased by $\geqslant$20%, systolic BP<90mmHg, cardiac arrhythmias[1, 19] . A successful weaning is defined as spontaneous breathing(SB) for >48 h following extubation without any level of ventilator support, including the case that noninvasive ventilation (NIV) is used as a prophylactic measure after exutbation for patients who are at high risk for reintubation but who do not develop acute respiratory failure (ARF). A failed weaning is defined as either SBT failure or the inability to maintain SB for at least 48 h after extubation, including the case that NIV is used as a treatment option for patients who have been extubated but developed ARF within 48 h. If a patient passes the

SBT but is not extubated, his weaning also fails. The time from the beginning to the end of mechanical ventilation is defined as total ventilation time. Weaning time is the time spent in partial support mode.

Diaphragm ultrasound

Three observers will be trainded before commencing the study. They will measure the diaphragm excursion and thickness by ultrasound as previously described [15, 26]. Briefly, all patients will be measured in a semi-recumbent position with the head of bed elevated between 20° and 40°. The right diaphragmatic excursion is measured with a 3.5-5MHz ultrasound probe (Mindray M9, China). The probe is placed immediately below the right costal margin along the mid-clavicular line and is directed medially, cephalad and dorsally, so that the ultrasound beam reaches perpendicularly the posterior third of the diaphragm. The liver is used as an acoustic window. Firstly, B-mode is used to get the best approach and to select the exploration line. Then M-mode is used to display the motion of the diaphragm along the selected line. During inspiration, the normal diaphragm contracts and moves caudally toward the probe; During expiration, the normal diaphragm moves cranially away from the probe; this is recorded as an upward motion of the M-mode tracing (Figure 1). The diaphragm excursion is measured on the vertical axis of the tracing from the baseline to the point of maximum height of inspiration on the graph. Three measurements will be recorded and averaged. All measurements will be performed during spontaneous breathing.

The diaphragm thickness is measured by a 10MHz linear ultrasound probe set to B mode placed perpendicularly to the chest wall, in the 9th or 10th intercostal spaces, between the anterior axillary and the midaxillary lines [27]. In this area, the diaphragm is imaged as three layer structure, including two parallel echoic lines (the diaphragmatic pleura and the peritoneal membrane) and a hypoechoic structure between them (the muscle itself) [27, 28]. We will freeze the image at end-expiration and end-inspiration. On frozen B-mode image, the distance from the middle of the

pleural line to the middle of the peritoneal line is the diaphragm thickness (Figure 2). We will measure the diaphragm thickness three times at the point of maximum thickening and three times at minimum thickening at functional residual capacity and make a mean. Diaphragm thickening fraction (DTF) = (Thickness at end inspiration − Thickness at end expiration)/ Thickness at end expiration. All examinations will be recorded on a personal computer for subsequent blind analysis.

Follow-up study

Diaphragm dysfunction is common in critically ill patients. It can be caused by multiple factors including prolonged mechanical ventilation. We first examine the serial changes in diaphragm function during an SBT with ultrasound in order to assess its value for predicting weaning outcome. Then we will measure the right diaphragm excursion and bilateral diaphragm thickening fraction during spontaneous breathing by bedside ultrasound in patients daily over the first seven days following extubation. This follow-up study will be performed in order to find whether the damage of diaphragm is reversible.

Statistical analysis

We use the following formula to calculate the sample size: Sample size=$\frac{Z^2_{1-\alpha/2}\times S_N\times(1-S_N)}{L^2\times Prevalence}$. α =0.05, $Z_{1-\alpha/2}$=1.96, L=0.1, S_N stands for sensitivity and Prevalence stands for extubation failure rate. According to the information from the latest literatures, the sample size is 165[23, 24, 29]. Date will be presented as the mean and standard error, or median and interquartile range for continuous variables as appropriate [30]. Means will be compared between the groups by independent Student's *t* test. Differences between the parameters with each group will be assessed by the paired Student's *t* test. The b^2 tests will be used to compare the categorical variables. Linear regression will be utilized to examine the correlation between variables. Multivariate logistic regression will be used to identify indexes that can independently predict the weaning outcome [31]. Sensitivity,

specificity, positive predictive value (PPV) and negative predictive value (NPV) will be calculated for diaphragm excursion, DTF and RSBI in predicting weaning outcome. Receiver operating characteristic (ROC) curves will be utilized to assess the diagnostic performance of diaphragm excursion, DTF and RSBI in predicting weaning outcome. Differences between ROC curves will be identified using nonparametric comparisons of area under the curve. Serial measured values will be analyzed using serial measurement analysis of variance. To assess intra-observer variability, the same observer will repeat the measurement 5 min after the initial measurement. To assess inter-observer variability, 20 patients will be measured by two different oprators. Pearson correlation analysis and Bland-Altman plotting will be performed to evaluate the reproducibility of diaphragm ultrasound studies. A two-tailed p value <0.05 is considered statistically significant. All statistical analyses will be performed using R software.

Discussion

There are approximately 20% of patients have difficulties in weaning from mechanical ventilation [2]. Weaning outcome can be affected by a variety of factors such as cardiac dysfunction, muscle weakness, pulmonary insufficiency and electrolyte disturbances [32]. As a result, single parameter is not enough to make an accurate prediction. RSBI is one of the most accurate weaning indices currently being used. This indicator reflects the contribution of all inspiratory muscles, rather than the function of the diaphragm. Therefore, diaphragm fatigue can be masked by compensatory action of the other inspiratory muscle during SBT. However, the compensatory effect of non-diaphragm inspiratory muscles cannot be maintained for a long time [33,34]. Thus, a substantial proportion of patients who pass RSBI test may still fail to wean from mechanical ventilation.

A patient's breathing may be stable at the start of an SBT, but deteriorate a few hours later. Chatila *et al*. reported that RSBI measured at 30 min of an SBT was better than RSBI measured at the start of the trial as a weaning predictor [35]. Another study concluded that RSBI measured at the end of

SBT had better diagnostic accuracy [36]. Also there was a study found that RSBI measured serially was more useful [8]. Segal *et al*. concluded that the percent change of RSBI during SBT was better in predicting the weaning outcome [37]. However, most reported weaning parameters were measured at the start of SBT. Our review of the literature failed to identify studies investigating the serial changes in diaphragm function during the SBT. We hypothesize that the changes of diaphragm function prior to RSBI is a better predictor of weaning outcome. The aim of this study is to explore weather the diaphragm function changes significantly during an SBT, as well as its impact on weaning outcome. Furthermore, we attempt to investigate the right time to measure the diaphragm function that is able to best predict the extubation outcome. Accordingly, we design this experiment to observe the serial changes in diaphragm function during the SBT with ultrasound, and access its value of predicting weaning outcome.

Currently the gold standard for evaluating the function of the diaphragm is trans-diaphragmatic pressure, other methods such as fluoroscopy and magnetic resonance imaging are also used widely [38,39]. However, these methods are invasive, costly and uncomfortable, and have to transport patients and expose patients to radiations. Bedside ultrasound is an alternative to evaluate the diaphragm function. It is simple, rapid and noninvasive, therefore it is increasingly used in ICU patients. A number of studies have indicated that diaphragm excursion and thickening fraction could reflect the function of the diaphragm and predict the weaning outcome [23,24,40]. So in this study, we use diaphragm excursion and thickening fraction measured by ultrasound to evaluate the function of diaphragm.

We hypothesize that diaphragm function measured by ultrasound during the SBT is an important predictor of weaning outcome and serial measurement have better predictive value than a single measurement.

Contributorship statement

All the listed authors have participated actively in the study, and have seen and approved the submitted manuscript. Pengmin Zhou, Yucai Hong and Peifeng Xu were responsible for the design of this study. Pengmin Zhou, Yiming Zhao, Xuchang Qin, Jiawei Guo, Yun Pan and Shengping Lin were responsible for the drafting of the article. Zhongheng Zhang, Huabo Cai, Hui Zhao and Junru Dai were responsible for the revision of the article.

Competing interests

The authors do not have any possible competing interest.

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Data sharing statement

No additional unpublished data are available.

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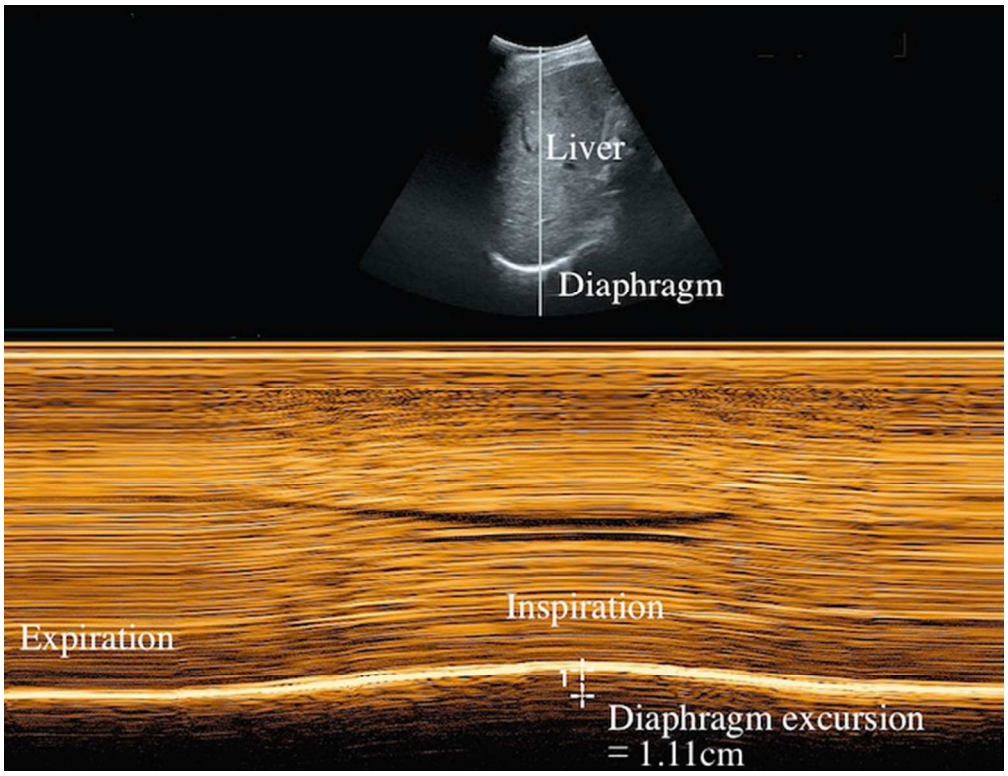

Figure 1 The liver is used as a window for the right diaphragm. During inspiration, the normal diaphragm contracts and moves caudally toward the probe; During expiration, the normal diaphragm moves cranially away from the probe; this is recorded as an upward motion of the M-mode tracing. The diaphragm excursion is measured on the vertical axis of the tracing from the baseline to the point of maximum height of inspiration on the graph. Three measurements will be recorded and averaged.

57x44mm (300 x 300 DPI)

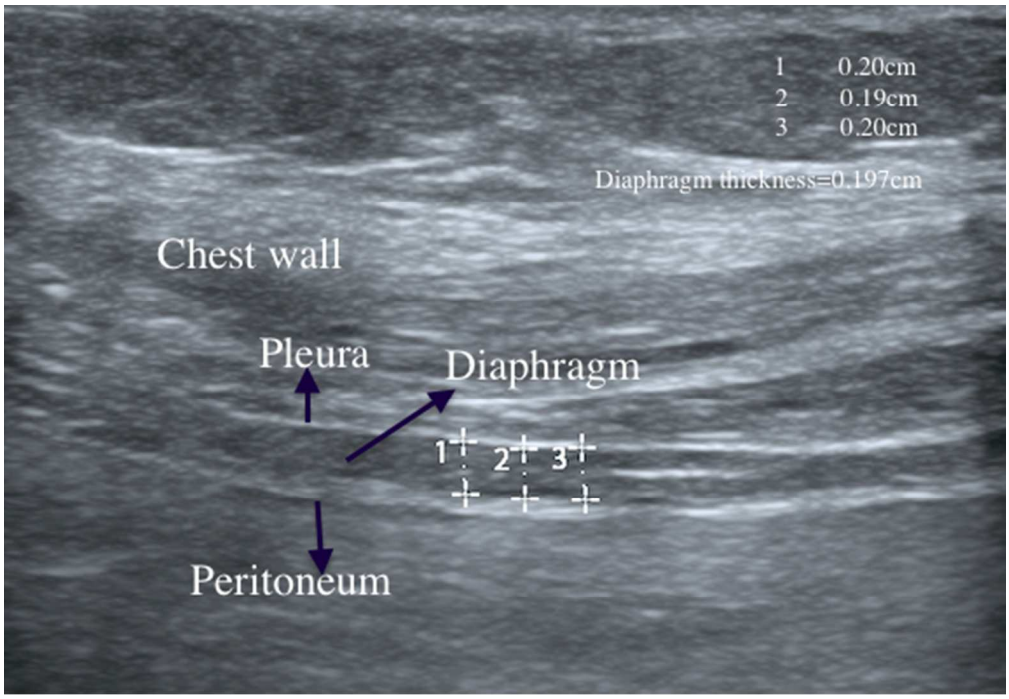

Figure 2 The diaphragm is imaged as three layer structure, including two parallel echoic lines (the pleura and the peritoneum) and a hypoechoic structure between them (the muscle itself). The distance from the middle of the pleural line to the middle of the peritoneal line is the diaphragm thickness. We measure the diaphragm thickness three times at the point of maximum thickening and three times at minimum thickening at functional residual capacity and make a mean.

48x33mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended Items to Address in Clinical Trial Protocol and Related Documents

Section/Item	Item Number	Description	Page number in the main document
Administrative information			
Title	1	The predictive value of serial changes in diaphragm function during the spontaneous breathing trial for weaning outcome: a study protocol	1
Trial registration	2a	Can serial changes in diaphragm function during the spontaneous breathing trial predict the weaning outcome?(ISRCTN42917473)	3
	2b	ISRCTN42917473	3
Protocol version	3	Date:1/11/2016 Version: 1.0	N/A
Funding	4	This study received the financial support from the Department of Health of Zhejiang Province.	11
Roles and responsibilities	5a	Pengmin Zhou, Yucai Hong and Peifeng Xu were responsible for the design of this study. Pengmin Zhou, Yiming Zhao, Xuchang Qin, Jiawei Guo, Yun Pan and Shengping Lin were responsible for the drafting of the article. Zhongheng Zhang, Huabo Cai, Hui Zhao and Junru Dai were responsible for the revision of the article.	11
	5b	Sponsor name: Department of Health of Zhejiang Province, Telephone number: (0571)87087342, E-mail: webmaster@zjwst.gov.cn, website: www.zjwst.gov.cn.	11
	5c	Department of Health of Zhejiang Province provide funding for this study. They will not have ultimate authority over any of these activities.	11
	5d	N/A	N/A
Introduction			
Background and rationale	6a	Excursion and thickening fraction of the diaphragm has been measured by bedside ultrasonography and were found to be able	3-4

		to quantitatively reflect diaphragm function, and thus was useful in predicting weaning outcome. However, these indices are usually measured at the start of an SBT. To the best of our knowledge, there is no report on the impact of serial changes in diaphragm excursion and thickening fraction during the SBT on weaning outcomes.	
	6b	There are no comparators in this study.	N/A
Objectives	7	The purpose of this study is to investigate whether the diaphragm function measured serially by ultrasonography during the SBT could be used to predict the weaning outcome.	2
Trial design	8	This is a prospective observational study.	2
Methods			
Participants,interventions , and outcomes			
Study setting	9	This study will be performed in an academic hospital in China.	2
Eligibility criteria	10	Inclusion criteria for participants: 1) endotracheal intubation for more than 48 hours; 2) adequate cough, absence of excessive tracheobronchial secretion, resolution of disease acute phase for which the patient was intubated; 3) alert and cooperative, no sedation; 4) hemodynamically stable (i.e. fC≤140 beats•min-1 , systolic BP 90-160mmHg, no or minimal vasopressors); 5) stable metabolic status and 6) improved respiratory function: SaO2 >90% on ≤FiO2 0.4(or PaO2/FiO2 ≥150mmHg), PEEP≤8 cmH2O, fR≤35 beats•min-1, no significant respiratory acidosis Exclusion criteria for participants: 1) diaphragm paralysis; 2) cervical spine injury; 3) neuromuscular diseases; 4) a current thoracostomy; 5) pneumothorax;	5

		6) pneumomediastinum.	
Interventions	11a	When the objects meet the above inclusion criteria, We will use the tube compensation mode (TC) to perform the spontaneous breathing trial (SBT) with the compensation ratio at 85%. The patients will undergo SBT for 2 hours in a semi-recumbent position. If the patient fails to tolerant the SBT, we will terminate the trial immediately and turn back to the control mode. Patients who pass the 2-hour SBT will be extubated. Right diaphragm excursion and bilateral diaphragm thickening fraction will be evaluated by ultrasonography during spontaneous breathing. Images will be obtained before to start the SBT and at 5 min, 30min, one hour and two hours after the initiation of SBT. Rapid shallow breathing index (RSBI) will be simultaneously calculated at the bedside by a respiratory nurse.	5
	11b	If the patient fails to tolerant the SBT, we will terminate the trial immediately. Criteria for failure to the SBT was the following: 1) agitation and anxiety, depressed mental status, diaphoresis, cyanosis, increased accessory muscle activity, facial signs of distress, dyspnea, 2) PaO2 $\leqslant$ 50-60mmHg on FiO2 $\geqslant$ 0.5 or SaO2<90%, PaCO2 >50mmHg or an increase in PaCO2 >8mmHg, pH<7.32 or a decrease in pH $\geqslant$ 0.07 pH units, fR>35 breaths • min-1L-1 or increased by $\geqslant$ 50%, fC>140 breaths • min-1L-1 or increased by $\geqslant$ 20%, systolic BP>180mmHg or increased by $\geqslant$ 20%, systolic BP<90mmHg, 3) cardiac arrhythmias	6
	11c	N/A	N/A
	11d	Relevant concomitant care and interventions are as usual.	N/A
Outcomes	12	Patients were divided into two groups according to their weaning outcomes. A successful weaning was defined as	6

		spontaneous breathing(SB) for >48 h following extubation without any level of ventilator support. A failed weaning was defined as either SBT failure or the inability to maintain SB for at least 48 h after extubation.	
Participant timeline	13	When the objects meet the above inclusion criteria, they will undergo SBT for 2 hours. If the patient fails to tolerant the SBT, we will terminate the trial immediately and turn back to the control mode. Patients who passed the 2-hour SBT were extubated. During the SBT, Right diaphragm excursion and bilateral diaphragm thickening fraction will be evaluated by ultrasonography during spontaneous breathing. Images will be obtained before to start the SBT and at 5 min, 30min, one hour and two hours after the initiation of SBT. Rapid shallow breathing index (RSBI) will be simultaneously calculated at the bedside by a respiratory nurse.	5
Sample size	14	We use the following formula to calculate the sample size: Sample size= (Z_(1-α/2)^2×S_N×(1-S_N))/(L^2×Prevalence). α =0.05, Z1-α/2 =1.96, L=0.1, SN stands for sensitivity and Prevalence stands for extubation failure rate. According to the information from the latest literatures, the sample size is 165.	8
Recruitment	15	Every patient in our EICU who met the inclusion criteria was enrolled.	5
Assignment of Interventions(for controlled trials)			
Allocation Sequence generation	16a	N/A	N/A
Allocation concealment mechanism	16b	N/A	N/A
Implementation	16c	N/A	N/A
Blinding(masking)	17a	N/A	N/A
	17b	N/A	N/A
Data collection,			

Management, and Analysis			
Data collection methods	18a	Right diaphragm excursion and bilateral diaphragm thickening fraction will be evaluated by ultrasonography during spontaneous breathing. Images will be obtained before to start the SBT and at 5 min, 30min, one hour and two hours after the initiation of SBT. Rapid shallow breathing index (RSBI) will be simultaneously calculated at the bedside by a respiratory nurse. Laboratory measurements including blood gas, pro-brain natriuretic peptide (pro-BNP), plasma protein, hemoglobin, chemistry profile, blood count and C reactive protein will be obtained and recorded before starting the SBT and at the end of the SBT. Cardiac function will be assessed by pro-BNP and echocardiography findings such as left ventricular ejection fraction (LVEF), E-point to septal separation(EPSS) and e'/a' at the end of the SBT.	5-6
	18b	All patients included into our cohort are followed up.	N/A
Data management	19	Double data entry	N/A
Statistical methods	20a	Date will be presented as the mean and standard error, or median and interquartile range for continuous variables as appropriate [30]. Means will be compared between the groups by independent Student's t test. Differences between the parameters with each group will be assessed by the paired Student's t test. The b2 tests will be used to compare the categorical variables. Linear regression will be utilized to examine the correlation between variables. Multivariate logistic regression will be used to identify indexes that can independently predict the weaning outcome [31]. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) will be calculated for diaphragm excursion, DTF and RSBI in predicting weaning outcome. Receiver operating characteristic (ROC) curves will be	8-9

		utilized to assess the diagnostic performance of diaphragm excursion, DTF and RSBI in predicting weaning outcome. Differences between ROC curves will be identified using nonparametric comparisons of area under the curve. Serial measured values will be analyzed using serial measurement analysis of variance. To assess intra-observer variability, the same observer will repeat the measurement 5 min after the initial measurement. To assess inter-observer variability, 20 patients will be measured by two different oprators. Pearson correlation analysis and Bland-Altman plotting will be performed to evaluate the reproducibility of diaphragm ultrasound studies. A two-tailed p value <0.05 is considered statistically significant. All statistical analyses will be performed using R software.	
	20b	N/A	N/A
	20c	N/A	N/A
Monitoring			
Data monitoring	21a	This is a prospective observational study. This study has no harm to the patient's physical, psychological and social relations. And this study accepted the supervision of Ethics Committee of Sir Run-Run Shaw Hospital, Zhejiang University School of Medicine. So a DMC is not needed.	3
	21b	N/A	N/A
Harms	22	This is a prospective observational study. This study has no harm to the patient's physical, psychological and social relations.	3
Auditing	23	This study was audited by Ethics Committee of Sir Run-Run Shaw Hospital, Zhejiang University School of Medicine every 12 months. And this process will be independent from investigators and the sponsor.	3
Ethics and dissemination			
Research ethics approval	24	The study protocol was approved by the ethics committee of Sir Runrun Shaw hospital, an affiliate of Zhejiang University, Medical College.	3

Protocol amendments	25	If our protocol has modifications, we will communicate to relevant parties(including investigators, trial participants, trial registries, journals and regulators) as soon as possible.	N/A
Consent or assent	26a	Investigator will obtain informed consent from potential trial participants or authorized surrogates.	N/A
	26b	N/A	N/A
Confidentiality	27	The personal information of the participants is confidential, and the participant's medical records will be kept in hospital.	N/A
Declaration of interests	28	The principal investigators have no financial and other competing interests for the overall trial and each study site.	11
Access to data	29	Corresponding author will have access to the final trial data set.	11
Ancillary and post-trial care	30	Ancillary and post-trial care is as usual.	N/A
Dissemination policy	31a	The results will be published in a peer-reviewed journal and shared with the worldwide medical community.	3
	31b	N/A	N/A
	31c	N/A	N/A
Appendices			
Informed consent materials	32	Our informed consent materials are approved by ethics committee of Sir Runrun Shaw hospital, an affiliate of Zhejiang University, Medical College. All participants gave informed written consent.	N/A
Biological specimens	33	N/A	N/A

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The predictive value of serial changes in diaphragm function during the spontaneous breathing trial for weaning outcome: a study protocol

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The predictive value of serial changes in diaphragm function during the spontaneous breathing trial for weaning outcome: a study protocol

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Abstract

Introduction: There is a variety of tools being used in clinical practice for the prediction of weaning success from mechanical ventilation. However, their diagnostic performances are less than satisfactory. The purpose of this study is to investigate the value of serial changes in diaphragm function measured by ultrasound during the spontaneous breathing trial (SBT) as a weaning predictor.

Methods and analysis: This is a prospective observational study conducted in a 10-bed medical emergency intensive care unit (EICU) in a university-affiliated hospital. The study will be performed from November 2016 to December 2017. All patients in the EICU who are expected to have mechanical ventilation for more than 48 hours through endotracheal tube are potentially eligible for this study. Patients will be included if they fulfill the criteria for SBT. All enrolled patients will be ventilated with an Evita-4 (Draeger, Lubeck, Germany) by using volume assist control mode prior to SBT. Positive end-expiratory pressure (PEEP) will be set to 5cmH_2O and fractional inspired oxygen (FiO_2) will be set to a value below 0.5 that guarantees oxygen saturation by pulse oximetry (SpO_2) greater than 90%. Enrolled patients will undergo SBT for 2 hours in semi-recumbent position. During the SBT, the patients will breathe through the ventilator circuit by using flow triggering (2L/min) with automatic tube compensation (ATC) of 100% and 5 cmH_2O PEEP [1]. The fractional inspired oxygen (FiO_2) will be set to the same value as used before SBT. If the patients fail to tolerate the SBT, the trial will be discontinued immediately and the ventilation

mode will be switched to that used before the trial. Patients who pass the 2-hour SBT will be extubated. Right diaphragm excursion and bilateral diaphragm thickening fraction will be measured by ultrasonography during spontaneous breathing. Images will be obtained immediately prior to the SBT, and at 5 min, 30min, 60min , 90min, and 120min after the initiation of SBT. Rapid shallow breathing index (RSBI) will be simultaneously calculated at the bedside by a respiratory nurse.

Ethics and dissemination: The study protocol is approved by the ethics committee of Sir Runrun Shaw hospital, an affiliate of Zhejiang University, Medical College. The results will be published in a peer-reviewed journal and shared with the worldwide medical community.

Trial registration: The study was registered at International Standard Registered Clinical/Social Study number (ISRCTN) registry (ISRCTN42917473).

Strengths and limitations of this study:

1. This is the first study to investigate the serial changes in diaphragm function during the SBT. It is possible to find a better predictor for weaning outcome.
2. Bedside ultrasound is used to assess the function of the diaphragm in this study, which is simple, rapid and noninvasive.
3. The study is limited by the fact that we do not compare the ultrasound result with trans-diaphragmatic pressure, and the latter is considered as the gold standard of diaphragm function.

Introduction

Approximately 20% of mechanically ventilated patients have difficulties in weaning from mechanical ventilation [2], and about 40% of ventilation time is spent on the mechanical ventilation weaning process [3]. Respiratory parameters such as breathing frequency, minute ventilation, maximum inspiratory pressure, tracheal airway occlusion pressure 0.1 s, and a combined index

named CROP (compliance, rate, O_2, pressure index) have been employed to determine the optimal timing of weaning from mechanical ventilation [4]. Yang and Tobin [5] found that the most accurate predictor of weaning outcome was rapid shallow breathing index (RSBI). However, the threshold of RSBI varied in different studies[6-8], owing to the differences in ventilate settings, age, gender, body position, underlying illness and endotracheal size[9-11]. It was reported that RSBI measured early during a spontaneous breathing trial (SBT) is inaccurate in predicting weaning success in patients with chronic obstructive pulmoanry diseases[12]. Also RSBI is not a good predictor for patients whose primary problem is related to poor cough, increased secretion and compromised airway protection. These conditions are commonly seen in neurosurgical patients [13,14].

Recently, some authors have proposed that ultrasonography might be useful during weaning from mechanical ventilation [15]. Excursion and thickening fraction of the diaphragm has been measured by bedside ultrasonography and was found to be able to quantitatively evaluate diaphragm function, which was useful in predicting weaning outcome [16-20]. However, these indices are usually measured at the start of SBT. To the best of our knowledge, there is no report on the impact of serial changes in diaphragm excursion and thickening fraction during the SBT on weaning outcomes. The purpose of this study is to investigate whether serial changes in diaphragm function measured by ultrasonography during the SBT could be used to predict the weaning outcome.

Study design

This is a prospective observational study conducted in a 10-bed medical intensive care unit in a hospital with about 2400 beds. The hospital is an academic teaching hospital affiliated to Zhejiang University. Our EICU enrolled approximately 600 critically ill patients annually, and patients with invasive mechanical ventilation accounted for 40% of the total admissions. The study will start

from November 2016, and is planned to end in November 2017. All patients will be consecutively enrolled and followed up for the entire hospital stay.

Study population

All patients in the ICU who are mechanically ventilated for more than 48 hours through endotracheal tube are potentially eligible for this study. Patients will be included if they meet all of the following criteria: 1) adequate cough, absence of excessive tracheobronchial secretion, resolution of underlying critical illness for which the patient is intubated; 2) the patient is alert and cooperative, without sedation; 3) hemodynamically stable (*i.e. heart rate*≤140 beats·min^{-1} , systolic blood pressure between 90 and 160mmHg, without vasopressors); 4) stable metabolic status and 5) improved respiratory function: arterial oxygen saturation (SaO_2) >90% on fractional inspired oxygen (FiO_2) ≤ 0.4 (or oxygen index (PaO_2/FiO_2)≥150mmHg), positive end-expiratory pressure (PEEP)≤8 cmH_2O, *respiratory rate*≤35 beats·min^{-1}, without respiratory acidosis[2]. After enrollment, the patients will undergo a SBT. Subjects will be excluded if they have a history of diaphragm paralysis, cervical spine injury, neuromuscular diseases, a current thoracostomy, pneumothorax, or pneumomediastinum.

Study protocol and some definitions

Patients who meet the above mentioned criteria will be followed up. Demographics such as sex, age on admission, body mass index(BMI), underlying diseases, reasons for tracheal intubation, Acute Physiology and Chronic Health Evaluation II (APACHE II), duration of mechanical ventilation, will be recorded. Electrocardiogram, heart rate, arterial blood pressure and oxygen saturation by pulse oximetry (SpO2) will be continuously monitored. All enrolled patients will be ventilated with an Evita-4 (Draeger, Lubeck, Germany) by using volume assist control mode prior to SBT. Positive

end-expiratory pressure (PEEP) will be set to 5cmH_2O and fractional inspired oxygen (FiO_2) will be titrated below 50%, while ensuring SpO_2 greater than 90%. When the subjects are suitable for SBT, they will proceed to undergo a SBT for 2 hours. During the SBT, the patients will breathe through the ventilator circuit by using flow triggering (2L/min) with automatic tube compensation (ATC) of 100% and 5 cmH2O PEEP. The FiO2 will be set to the same value as used before SBT [1]. During the two-hour periods, we will terminate the trial immediately and turn back to the ventilation mode used before the trial if the SBT fails. Patients who pass the 2-hour SBT will be extubated and followed up for the presence of postextubation respiratory distress for 48 hours. Right diaphragm excursion and bilateral diaphragm thickening fraction will be evaluated by ultrasonography during spontaneous breathing immediately prior to SBT, and at 5 min, 30min, 60min, 90min, and 120min after the initiation of SBT. RSBI, which is calculated as the ratio of respiratory frequency to tidal volume, will be simultaneously recorded at the bedside by a respiratory nurse. The values of respiratory frequency and tidal volume can be obtained from the ventilator screen. All measurements of RSBI during one minute will be obtained, and the mean value will be used for analysis. Laboratory measurements including arterial blood gas, pro-brain natriuretic peptide (pro-BNP), plasma protein, hemoglobin, chemistry profile, blood count and C-reactive protein will be obtained and recorded prior to the SBT and at the end of the SBT. If the patients present signs and symptoms such as agitation, anxiety, depressed mental status, diaphoresis, cyanosis, increased accessory muscle activity, facial signs of distress and dyspnea during SBT, his or her arterial blood gas will also be obtained and recorded immediately. Cardiac function will be assessed by pro-BNP and echocardiography findings such as left ventricular ejection fraction (LVEF), E-point to septal separation (EPSS) and e'/a' at the end of the SBT. Attending physicians are blinded to ultrasound measurements. According to the weaning outcome, subjects will be divided into two groups, the success and the failure groups.

Criteria for passing SBT are as follows: 1) the patients are cooperative; 2) SpO_2 greater than 90%, *respiratory rate*⩽35 breaths·min^{-1}, *heart rate*⩽140 breaths·min^{-1}; 3) there is no discomfort, diaphoresis, or signs of increased breathing work. Criteria for a failure of SBT are as follows: agitation and anxiety, depressed mental status, diaphoresis, cyanosis, increased accessory muscle activity, facial signs of distress, dyspnea, PaO_2⩽50-60mmHg on FiO_2⩾0.5 or SpO_2<90%, arterial partial pressure of carbon dioxide ($PaCO_2$) >50mmHg or an increase in $PaCO_2$ >8mmHg, pH<7.32 or a decrease in pH⩾0.07 pH units, *respiratory rate*>35 breaths·min^{-1} or increased by ⩾50%, *heart rate*>140 breaths·min^{-1} or increased by ⩾20%, systolic blood pressure>180mmHg or increased by ⩾20%, systolic blood pressure<90mmHg, cardiac arrhythmias[2, 21] . A successful weaning is defined as spontaneous breathing(SB) for >48 h following extubation without any level of ventilator support, including noninvasive ventilation (NIV) used as a prophylactic measure after exutbation. A failed weaning is defined as either SBT failure or the inability to maintain SB for at least 48 h after extubation, including the situation in which NIV is used for patients who have been extubated but developed acute respiratory failure (ARF) within 48 h. A patient fails the weaning if he or she passes the SBT but requires endotracheal tube to protect the airway. The time from the beginning to the end of mechanical ventilation is defined as the total ventilation time. Weaning time is the time spent in partial support mode.

Diaphragm ultrasound

Three observers will be trained prior to the study. They will measure the diaphragm excursion and thickness by ultrasound as previously described [15, 22]. Briefly, all patients will be measured in semi-recumbent position with the head of bed elevated between 20° and 40°. The right diaphragmatic excursion is measured with a 3.5-5MHz ultrasound probe (Mindray M9, China). The probe is placed immediately below the right costal margin along the mid-clavicular line and is directed medially, cephalad and dorsally, so that the ultrasound beam reaches perpendicularly the

posterior third of the diaphragm. The liver is used as an acoustic window. Firstly, B-mode is used to get the best image and to select the exploration line. Then M-mode is used to display the motion of the diaphragm along the selected line. During inspiration, the normal diaphragm contracts and moves caudally toward the probe; During expiration, the normal diaphragm moves cranially away from the probe; this is recorded as an upward motion of the M-mode tracing (Figure 1). The diaphragm excursion is measured on the vertical axis of the tracing from the baseline to the point of maximum height of inspiration on the image. Three measurements will be recorded and averaged. All measurements will be performed during spontaneous breathing.

The diaphragm thickness is measured by a 10MHz linear ultrasound probe which is placed in the 9th or 10th intercostal spaces and between the anterior axillary and the midaxillary lines, perpendicularly to the chest wall [23]. The ultrasound image is switched to the B-mode. In this area, the diaphragm is imaged as three-layer structure, including two parallel echoic lines (the diaphragmatic pleura and the peritoneal membrane) and a hypoechoic structure between them (the muscle itself) [23, 24]. We will freeze the image at end-expiration and end-inspiration. On the frozen images, the distance from the middle of the pleural line to the middle of the peritoneal line is the diaphragm thickness (Figure 2). We will measure the diaphragm thickness three times on the same scan and the values will be averaged. Diaphragm thickening fraction (DTF) will be estimated by the following equation:

DTF = (Thickness at end inspiration − Thickness at end expiration)/ Thickness at end expiration.

The DTF for each patient will be calculated as the mean of the values measured in three breaths.

All examinations will be recorded on a personal computer for subsequent blinded analysis.

Follow-up study

Diaphragm dysfunction is common in critically ill patients. It can be caused by multiple factors including prolonged mechanical ventilation. We first examine the serial changes in diaphragm function during an SBT with ultrasound in order to assess its value for predicting weaning outcome. Then we will measure the right diaphragm excursion and bilateral diaphragm thickening fraction during spontaneous breathing with bedside ultrasound on a daily basis over the first seven days following extubation. This follow-up study will be performed in order to find whether the damage of diaphragm is reversible.

Data collection

Demographics such as sex, age on admission, body mass index(BMI), underlying diseases, reasons for tracheal intubation, Acute Physiology and Chronic Health Evaluation II (APACHE II) and duration of mechanical ventilation will be recorded. Laboratory measurements including arterial blood gas, pro-brain natriuretic peptide (pro-BNP), plasma protein, hemoglobin, chemistry profile, blood count and C-reactive protein will be obtained and recorded prior to the SBT and at the end of the SBT. If the patients present signs and symptoms such as agitation, anxiety, depressed mental status, diaphoresis, cyanosis, increased accessory muscle activity, facial signs of distress and dyspnea during SBT, his or her arterial blood gas will also be obtained and recorded immediately. Cardiac function will be assessed and recorded by pro-BNP and echocardiography findings such as left ventricular ejection fraction (LVEF), E-point to septal separation (EPSS) and e'/a' at the end of the SBT. The RSBI, diaphragm excursion and diaphragm thickening fraction will be recorded at the 5th minute, 30th minute, 60th minute, 90th minute and 120th minute of SBT. Changes in diaphragm function will be assessed by diaphragm excursion fraction (DEF) and DTF fraction (DTFF), which is calculated as the percent change of diaphragm excursion and DTF (relative to baseline) during the SBT. DEF at time t can be calculated by the following equation:

$DEF_t = (DE_t\text{-}DE_5)/DE_5$,

where t equals 30min, 60min, 90min and 120min, DE_5 is the diaphragm excursion at 5 minutes after the initiation of SBT.

DTF fraction (DTFF) at time t can be calculated by the following equation:

$DTFF_t = (DTF_t\text{-}DTF_5)/DTF_5$,

where t equals 30min, 60min, 90min and 120min, DTF_5 is the DTF at 5 minutes after the initiation of SBT.

The percent change of RSBI (relative to baseline) at time t can be calculated by the following equation:

$RSBIF_t = (RSBI_t\text{-}RSBI_5)/RSBI_5$,

where t equals 30min, 60min, 90min and 120min, $RSBI_5$ is the RSBI at 5 minutes after the initiation of SBT.

Maximum percent change in diaphragm excursion, DTF and RSBI during SBT will be recorded. The outcome is extubation failure or successful extubation.

Statistical analysis

The study aims to investigate the diagnostic performance of diaphragmatic parameters in predicting extubation failure. Thus, the estimated sensitivity and prevalence are needed for the calculation of sample size. Furthermore, we define the type I error to be 0.05. The following equation is used to calculate the sample size:

Sample size$=\frac{Z^2_{1-\alpha/2}\times S_N\times(1-S_N)}{L^2\times Prevalence}$,

where α is the type I error which is assumed to be =0.05, $Z_{1-\alpha/2}$ is =1.96, L is likelihood ratio which is assumed to be =0.1, S_N stands for sensitivity and is assumed to be 83% [19], and Prevalence stands for extubation failure rate and is assumed to be 33.1% [25]. A total of 164 patients are required for the study. Data will be presented as the mean and standard error, or median and interquartile range for continuous variables as appropriate [26]. Continuous variables will be compared between the weaning success and failure groups by independent Student's *t* test. Differences between the parameters with each group will be assessed by the paired Student's *t* test. The Chi-square tests will be used to compare the differences for categorical variables. Multivariate logistic regression will be used to identify variables that can independently predict the weaning outcome [27], adjusting for variables of cardiac functions such as diastolic function and systolic function. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) will be calculated for diaphragm excursion, DTF, RSBI, maximum percent change in diaphragm excursion, DTF and RSBI in predicting weaning outcome. Receiver operating characteristic (ROC) curves will be utilized to assess the diagnostic performance of diaphragm excursion, DTF, RSBI, maximum percent change in diaphragm excursion, DTF and RSBI in predicting weaning outcome. Differences of the area under ROC curves will be compared using nonparametric method. Serial measured values will be analyzed using serial measurement analysis of variance. To assess inter-observer variability, 20 patients will be measured by two different operators. Pearson correlation analysis and Bland-Altman plotting will be performed to evaluate the reproducibility of diaphragm ultrasound studies. A two-tailed p value <0.05 is considered statistically significant. All statistical analyses will be performed using R software.

Discussion

There are approximately 20% of patients have difficulties in weaning from mechanical ventilation [2]. Weaning outcome can be affected by a variety of factors such as cardiac dysfunction, muscle weakness, pulmonary insufficiency and electrolyte disturbances [28]. As a result, single parameter

is inadequate to make an accurate prediction. RSBI has been demonstrated to be accurate in predicting weaning outcome, and is now widely used in clinical practice. This index reflects the contribution of all inspiratory muscles, rather than the function of the diaphragm. Therefore, diaphragm fatigue can be masked by compensatory action of the other inspiratory muscle during SBT. However, the compensatory effect of non-diaphragm inspiratory muscles cannot be maintained for a long time [29,30]. Thus, a substantial proportion of patients who pass RSBI test may be subject to weaning failure.

It is common that a patient presents a normal respiratory pattern at the beginning of an SBT, but the respiration deteriorates a few hours later. Chatila *et al*. reported that RSBI measured at 30 min after initiation of SBT was superior to RSBI measured at the start of the trial as a weaning predictor [31]. Another study demonstrated that RSBI measured at the end of SBT had better diagnostic accuracy [32]. Also there was a study found that RSBI measured serially was more reliable to predict weaning outcomes [8]. Segal *et al*. showed that the percent change of RSBI during SBT was better in predicting the weaning outcome [33]. They concluded that the evolution of respiratory pattern, as assessed by change in RSBI, was a marker of the dynamic changes that occur during weaning that may improve the ability to predict extubation outcome [33]. However, to the best of our knowledge, there is no study investigating the serial changes in diaphragm function during the SBT. We hypothesize that the changes of diaphragm function prior to RSBI is a better predictor of weaning outcome. The aim of this study is to explore whether changes in diaphragm function during SBT is a good predictor of weaning outcome. Furthermore, we attempt to investigate the timing of diaphragm function measurement which has the best diagnostic performance.

Currently the gold standard for evaluating the diaphragm function is trans-diaphragmatic pressure. Other methods such as fluoroscopy and magnetic resonance imaging have also been reported [34,35]. However, these methods are invasive, costly and uncomfortable to patients. Also, these methods involve transportation of patients to radiology department and radiation exposure. The

transportation imposes considerable risk on critically ill patients. Bedside ultrasound is an alternative to evaluate the diaphragm function, which is simple, rapid and noninvasive, and has been increasingly used in critical care setting. A number of studies have demonstrated that diaphragm excursion and thickening fraction could reflect the function of the diaphragm and predict the weaning outcome [19,20,36]. Thus, our study will employ diaphragm excursion and thickening fraction measured by ultrasound to evaluate the function of diaphragm. And we will evaluate the changes in diaphragm function by maximum percent change in diaphragm excursion and thickening fraction during the SBT.

We hypothesize that serial changes in diaphragm function measured by ultrasound during SBT is a reliable predictor of weaning outcome.

Contributorship statement

All the listed authors have participated actively in the study, and have seen and approved the submitted manuscript. Pengmin Zhou, Yucai Hong and Peifeng Xu were responsible for the design of this study. Pengmin Zhou, Yiming Zhao, Xuchang Qin, Jiawei Guo, Yun Pan and Shengping Lin were responsible for the drafting of the article. Zhongheng Zhang, Huabo Cai, Hui Zhao and Junru Dai were responsible for the revision of the article.

Competing interests

The authors do not have any possible competing interest.

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Data sharing statement

No additional unpublished data are available.

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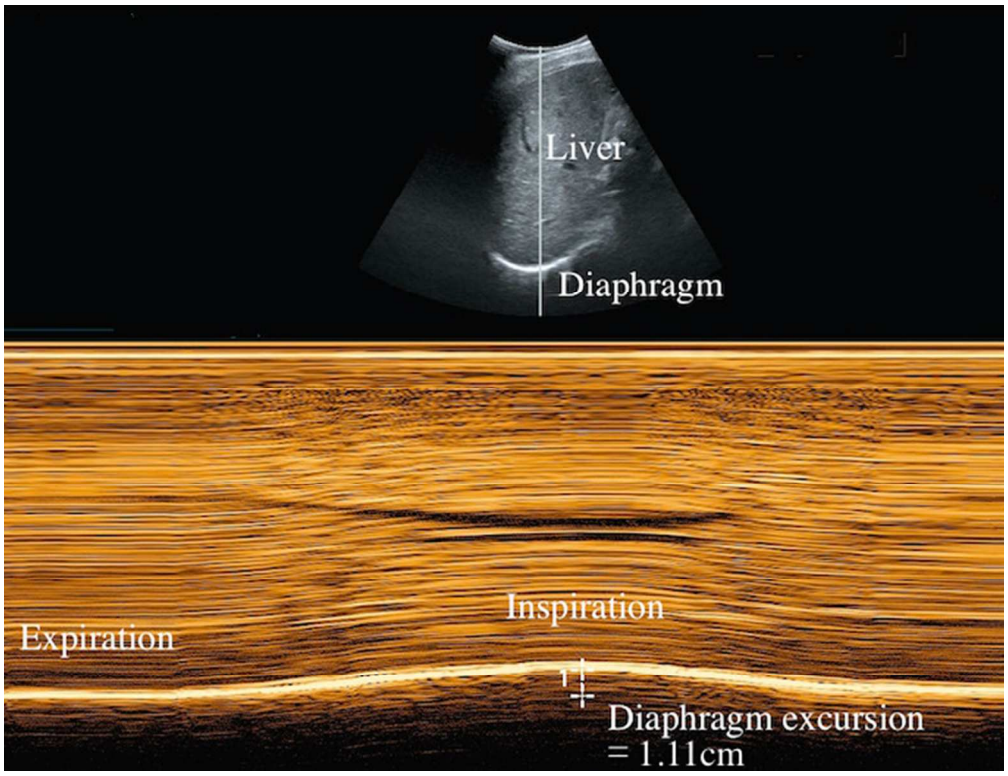
Figure 1 The liver is used as a window for the right diaphragm. During inspiration, the normal diaphragm contracts and moves caudally toward the probe; During expiration, the normal diaphragm moves cranially away from the probe; this is recorded as an upward motion of the M-mode tracing. The diaphragm excursion is measured on the vertical axis of the tracing from the

baseline to the point of maximum height of inspiration on the graph. Three measurements will be recorded and averaged.

Figure 2 The diaphragm is imaged as three layer structure, including two parallel echoic lines (the pleura and the peritoneum) and a hypoechoic structure between them (the muscle itself). The distance from the middle of the pleural line to the middle of the peritoneal line is the diaphragm thickness. We will measure the diaphragm thickness three times on the same scan and the values will be averaged. Diaphragm thickening fraction (DTF) will be estimated by the following equation:

DTF = (Thickness at end inspiration − Thickness at end expiration)/ Thickness at end expiration.

The DTF for each patient will be calculated as the mean of the values measured in three breaths.



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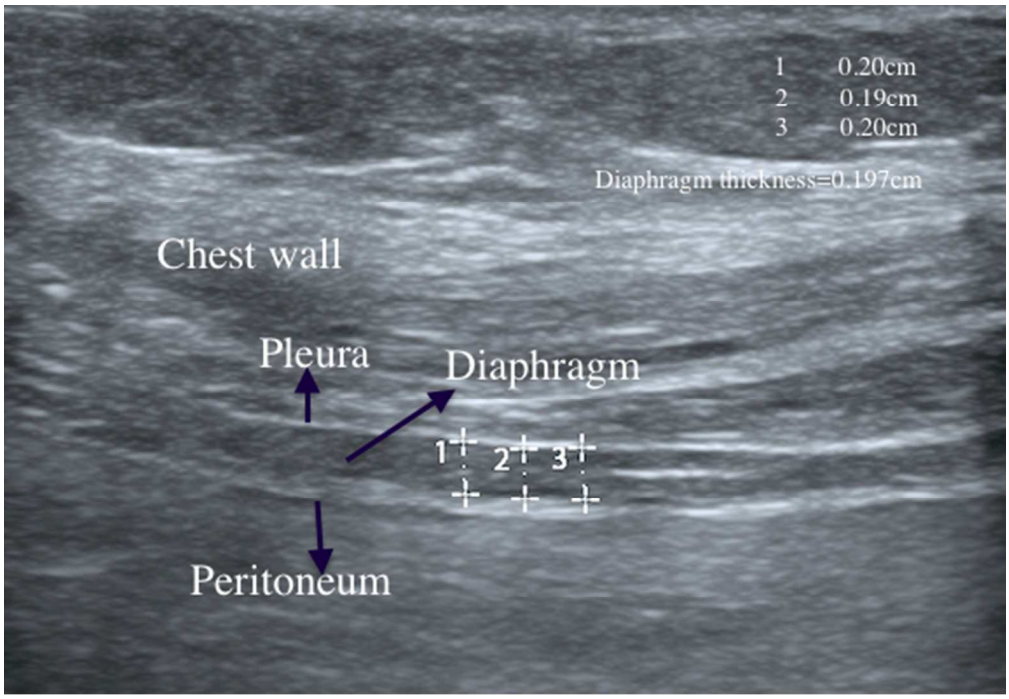
57x44mm (300 x 300 DPI)



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48x33mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended Items to Address in Clinical Trial Protocol and Related Documents

Section/Item	Item Number	Description	Page number in the main document
Administrative information			
Title	1	The predictive value of serial changes in diaphragm function during the spontaneous breathing trial for weaning outcome: a study protocol	1
Trial registration	2a	Can serial changes in diaphragm function during the spontaneous breathing trial predict the weaning outcome?(ISRCTN42917473)	3
	2b	ISRCTN42917473	3
Protocol version	3	Date:1/11/2016 Version: 1.0	N/A
Funding	4	This study received the financial support from the Department of Health of Zhejiang Province.	13
Roles and responsibilities	5a	Pengmin Zhou, Yucai Hong and Peifeng Xu were responsible for the design of this study. Pengmin Zhou, Yiming Zhao, Xuchang Qin, Jiawei Guo, Yun Pan and Shengping Lin were responsible for the drafting of the article. Zhongheng Zhang, Huabo Cai, Hui Zhao and Junru Dai were responsible for the revision of the article.	13
	5b	Sponsor name: Department of Health of Zhejiang Province, Telephone number: (0571)87087342, E-mail: webmaster@zjwst.gov.cn, website: www.zjwst.gov.cn.	13
	5c	Department of Health of Zhejiang Province provide funding for this study. They will not have ultimate authority over any of these activities.	13
	5d	N/A	N/A
Introduction			
Background and rationale	6a	Excursion and thickening fraction of the diaphragm has been measured by bedside ultrasonography and were found to be able to quantitatively reflect diaphragm	3-4

		function, and thus was useful in predicting weaning outcome. However, these indices are usually measured at the start of an SBT. To the best of our knowledge, there is no report on the impact of serial changes in diaphragm excursion and thickening fraction during the SBT on weaning outcomes.	
	6b	There are no comparators in this study.	N/A
Objectives	7	The purpose of this study is to investigate whether the diaphragm function measured serially by ultrasonography during the SBT could be used to predict the weaning outcome.	2
Trial design	8	This is a prospective observational study.	2
Methods			
Participants,interventions, and outcomes			
Study setting	9	This study will be performed in an academic hospital in China.	2
Eligibility criteria	10	All patients in the ICU who are mechanically ventilated for more than 48 hours through endotracheal tube are potentially eligible for this study. Patients will be included if they meet all of the following criteria: 1) adequate cough, absence of excessive tracheobronchial secretion, resolution of underlying critical illness for which the patient is intubated; 2) the patient is alert and cooperative, without sedation; 3) hemodynamically stable (i.e. heart rate≤140 beats • min-1 , systolic blood pressure between 90 and 160mmHg, without vasopressors); 4) stable metabolic status and 5) improved respiratory function: arterial oxygen saturation (SaO2) >90% on fractional inspired oxygen (FiO2) ≤ 0.4 (or oxygen index (PaO2/FiO2)≥150mmHg), positive end-expiratory pressure (PEEP)≤8 cmH2O, respiratory rate≤35 beats • min-1, without respiratory acidosis[2]. Subjects will be excluded if they have a	5

		history of diaphragm paralysis, cervical spine injury, neuromuscular diseases, a current thoracostomy, pneumothorax, or pneumomediastinum.	
Interventions	11a	All enrolled patients will be ventilated with an Evita-4 (Draeger, Lubeck, Germany) by using volume assist control mode prior to SBT. Positive end-expiratory pressure (PEEP) will be set to 5cmH2O and fractional inspired oxygen (FiO2) will be set to a value below 0.5 that guarantees oxygen saturation by pulse oximetry (SpO2) greater than 90%. Enrolled patients will undergo SBT for 2 hours in semi-recumbent position. During the SBT, the patients will breathe through the ventilator circuit by using flow triggering (2L/min) with automatic tube compensation (ATC) of 100% and 5 cmH2O PEEP [1]. The fractional inspired oxygen (FiO2) will be set to the same value as used before SBT. If the patients fail to tolerate the SBT, the trial will be discontinued immediately and the ventilation mode will be switched to that used before the trial. Patients who pass the 2-hour SBT will be extubated. Right diaphragm excursion and bilateral diaphragm thickening fraction will be measured by ultrasonography during spontaneous breathing. Images will be obtained immediately prior to the SBT, and at 5 min, 30min, 60min , 90min, and 120min after the initiation of SBT. Rapid shallow breathing index (RSBI) will be simultaneously calculated at the bedside by a respiratory nurse.	5
	11b	If the patient fails to tolerant the SBT, we will terminate the trial immediately. Criteria for failure to the SBT was the following: 1) agitation and anxiety, depressed mental status, diaphoresis, cyanosis,	6

		increased accessory muscle activity, facial signs of distress, dyspnea, 2) PaO2≤50-60mmHg on FiO2≥0.5 or SaO2<90%, PaCO2 >50mmHg or an increase in PaCO2 >8mmHg, pH<7.32 or a decrease in pH≥0.07 pH units, fR>35 breaths•min-1L-1 or increased by ≥50%, fC>140 breaths•min-1L-1 or increased by ≥ 20%, systolic BP>180mmHg or increased by ≥20%, systolic BP<90mmHg, 3) cardiac arrhythmias	
	11c	N/A	N/A
	11d	Relevant concomitant care and interventions are as usual.	N/A
Outcomes	12	Patients were divided into two groups according to their weaning outcomes. A successful weaning was defined as spontaneous breathing(SB) for >48 h following extubation without any level of ventilator support. A failed weaning was defined as either SBT failure or the inability to maintain SB for at least 48 h after extubation.	6
Participant timeline	13	When the objects meet the above inclusion criteria, they will undergo SBT for 2 hours. If the patient fails to tolerant the SBT, we will terminate the trial immediately and turn back to the control mode. Patients who passed the 2-hour SBT were extubated. During the SBT, Right diaphragm excursion and bilateral diaphragm thickening fraction will be evaluated by ultrasonography during spontaneous breathing. Images will be obtained before to start the SBT and at 5 min, 30min, 60min, 90min and 120min after the initiation of SBT. Rapid shallow breathing index (RSBI) will be simultaneously calculated at the bedside by a respiratory nurse.	5
Sample size	14	The study aims to investigate the diagnostic performance of diaphragmatic parameters in predicting extubation	10

		failure. Thus, the estimated sensitivity and prevalence are needed for the calculation of sample size. Furthermore, we define the type I error to be 0.05. The following equation is used to calculate the sample size: Sample size=$\frac{Z^2_{1-\alpha/2}\times S_N\times(1-S_N)}{L^2\times Prevalence}$, where α is the type I error which is assumed to be =0.05, Z1-α/2 is =1.96, L is likelihood ratio which is assumed to be =0.1, SN stands for sensitivity and is assumed to be 83% [19], and Prevalence stands for extubation failure rate and is assumed to be 33.1% [25]. A total of 164 patients are required for the study.	
Recruitment	15	Every patient in our EICU who met the inclusion criteria was enrolled.	5
Assignment of Interventions(for controlled trials)			
Allocation Sequence generation	16a	N/A	N/A
Allocation concealment mechanism	16b	N/A	N/A
Implementation	16c	N/A	N/A
Blinding(masking)	17a	N/A	N/A
	17b	N/A	N/A
Data collection, Management, and Analysis			
Data collection methods	18a	Demographics such as sex, age on admission, body mass index(BMI), underlying diseases, reasons for tracheal intubation, Acute Physiology and Chronic Health Evaluation II (APACHE II) and duration of mechanical ventilation will be recorded. Laboratory measurements including arterial blood gas, pro-brain natriuretic peptide (pro-BNP), plasma protein, hemoglobin, chemistry profile,	9-10

		blood count and C-reactive protein will be obtained and recorded prior to the SBT and at the end of the SBT. If the patients present signs and symptoms such as agitation, anxiety, depressed mental status, diaphoresis, cyanosis, increased accessory muscle activity, facial signs of distress and dyspnea during SBT, his or her arterial blood gas will also be obtained and recorded immediately. Cardiac function will be assessed and recorded by pro-BNP and echocardiography findings such as left ventricular ejection fraction (LVEF), E-point to septal separation (EPSS) and e'/a' at the end of the SBT. The RSBI, diaphragm excursion and diaphragm thickening fraction will be recorded at the 5th minute, 30th minute, 60th minute, 90th minute and 120th minute of SBT. Changes in diaphragm function will be assessed by diaphragm excursion fraction (DEF) and DTF fraction (DTFF), which is calculated as the percent change of diaphragm excursion and DTF (relative to baseline) during the SBT. DEF at time t can be calculated by the following equation: DEFt = (DEt-DE5)/DE5, where t equals 30min, 60min, 90min and 120min, DE5 is the diaphragm excursion at 5 minutes after the initiation of SBT. DTF fraction (DTFF) at time t can be calculated by the following equation: DTFFt = (DTFt-DTF5)/DTF5, where t equals 30min, 60min, 90min and 120min, DTF5 is the DTF at 5 minutes after the initiation of SBT. The percent change of RSBI (relative to baseline) at time t can be calculated by the following equation:	

		RSBIFt = (RSBIt-RSBI5)/RSBI5, where t equals 30min, 60min, 90min and 120min, RSBI5 is the RSBI at 5 minutes after the initiation of SBT. Maximum percent change in diaphragm excursion, DTF and RSBI during SBT will be recorded. The outcome is extubation failure or successful extubation.	
	18b	All patients included into our cohort are followed up.	N/A
Data management	19	Double data entry	N/A
Statistical methods	20a	Data will be presented as the mean and standard error, or median and interquartile range for continuous variables as appropriate [26]. Continuous variables will be compared between the weaning success and failure groups by independent Student's t test. Differences between the parameters with each group will be assessed by the paired Student's t test. The Chi-square tests will be used to compare the differences for categorical variables. Multivariate logistic regression will be used to identify variables that can independently predict the weaning outcome [27], adjusting for variables of cardiac functions such as diastolic function and systolic function. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) will be calculated for diaphragm excursion, DTF, RSBI, maximum percent change in diaphragm excursion, DTF and RSBI in predicting weaning outcome. Receiver operating characteristic (ROC) curves will be utilized to assess the diagnostic performance of diaphragm excursion, DTF, RSBI, maximum percent change in diaphragm excursion, DTF and RSBI in predicting weaning outcome. Differences of the area under ROC curves will be	10-11

		compared using nonparametric method. Serial measured values will be analyzed using serial measurement analysis of variance. To assess inter-observer variability, 20 patients will be measured by two different operators. Pearson correlation analysis and Bland-Altman plotting will be performed to evaluate the reproducibility of diaphragm ultrasound studies. A two-tailed p value <0.05 is considered statistically significant. All statistical analyses will be performed using R software.	
	20b	N/A	N/A
	20c	N/A	N/A
Monitoring			
Data monitoring	21a	This is a prospective observational study. This study has no harm to the patient's physical, psychological and social relations. And this study accepted the supervision of Ethics Committee of Sir Run-Run Shaw Hospital, Zhejiang University School of Medicine. So a DMC is not needed.	3
	21b	N/A	N/A
Harms	22	This is a prospective observational study. This study has no harm to the patient's physical, psychological and social relations.	3
Auditing	23	This study was audited by Ethics Committee of Sir Run-Run Shaw Hospital, Zhejiang University School of Medicine every 12 months. And this process will be independent from investigators and the sponsor.	3
Ethics and dissemination			
Research ethics approval	24	The study protocol was approved by the ethics committee of Sir Runrun Shaw hospital, an affiliate of Zhejiang University, Medical College.	3
Protocol amendments	25	If our protocol has modifications, we will communicate to relevant parties(including investigators, trial participants, trial registries, journals and regulators) as soon	N/A

		as possible.	
Consent or assent	26a	Investigator will obtain informed consent from potential trial participants or authorized surrogates.	N/A
	26b	N/A	N/A
Confidentiality	27	The personal information of the participants is confidential, and the participant's medical records will be kept in hospital.	N/A
Declaration of interests	28	The principal investigators have no financial and other competing interests for the overall trial and each study site.	13
Access to data	29	Corresponding author will have access to the final trial data set.	13
Ancillary and post-trial care	30	Ancillary and post-trial care is as usual.	N/A
Dissemination policy	31a	The results will be published in a peer-reviewed journal and shared with the worldwide medical community.	3
	31b	N/A	N/A
	31c	N/A	N/A
Appendices			
Informed consent materials	32	Our informed consent materials are approved by ethics committee of Sir Runrun Shaw hospital, an affiliate of Zhejiang University, Medical College. All participants gave informed written consent.	N/A
Biological specimens	33	N/A	N/A