Supplementary Online Content 1

Writing Committee for the RELAx Collaborative Group. Effect of a low vs high positive end-expiratory pressure (PEEP) strategy on ventilator-free days in intensive care unit patients without ARDS - a randomized clinical trial.

Supplement 1. Trial protocol

This supplementary material has been provided by the authors to give readers additional information about their work.

PROTOCOL TITLE: REstricted versus Liberal positive end–expiratory pressure in patients without Acute respiratory distress syndrome – RELAx, a Randomized Controlled Trial

Protocol ID	RELAx
Short title	Restricted versus liberal positive end–expiratory pressure
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ABBREVATIONS AND DEFINITIONS

ARDS	Acute Respiratory Distress Syndrome
CE	Cost–Effective
DSMB	Data Safety Monitoring Board
ICU	Intensive Care Unit
LOS	Length Of Stay
METC	Medical Research Ethical Committee (MREC) in Dutch: *Medische Ethische Toetsings Commissie*
NAS	Nurse & Activity Score
PBW	Predicted Body Weight
PEEP	Positive End–Expiratory Pressure
RCT	Randomized Controlled Trial
SAE	Serious Adverse Event
Sponsor	The party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization or investigator; a party that provides funding for a study bus does not commission is not regarded as the sponsor, but referred to as a subsidizing party
TISS	Therapeutic Intervention Scoring System
VFD–28	Ventilator–Free Days and alive at day 28

SUMMARY

Rationale: While there is sufficient randomized controlled trial–evidence for benefit of higher levels of positive end–expiratory pressure (PEEP) during ventilation of intensive care unit (ICU) patients with acute respiratory distress syndrome (ARDS), evidence for benefit of PEEP, at any level, during ventilation of ICU patients without ARDS is still insufficient. One recent metaanalysis suggests no benefit of PEEP in ICU patients without ARDS. Nevertheless, there is a trend to use higher PEEP levels in these patients in recent years.

Hypothesis: We hypothesize that ventilation with the lowest possible PEEP level ('restricted PEEP', i.e., the lowest PEEP level resulting in an acceptable level of oxygenation) is as effective and safe as ventilation with the PEEP level currently practiced ('liberal PEEP', i.e., a PEEP level of 8 cm H_2O, the median PEEP level applied in these patients in the Netherlands) in ICU patients without ARDS.

Objective: To compare ventilation with the lowest possible PEEP level to ventilation with the PEEP level currently practiced in ICU patients without ARDS.

Study design: National multicenter, non–inferiority, open, randomized controlled trial in intubated and ventilated adult ICU patients without ARDS.

Study population: Consecutive intubated and ventilated adult ICU patients without ARDS with an anticipated duration of ventilation of at least 24 hours.

Procedure: Patients are randomly assigned in a 1:1 ratio to the 'restricted PEEP'–arm or to the 'liberal PEEP'–arm of this trial.

Study endpoints: The primary endpoint is the number of ventilator–free days and alive at day 28. Secondary endpoints include ICU– and hospital length of stay (LOS), ICU– and hospital, and 90–day mortality, incidence of severe hypoxemia, severe atelectasis and the need for rescue therapies, pneumonia, pneumothorax, the incidence and development of ARDS and days with use of hemodynamic support and with use of sedation. Also, therapeutic intervention scoring system (TISS)/ Nursing Activities Score (NAS) and related healthcare costs will be estimated and compared.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Differences in burden and risk of the two ventilation strategies are uncertain. Ventilation with the lowest possible PEEP level could increase the risk of atelectasis and also the risk of potentially dangerous hypoxemia, which can be adequately treated within the ICU setting. Ventilation with the PEEP

level currently practiced could increase the amount of overdistended lung tissue and increase hemodynamic compromise. No other study interventions are performed. Collection of demographic data, ventilation data and outcome data causes no harm for the patients.

1. INTRODUCTION AND RATIONALE

1.1 Mechanical ventilation associated lung injury

Mechanical ventilation is typically seen as a life–saving intervention in critically ill patients, despite increasing and unequivocal evidence that it can aggravate and even initiate lung injury.[1] Indeed, ventilation may contribute to development of atelectasis,[2,3] increasing the risk of repetitive opening and closing of lung tissue, a phenomenon frequently referred to as 'atelectrauma'.[1] Results from preclinical studies using animals[4,5] and studies in humans[6,7] support the use of positive end–expiratory pressure (PEEP) during ventilation to prevent, or at least minimize the risk of atelectrauma. Ventilation with PEEP, however, can also lead to lung injury due to overdistension,[8,9] frequently referred to as 'volutrauma'.[1]

1.2 Pulmonary effects of PEEP

Atelectasis is more extensive in patients with the acute respiratory distress syndrome (ARDS) than in patients without lung injury, and are more frequently seen with mandatory than spontaneous forms of ventilation.[10,11] In patients with ARDS, seen the balance between the positive effects of higher PEEP levels (i.e., reduction in atelectrauma, by reducing atelectasis) and negative effects of higher PEEP levels (i.e., increase in volutrauma, by increasing overdistension), ventilation with a higher PEEP level could result in a net beneficial effect. In patients without ARDS, however, patients who also more frequently receive spontaneous forms of ventilation, the balance between benefit and harm could go into the other direction, as the reduction in atelectrauma could be minimal or negligible, at a price of more volutrauma.

The results of one metaanalysis using the individual patient data from three large randomized controlled trials (RCTs) comparing higher to lower PEEP levels during ventilation of patients with ARDS suggests benefit of higher PEEP levels (albeit only in patients with more severe form of ARDS).[12-15] Sufficiently large RCTs comparing higher to lower PEEP levels during ventilation of patients without ARDS are presently lacking, and the available data does not allow individual patient data metaanalyses.[16]

1.3 Non–pulmonary effects of PEEP

Besides increasing lung aeration, ventilation with PEEP could also have extrapulmonary effects. Ventilation with PEEP affects the loading conditions of the heart,[17] as every increase in intrathoracic pressure reduces the preload of the heart

and might increase as well as decrease the afterload of the right ventricle depending on whether lung tissue is recruited by PEEP.[17] The effects of ventilation with PEEP on cardiac performance could also differ between patients with ARDS and patients without lung injury. Ventilation with higher PEEP levels could reduce right ventricle afterload through the prevention of atelectases in ARDS patients, while it could increase right ventricle afterload and reduce left ventricle preload through increases in overdistended lung tissue in patients without ARDS. RCTs evaluating the extrapulmonary effects of PEEP are lacking, both in ventilated patients with ARDS, and ventilated patients without ARDS.

1.4 Systematic review and metaanalysis of RCTs of PEEP

A recent systematic review and metaanalysis of RCTs in patients without ARDS did not find benefit from ventilation with higher PEEP levels with regard to mortality and duration of ventilation, neither in surgical ICU patients nor in medical ICU patients.[16] The analysis even suggested no benefit of any level of PEEP in these patients. There were no differences found in the incidence of hypotension and blood pressure levels between ventilation with higher PEEP levels versus lower PEEP levels.

1.5 Is there benefit of intraoperative PEEP?

The effects of PEEP during ventilation gained also interest from anesthesiologists, who struggle with the same question of whether or not to use PEEP in surgery patients without lung injury. Three RCTs showed that ventilation with PEEP combined with low tidal volumes was associated with better outcomes compared to ventilation without or a low level of PEEP combined with high tidal volumes.[18-20] These RCTs thus studied the effect of a bundle of ventilator settings that are both expected to have an effect on the lungs, and it is impossible to conclude which part of the bundle was responsible for the benefit found. A more recent RCT, however, showed no difference in the incidence of pulmonary complication when no PEEP was compared to PEEP during ventilation at low tidal volumes.[21] Furthermore, one individual patient metaanalysis using data from all four RCTs mentioned above suggests that benefit seemed to come mainly from restrictions in tidal volume size, and not from using higher levels of PEEP, in patients undergoing intraoperative ventilation during general anesthesia for surgery.[22]

1.6 An historical perspective

In the early years of mechanical ventilation, PEEP was seldom used because of its alleged negative effects on hemodynamics.[23] Most RCTs of PEEP in ICU patients without ARDS compared ventilation with some level of PEEP to no PEEP (figure 1). In the 1960s, Ashbaugh observed that PEEP improved oxygenation in mechanically ventilated patients with ARDS, triggering the use of PEEP in patients with this life–threatening complication of critical illness.[24] In the 1970s, animal experiments suggested that prophylactic PEEP could be beneficial as well,[25-27] maybe even preventing development of ARDS.[28,29] Since then PEEP is increasingly used, also in patients without ARDS, despite evidence for benefit of this strategy.

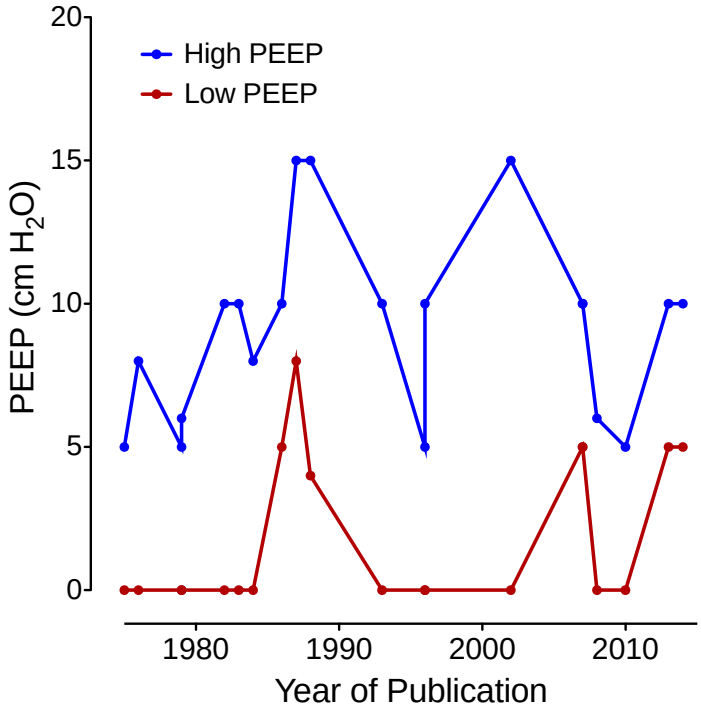

Figure 1. PEEP levels in randomized controlled trials in patients without ARDS.[16]

1.7 Current PEEP practice in ICU patients without ARDS

Due to absence of RCT–evidence, it is highly uncertain what the best PEEP level is in ICU patients without ARDS. Interestingly, there is a salient tendency to use higher PEEP levels in these patients.[30-32] Even more surprising, in the Netherlands ICU patients without ARDS are ventilated with a median PEEP level of 8 cm H_2O, higher compared to a PEEP level of 6 cm H_2O in surrounding countries,[33] and what is reported to be used worldwide.[34]

1.8 Need for a new RCT of PEEP in patients without ARDS

While guidelines recommend using higher PEEP levels in ICU patients with ARDS, recommendations regarding the PEEP level to use in ICU patients without lung injury are lacking. Often a minimum PEEP level of 5 cm H_2O is recommended, though this is without any scientific support. Consequently, the ICU community requests a well–powered high–quality RCT comparing ventilation with higher versus lower PEEP levels in ICU patients without ARDS.[16] This RCT should use objective and patient–relevant outcomes, such as duration of ventilation and ICU– and hospital length of stay (LOS), amongst others.

1.9 The RELAx trial

The 'REstricted versus Liberal positive end–expiratory pressure in patients without Acute respiratory distress syndrome' (RELAx) trial is a national multicenter open randomized controlled trial in ICU patients without ARDS at start of ventilation. It will be the first RCT comparing ventilation with the lowest possible PEEP level with ventilation with the median PEEP level currently practiced in the Netherlands that recruits a sufficient number of patients to test the hypothesis that ventilation with the lowest possible PEEP level is non–inferior to ventilation with a PEEP level of 8 cm H_2O with regard to objective and patient–relevant clinical endpoints.

2. OBJECTIVES AND HYPOTHESIS

2.1 Objectives

2.1.1 Primary objective

The aim of the RELAx trial is to compare ventilation with the lowest possible PEEP level ('restricted PEEP', i.e., the lowest PEEP level resulting in an acceptable level of oxygenation) to ventilation with the PEEP level currently practiced ('liberal PEEP', i.e., a PEEP level of 8 cm H_2O, the median PEEP level in these patients in the Netherlands) in intubated and ventilated ICU patients not fulfilling the consensus definition for ARDS at start of ventilation.

2.1.2. Secondary objectives

Secondary objectives are to compare the effects of '*restricted PEEP' vs. 'liberal PEEP'* on ICU– and hospital length of stay (LOS), ICU– and hospital, and 90–day mortality, the incidence of severe hypoxemia, severe atelectasis, and the need for rescue therapies including recruitment maneuvers, bronchoscopy and prone position, pneumonia, pneumothorax, the incidence and development of ARDS, days with use of hemodynamic support and with use of sedation, therapeutic intervention scoring system (TISS)/ Nursing Activities Score (NAS) and related healthcare costs.

2.2 Hypothesis

2.2.1 Primary hypothesis

We hypothesize that ventilation with the lowest possible PEEP level results in a similar number of ventilator–free days at day 28 as ventilation with the PEEP level currently practiced in ICU patients without ARDS.

2.2.2. Secondary hypotheses

The secondary hypotheses are that ventilation with the lowest possible PEEP level is equal to ventilation with the PEEP level currently practiced in ICU patients without ARDS, with regard to the other endpoints mentioned above.

3. STUDY DESIGN

The RELAx trial is a national multicenter, non–inferiority, open, randomized controlled trial in intubated and ventilated adult ICU patients without ARDS expected to need ventilation for at least 24 hours. A total of 980 ICU patients in 12 participating academic as well as non–academic centers will be included.

4. STUDY POPULATION

4.1 Population

The RELAx trial will recruit consecutive intubated and mechanically ventilated ICU patients without ARDS at onset of ventilation and who are expected to need ventilation > 24 hours. Patients are included in the ICUs of 3 academic and 9 non–academic centers in the Netherlands. Patients are screened for eligibility and randomized within one hour after initiation of invasive ventilation or, if already intubated and ventilated before admission, on ICU admission. A total of 980 patients will be randomized; approximately 82 patients per center.

4.2 Inclusion criteria

In order to be eligible to participate in this trial, patients must meet all of the following criteria:

- Admission to one of the participating ICUs
- Need for and start of invasive ventilation
- An expected duration of ventilation > 24 hours

4.3 Exclusion criteria

Patients who meet any of the following criteria will be excluded:

- Age less than 18 years
- Patients with a clinical diagnosis of ARDS or possible ARDS with a PaO_2/FiO_2 < 200 mmHg (as the benefit of ventilation with higher PEEP levels has been proven in these patients; see **text box 1**)
- Patients with ongoing cardiac ischemia due to cardiac infarction and failed revascularization, patients with increased and uncontrollable intracranial pressure (of ≥ 18 mmHg), patients with delayed cerebral ischemia after subarachnoid hemorrhage, patients with necrotizing fasciitis, and severe untreatable anemia such as in case of Jehovah's Witnesses (as these patients can be considered to be vulnerable to the potentially dangerous hypoxemia which could develop more often, even for a short time, in the 'restricted PEEP'–arm of this trial; see **text box 2**)
- Patients previously randomized in this RCT
- Patients participating in another RCT with the same clinical endpoint, or interventions possibly compromising the primary outcome

- Invasive ventilation longer than 12 hours directly preceding the present ICU admission
- Invasive ventilation longer than 1 hour before randomization
- Patients with suspected or confirmed pregnancy
- Patients with morbid obesity (body mass index > 40)
- Patients with GOLD classification III or IV chronic obstructive pulmonary disease (COPD)
- Patients with premorbid restrictive pulmonary disease (evidence of chronic interstitial infiltration on chest radiographs)
- Patients in whom pulse oximetry is known to be unreliable, e.g., patients with carbon monoxide poisoning
- Any neurologic diagnosis that can prolong duration of mechanical ventilation, e.g., patients with Guillain–Barré syndrome, high spinal cord lesion or amyotrophic lateral sclerosis, multiple sclerosis, or myasthenia gravis
- Patients receiving veno-venous, veno-arterial or arterio-venous extracorporeal membrane oxygenation (ECMO)
- No informed consent

Text Box 1 – Diagnosing ARDS

The diagnosis of ARDS is clinical, requiring (a) a medical history, (b) the presence of bilateral opacities on the chest radiograph that are fully explained by effusions, lobar/lung collapse or nodules, and (c) respiratory failure not fully explained by cardiac failure or fluid overload. The PaO_2/FiO_2 is used to classify ARDS severity, with a PaO_2/FiO_2 between 200 and 300 mmHg indicating mild ARDS, and a PaO_2/FiO_2 < 200 mm Hg indicating moderate or severe ARDS.

The diagnostic approach, however, could be difficult if not impossible in ICU patients within the first hour after intubation and start of ventilation: they frequently suffer from temporary post–intubation atelectasis as a reason for a low PaO_2/FiO_2, the medical history is often not yet complete, and imaging studies are usually not yet performed or the results available. The risk is that only the PaO_2/FiO_2 is used to diagnose ARDS in the short time frame after intubation, which could induce severe bias, as many of these patients do not have ARDS.

Thus, we exclude all patients that are clinically diagnosed with ARDS. **Patients with a PaO_2/FiO_2 < 200 mm Hg are also excluded** since we consider these patients at high risk of having ARDS; only when the attending physician explicitly states the patients has no ARDS *and* no direct risk factor for ARDS is present, the patient can be included. Patients without ARDS and with a PaO_2/FiO_2 between 200 and 300 mmHg can be included: as these patients very seldom have ARDS.

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Text Box 2 – Potentially vulnerable patients

Oxygen delivery to the tissues (DO_2) depends on cardiac output and arterial blood oxygen content,[35] the latter being dependent on hemoglobin saturation, arterial blood oxygen saturation (SaO_2) and partial pressure of oxygen (PaO_2). The understanding of the importance of the several components of DO_2 has led to emphasize early identification and prevention of hypovolemia (to prevent a low cardiac output) and anemia, but also prevention of hypoxemia for critically ill patients.

Administration of fluids, packed red blood cells, and additional oxygen could all be useful, though the effect size on DO_2 differs substantially. Indeed, a 50%–decrease in hemoglobin concentration (e.g., from 9 to 4,5 mmol/l) results in a 50% reduction of DO_2, whereas a 50%–reduction in the PaO_2 (e.g., from 12 to 6 kPa, or SaO_2 (from 98 to 78%) results only in no more than 20% decrease in DO_2. Thus, the influence of a drop in hemoglobin concentration is of greater influence on DO_2 as compared to a drop in PaO_2 or SaO_2.

Nevertheless, the targeted O_2 saturation proposed in this RCT could potentially be harmful in certain patient groups, like those with proven ongoing cardiac ischemia or delayed cerebral ischemia, or necrotizing fasciitis, or severe untreatable anemia such as in case of Jehovah's Witnesses. Therefore, these patients should be excluded form participation in this trial.

4.4 Sample size

Group size calculation is focused on demonstrating non–inferiority. When the sample size in each is 445, an one–sided non–inferiority t–test (targeted at 0.05 significance level) for the difference in means of log–transformed normalized data has a 80% power to reject the null hypothesis that the number of VFD–28 in the 'restricted PEEP'–arm is inferior to the number of VFD–28 in the 'liberal PEEP'–arm by a margin of 10% anticipating on a coefficient of a variation of 0.70 (www.stichting–nice.nl), in favor or the alternative hypothesis that the number of VFD–28 in the 'restricted PEEP'–arm is non–inferior.

The choice for a margin of 10% is motivated by what we consider acceptable from a clinical point of view as the maximal acceptable reduction of the ventilator–free period for non–inferiority. Clinically this margin means that an increase of > 10%

371 in the duration of mechanical ventilation will reduce the VFD–28 with > 12 hours
372 (calculated over the expected mean duration of mechanical ventilation of 5 days)
373 (http://www.stichting–nice.nl) which will be considered inferior. To allow for an
374 anticipated drop out of 10% a total of 980 patients will be included.
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5. INTERVENTIONAL TREATMENT OF SUBJECTS

5.1 Randomization to the 'restricted PEEP'–arm or the 'liberal PEEP'–arm

Patients are randomly assigned in a 1:1 ratio to the 'restricted PEEP'–arm or to the 'liberal PEEP'–arm of this trial.

5.2 The 'restricted PEEP'–arm

Directly after start of invasive ventilation the PEEP level is set at 5 cm H_2O with an inspired oxygen fraction (FiO_2) between 0.21 and 0.6. The goal is to ventilate with the lowest possible PEEP level resulting in an acceptable level of oxygenation. For this, the operator, usually the attending ICU nurse, will reduce the level of PEEP in steps of 1 cm H_2O to a minimum level of 0 cm H_2O. Every 15 minutes the PEEP level is reduced with 1 cm H_2O, as long as the pulse oximetry reading shows a SpO_2 > 92% or the arterial blood gas shows a PaO_2 > 8 kPa, as illustrated in the flowchart (see **Figure 1**). Thereafter, ventilation continues with the lowest PEEP level at which the SpO_2 > 92% or PaO_2 > 8 kPa, using a FiO_2 of between 0.21 and 0.6. In case the SpO_2 drops below 92% or the PaO_2 drops below 8 kPa, brief periods of 5 minutes may be tolerated, first FiO_2 is increased up to maximum 0.6 before the level of PEEP is increased in steps of 1 cm H_2O until 5 cm H_2O. As soon as the patient stabilizes, again the level of PEEP is reduced in steps of 1 cm H_2O to a minimum level of 0 cm H_2O.

So–called 'down–titrations' of the PEEP level are allowed as often as wanted, but with a minimum of three 'down–titrations' per ICU nurse shift (i.e., every eight hours). This number is chosen to push nurses towards using the lowest possible PEEP level. We deliberately chose not to state a maximum for these 'down–titrations', as adjustments in ventilator settings, like FiO_2 and driving pressure, in the Dutch ICU setting are very frequent, occurring many more times than three times per shift – this is a safe process, and we assume it is the same for the PEEP level adjustments.

Patients are weaned from the ventilator (see: weaning) and tracheally extubated using the lowest PEEP level. In other words, the lowest PEEP level is used throughout the complete period of invasive ventilation. However, during pulmonary toileting and tracheal suctioning, bronchoscopic procedures, intra– or inter–ICU transport or any maneuver during which 'pre–oxygenation' with high FiO_2 is

deemed beneficial, ICU nurses are allowed to increase the FiO_2 > 0.6, and preferably not the level of PEEP.

Pulmonary rescue: in case of severe hypoxemia, defined as a drop in SpO_2 below 88% or a drop in PaO_2 below 7.3 kPa, common causes such as a mucus plug requiring pulmonary toilet should be considered and treated, the FiO_2 level is increased up to 1.0 and the PEEP level is set back at 5 cm H_2O or more, both to a level left to the discretion of the attending physician. After solving the cause for the drop in SpO_2 or PaO_2, the PEEP level is again 'down–titrated', following the same steps as described above. Development of atelectasis, or increases in the amount of atelectasis is not necessarily a reason for using a higher PEEP level, unless the SpO_2 drops below 92% or the PaO_2 drops below 8 kPa, and does not respond to increases in FiO_2 to maximal 0.6. If a patient develops ARDS, according to the Berlin definition for ARDS,[36,37] the level of PEEP should always be increased to 10 cm H_2O, or more.

Hemodynamic rescue: in case a patient becomes hemodynamic unstable, meaning that more inotropes and/or vasoactive agents are needed, hemodynamic compromise due to increases in atelectasis could be considered. Then, for a short period of time (e.g., for 1 to 2 hours) the PEEP level can be set at 5 cm H_2O. After solving the hemodynamic problem, the PEEP level is again 'down–titrated'.

5.3 The 'liberal PEEP'–arm

Directly after start of invasive ventilation the PEEP level is set at 8 cm H_2O with a FiO_2 between 0.21 and 0.6. The goal is to ventilate the patient mainly at this level of PEEP till tracheal extubation. For this, the operator will increase the level of PEEP, if a level of < 8 cm H_2O was used, to 8 cm H_2O in one single step (see **Figure 1**). Thereafter, ventilation continues with the PEEP level at 8 cm H_2O using a FiO_2 of between 0.21 and 0.6. In case the SpO_2 drops below 92% or the PaO_2 drops below 8 kPa, first FiO_2 is increased to maximum 0.6 before the level of PEEP is further increased.

Patients are weaned of the ventilator (see: weaning) and tracheally extubated using a PEEP level of 8 cm H_2O. However, during pulmonary toileting and tracheal suctioning, bronchoscopic procedures, intra– or inter–ICU transport or any maneuver during which 'pre–oxygenation' with high FiO_2 is deemed beneficial, ICU nurses are allowed to increase the FiO_2 > 0.6, and preferably not the level of PEEP. If preferred,

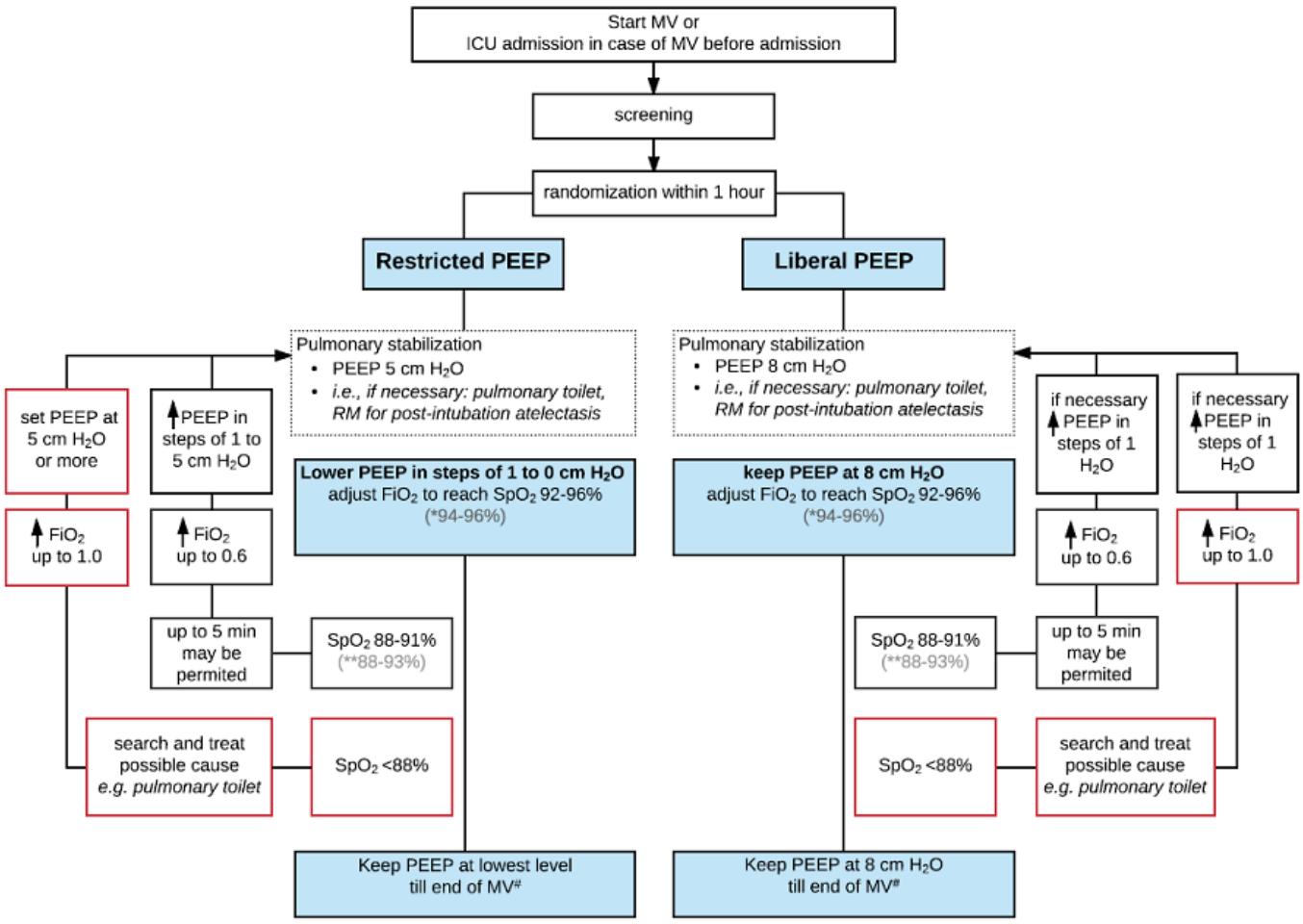
the level of PEEP can be set at 5 cm H_2O for one to two hours directly before tracheal extubation, left to the discretion of the attending physician.

Pulmonary rescue: in case of severe hypoxemia, defined as a drop in SpO_2 below 88% or a drop in PaO_2 below 7.3 kPa, common causes such as a mucus plug requiring pulmonary toilet can be considered and treated, FiO_2 level is increased up to 1.0 to a level left to the discretion of the attending physician, if necessary the PEEP level can be increased. After solving the cause for the drop in SpO_2 or the drop in PaO_2, FiO_2 and the level of PEEP is set back.

Hemodynamic rescue: in case a patient becomes hemodynamic unstable, meaning that more inotropes and/or vasoactive agents are needed, hemodynamic compromise due to increases in overdistension could be considered. Then, for a short period of time (e.g., for 1 to 2 hours) the PEEP level can be set at 5 cm H_2O. After solving the hemodynamic problem, the level of PEEP is again set back to 8 cm H_2O.

The goal is to ventilate patients in this arm with a PEEP level of 8 cm H_2O and only to adjust the PEEP level when deemed necessary. This reflects current ventilation practice in the Dutch setting, where the PEEP level is further increased to improve oxygenation, but decreased in case of hemodynamic compromise (see **Figure 1**).

Figure 1. Flowchart ventilator settings in the 'restricted PEEP'–arm and in the 'liberal PEEP'–arm



* = 94-96%, ** = 88-93%, higher oxygen targets are allowed for patients who develop:
1) ongoing cardiac ischemia due to cardiac infarction and failed revascularisation
2) delayed cerebral ischemia after subarachnoid hemorrhage
3) increased and uncontrollable intracranial pressure (> 18 mmHg)
4) necrotizing fasciitis
5) severe untreatable anemie, such as in case of Jehovah's Witnesses
#, short periods of PEEP at 5 cm H_2O are allowed to evaluate whether the patient can be extubated

Abbreviations: PEEP, positive end–expiratory pressure; MV, mechanical ventilation; PBW, predicted body weight; ARDS, acute respiratory distress syndrome.

In APPENDIX III a few patient examples are shown to clarify and explain the proposed ventilation strategy in the 'restricted PEEP'–arm.

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6. STANDARD TREATMENT OF SUBJECTS

6.1 Standard ventilatory management

The RELAx trial allows the following ventilatory modes: volume–controlled or pressure–controlled ventilation, and pressure support ventilation. Automated modes, in particular those that automatically change the PEEP level and FiO_2, are never allowed.

With volume–controlled and pressure–controlled ventilation the inspiration–to–expiration ratio is set at 1:2. With volume–controlled ventilation the inspiration time and pause are set at 25% and 10%, respectively. With pressure support ventilation, the highest possible pressure rise is chosen and cycling off is set at 25%.

Tidal volume size is between 6–8 ml/kg predicted body weight (PBW), which is calculated according to the following formula[38] 50 + 0.91 x (centimeters of height – 152.4) for males and 45.5 + 0.91 x (centimeters of height – 152.4) for females. The respiratory rate is adjusted to obtain a normal arterial blood pH (7.35 to 7.45). In case of metabolic acidosis or alkalosis, a lower or higher than normal $PaCO_2$ can be accepted, which is left to the discretion of the attending physician. Recruitment maneuvers are allowed when deemed necessary, but the decision to perform a recruitment maneuver is also left to the discretion of the attending physician.

6.2 Oxygenation targets

The oxygenation target ranges for SpO_2 and PaO_2 are 92% to 96%, and 8 kPa to 11.5 kPa, respectively.[39-43] Oxygenation will be maintained in the target ranges primarily by adjusting the FiO_2, which is typically set between 0.21 and 0.6. The oxygenation target is primarily assessed by peripheral saturation (SpO_2) as measured by pulse oximetry and only in case of unreliable reading the oxygenation will be assessed by the arterial blood oxygen pressure (PaO_2).

For patients in whom the risk of potentially dangerous hypoxemia could be become unacceptable during the trial (e.g., in patients who develop: ongoing cardiac ischemia due to cardiac infarction and failed revascularization, delayed cerebral ischemia after subarachnoid hemorrhage, increased and uncontrollable intracranial pressure (of ≥ 18 mmHg), necrotizing fasciitis or severe untreatable anemia such as with Jehovah's Witnesses), the oxygenation target ranges can be increased to SpO_2 and PaO_2 of 94% to 96%, and 9 kPa to 11.5 kPa, respectively.

6.3 Ventilator settings when a patient develops ARDS

In case a patient develops ARDS, ventilation should be continued according to existing guidelines for patients with ARDS. This at least consists of low tidal volumes (6 ml/kg PBW or lower), and higher PEEP levels (10 cm H_2O or higher). Also, a low driving pressure could be considered.

6.4 Ventilator settings when a patient requires ECMO

In the unlikely event that a patient receives ECMO, the ventilator is set according to the local protocol for ventilation under ECMO. This means that PEEP is *no longer* titrated according to the study protocol.

6.5 Weaning

In all patients who receive assist ventilation, three times a day it should be tested whether the patient accepts assist ventilation; this should also be tried when the patient shows respiratory muscle activity during assist ventilation.

The attending physician decides when to tracheally extubate a patient, based on general extubation criteria (i.e. responsive and cooperative, adequate cough reflex, adequate oxygenation with $FiO_2 \leq 0.4$, hemodynamically stable, no uncontrolled arrhythmia and a rectal temperature > 36 Celsius and after successfully passing a spontaneous breathing trial (SBT) with a T–piece *or* ventilation with minimal support (pressure support level < 10 cm H_2O) and $FiO_2 \leq 0.4$. In case SBTs are used, an SBT is judged as successful when the following criteria are met for at least 30 minutes, the attending physician takes the final decision for extubation:

- Respiratory rate < 35/min
- Peripheral oxygen saturation > 90%
- Increase < 20% of Heart rate and blood pressure
- No signs of anxiety and diaphoresis

In case a patient needs to be re–intubated and ventilated, the PEEP level is set as described above.

6.6 Tracheostomy

Early tracheostomy has no advantage over late tracheotomy.[44] Therefore, tracheostomy is only to be performed on strict indications and preferably not earlier than 10 days after intubation. Strict indications for tracheostomy:

- Expected duration of ventilation > 14 days

- Glasgow Coma Score < 7 and/or inadequate swallow or cough reflex with retention of sputum
- Severe ICU–acquired weakness
- Repeated respiratory failure after extubation
- Pre–existent diminished pulmonary reserves
- Failure to intubate
- Prolonged or unsuccessful weaning

Weaning with a tracheostomy follows recommendations as described under 'weaning', a suggested scheme for unassisted ventilation with a tracheostomy is described in APPENDIX II.

6.7 Sedation protocol

Sedation follows the local guidelines for sedation in each participating unit. In general, these guidelines favor the use of analgo–sedation over hypno–sedation, use of bolus over continuous infusion of sedating agents, and the use of sedation scores.

Nurses determine the level of sedation at least 3 times per day. The adequacy of sedation in each patient is evaluated using a Richmond Agitation Sedation Scale (RASS).[45,46] A RASS score of –2 to 0 is seen as adequate sedation. The goals of sedation are to reduce agitation, stress and fear; to reduce oxygen consumption (heart rate, blood pressure and minute volume are measured continuously); and to reduce physical resistance to– and fear of daily care and medical examination. Patient comfort is the primary goal.

Level of pain is determined using scales such as Numeric Rating Scale (NRS), Visual Analogue Scale (VAS), Critical Care Pain Observation Tool (CCPOT) or Behavioral Pain Scale (BPS).

6.8 Non–ventilatory management

6.8.1 Selective oropharyngeal– or digestive tract decontamination

To prevent nosocomial infections, selective oropharyngeal decontamination (SOD) or selective decontamination of the digestive tract (SDD) is performed in all patients who are expected to need ventilation for longer than 48 hours, and/or are expected to stay in ICU for longer than 72 hours.[47]

6.8.2 Thrombosis prophylaxis

Thrombosis prophylaxis is indicated for all patients who are not treated with anticoagulants, e.g. for therapeutic reasons or systemic prophylaxis because of an

implanted device or extracorporal circulation like for renal replacement therapy. Thrombosis prophylaxis will be given according to local guidelines.

6.8.3 Fluid regimens

A fluid balance targeted at normovolemia and a diuresis of ≥ 0.5 ml/kg/hour should be maintained. Crystalloid infusions are preferred over colloid infusions.

6.8.4 Nutrition

A hypo–caloric, protein–rich diet (1.2–1.7 gr/kg bodyweight /24 hours) is started as soon as possible after ICU admission. Enteral nutrition with a feeding gastric tube is preferred over intravenous feeding. If stomach retention occurs, a duodenal tube can be used if administration of prokinetic drugs is not sufficient, according to local guidelines. When optimal protein intake cannot be reached within 4 days, additional parenteral nutrition can be started.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter

The primary endpoint is the number of ventilator–free days and alive at day 28, defined as the number of days from day 1 to day 28; the patient is alive and breathes without assistance of the mechanical ventilator, if the period of unassisted breathing lasted at least 24 consecutive hours.

7.1.2 Secondary study parameters

Secondary study parameters include:

- ICU length of stay (LOS)
- Hospital LOS
- ICU mortality
- Hospital mortality
- 90–day mortality
- Incidence of development ARDS (APPENDIX I)
- Incidence of severe hypoxemia (APPENDIX I)
- Incidence of severe atelectasis, if a chest radiograph is obtained (APPENDIX I)
- Rescue therapies for severe hypoxemia or severe atelectasis
    - Recruitment maneuver (APPENDIX I)
    - Prone positioning
    - Bronchoscopy for opening atelectasis
- Incidence of pneumothorax, if a chest radiograph is obtained or other kind of imaging suitable for diagnosing pneumothorax is obtained (APPENDIX I)
- Incidence of pneumonia (APPENDIX I)
- The level of PEEP in the ‘restricted PEEP’–arm and the ‘liberal PEEP’–arm
- Days with use of hemodynamic support, defined as the number of ICU days with any use of vasopressors/inotropes for > 1 hour on a day
- Days with use of sedation, defined as the number of ICU days with any use of sedatives for > 1 hour on a day
- Therapeutic intervention scoring system (TISS)/ Nursing Activities Score (NAS)

7.1.3 Other study parameters

Health care related costs will be estimated from the health systems perspective over the time horizon of this trial. Costs include costs of ventilation, costs of stay in ICU, costs of stay in hospital, costs of use of inotropes and vasopressors, costs of use of sedatives, costs of use of tracheostomies, costs of ventilator–associated pneumonia. Costs will be determined for both PEEP arms during the 28 days follow up period after initial ICU admission. These are used to calculate incremental cost per mechanical ventilation–day avoided.

Lung ultrasound (LUS): within 12 hours after enrolment in the RELAx study, after 24-48 hours after enrolment and within 24 hours after detubation, a LUS will be performed to monitor changes in lung aeration. This is only done in patients admitted to the AMC (see appendix IV: RELAxLUS).

Cardiac ultrasound (ECHO): 24-48 hours after enrolment in the RELAx study, a transthoracic echocardiography (TTE) will be performed to assess the cardiac function. This is only done in a total of 68 patients admitted to the AMC (see appendix V: RELAxECHO).7.2 Randomization, blinding and treatment allocation

Randomization will be performed using a dedicated, password protected, SSL–encrypted website. Randomization sequence is generated by a dedicated computer randomization software program, ALEA, using random block sizes (4, 6, up to maximal 8). Due to the nature of the treatment, blinding is not possible.

Patients are randomly assigned in a 1:1 ratio to the ‘restricted PEEP’–arm or to the ‘liberal PEEP’–arm of this trial.

7.3 Study procedures

Patients in participating intensive care units (ICU) are screened and randomized within 1 hour after start of mechanical ventilation. Demographic data of all screened patients, regardless of meeting the enrollment criteria will be recorded (age, gender, expected duration of ventilation > or < than 24 hours).

The oxygenation target ranges for SpO_2 and PaO_2 are 92% to 96%, and 8 kPa to 11.5 kPa, respectively.[39-43] Oxygenation will be maintained in the target ranges primarily by adjusting the FiO_2, which is typically set between 0.21 and 0.6. The oxygenation target is primarily assessed by SpO_2, as measured by pulse oximetry and only in case of discrepancy unreliable reading the oxygenation will be assessed by the PaO_2. Therefore, no extra arterial blood gasses need to be obtained, besides the normally, 3–4 daily conducted arterial blood samples.

7.4 Data collection

- On admission and within the first 24 hours:
  - Gender and age (male + years)
  - Height and weight (cm + kg)
  - Reason for ICU admission
  - Reason for ventilation support
  - Cause of respiratory failure
  - APACHE II score and SAPS II score
- Respiratory status, on admission, and every day at a fixed time point until day 28:
  - Intubation status (if extubated: time of extubation)
  - Tracheostomy status (if tracheostomized: time of tracheostomy)
  - Invasiveness of ventilation (invasive, non–invasive, or intermittent ventilation via tracheostomy)
- Location of patient, every day at a fixed time point until day 28, and at day 90 (in ICU, hospital, other facility, or home) and life status (alive of deceased)
- Pulmonary complication, every day at a fixed time point until day 28 or discharge from ICU, whatever comes first:
  - ARDS (yes or no) (APPENDIX I)
  - Severe hypoxemia (yes or no) (APPENDIX I)
  - Pneumonia (yes or no) (APPENDIX I)
  - Severe atelectasis (yes or no) (APPENDIX I)
  - Pneumothorax (yes or no) (APPENDIX I)
- Need for rescue therapies for severe hypoxemia or severe atelectasis, every day at a fixed time point until day 28 or discharge from ICU, whatever comes first
  - Recruitment maneuver (yes or no) (APPENDIX I)
  - Prone positioning (yes or no)
  - Bronchoscopy for opening atelectasis (yes or no)
- Days with use hemodynamic support, every day at a fixed time point until day 28 or discharge from ICU, whatever comes first. Defined as the number of ICU days with any use of vasopressors/inotropes use for > 1 hour on a day (yes or no)
- Days with use of sedation, every day at a fixed time point until day 28 or discharge from ICU, whatever comes first. Defined as the number of ICU days with any use of sedatives for > 1 hour on a day (yes or no)

- ICU–acquired weakness, every day until day 28 or discharge form ICU, whatever comes first: Medical Research Council (MRC) score (APPENDIX I)[48]

7.4.1. Other data to be collected

- Mechanical ventilation parameters, 1 hour before and 1 hour after randomization and every day at a fixed time point until liberation from the ventilator:
  - Mode of ventilation
  - Tidal volume
  - Respiratory Rate
  - Level of positive end–expiratory pressure (PEEP, cm H_2O)
  - Peak and plateau pressures, or level of pressure support (level above PEEP, and maximal airway pressure, cm H_2O)
  - Inspiration to expiration ratio
  - Inspired oxygen fraction (%)
  - Minute volume (liters/minute)
- Respiratory parameters, 1 hour before and 1 hour after randomization, and every day at a fixed time point until liberation from the ventilator:
  - Peripheral oxygen saturation (%)
  - End–tidal fractions CO_2 (kPa)
  - PaO_2 (kPa)
  - $PaCO_2$ (kPa)
  - Arterial bicarbonate (mmol/L)
  - Arterial pH
  - Arterial base excess (mmol/L)
- Non–respiratory parameters, every day at fixed time point until liberation from the ventilator:
  - Cumulative fluid balance (ml)
  - Transfusion of blood products (type and ml)
  - Infusion of colloids (type and ml)
  - Infusion of (artificial) colloids (type and ml)
  - Sequential Organ Failure Assessment score (SOFA) score
  - Extra pulmonary infection, sepsis, re–operation, cardiac arrest
  - Therapeutic intervention scoring system (TISS)/ Nursing Activities Score (NAS)

7.5 Withdrawal of individual subject

Subjects can leave the trial at any time for any reason if they wish to do so without any consequences.

7.6 Follow up of subject withdrawn from the study

Patients withdrawn from the trial will not be subjected to follow up.

7.7 Replacement of individual subjects when deferred consent could not be obtained

When deferred consent is not obtained after randomization and provisional inclusion of a patient, the randomized subject will be replaced. In the randomization log these cases will be recorded without patient–specific data. The randomization subjects will be replaced in order to retain properly distributed randomization groups.

In the sample size calculation, a dropout rate of 10 % has been taken into account.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the trial if there is sufficient ground that continuation of the trial will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The trial will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

8.2 Secondary endpoints for safety

Since we compare two ventilation strategies that are currently used in standard care, additional risks are not expected. Furthermore, the study population consists of critically ill patients, with a high incidence of death or life–threatening events due to the severity of their illness (the hospital mortality in ventilated ICU patients is 21% [34]). Therefore, we propose to report the secondary endpoints of this trial, which incorporate ventilation specific complications, in a line listing two times per year to the METC to monitor safety of both treatment strategies. The METC will receive a line listing of the secondary endpoints incorporating ventilation specific ventilation complications (see below). These endpoints will be specified per study arm in the line listing without disclosing the specific arms.

Those ventilation specific complications include:

- ICU mortality
- Incidence of development of ARDS
- Incidence of severe hypoxemia
- Incidence of rescue therapy for severe hypoxemia and/or severe atelectasis:
    - Recruitment strategies
    - Prone positioning
    - Bronchoscopy for opening atelectasis

8.3 Data Safety Monitoring Board (DSMB)

An DSMB will be installed to monitor safety and the overall conduct of the trial. The DSMB will compose of 4 individuals who will be invited, one of which will be the chairman.

- The DSMB will first meet after inclusion of the first 150 patients, approximately 6 months after the first patient is enrolled.

- Subsequent to this meeting the DSMB will meet virtually every 6 months
- The DSMB will review the overall status of the program, number of patients enrolled overall and in each center, adherence to the protocol overall and by each center.
- The DSMB will monitor safety of both ventilation strategies by monitoring the secondary endpoints of ventilation specific complications.
- The following DSMB individuals will be invited:
    - I. Martin-Loeches, MD PhD, St James's University Hospital, Dublin, Ireland
    - P. Severgnini, MD, Universita degli Studi dell'Insubria, Varese, Italy
    - F. van Haren, MD PhD, Canberra Hospital, Garran, Australia
    - Prof. A. Artigas, MD PhD, Hospital de Sabadell, Sabadell, Spain

The report and/or advice of the DSMB will only be sent to the sponsor of the study, the Academic Medical Center. Should the sponsor decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

9. STATISTICAL ANALYSIS

9.1 General considerations

The statistical analysis will be based on the intention–to–treat principle. In addition, we will perform a per–protocol analysis to check for robustness of results. The intention–to–treat analysis includes all patients as randomized regardless of whether they received the randomized treatment or other protocol deviations. Per–protocol group analysis only considers those patients who completed the treatment according to the originally allocated protocol. In this non–inferiority trial we include a superiority, primary effect analysis. If the non–inferiority criterion is satisfied, a secondary analysis of the primary endpoint for superiority will be conducted. When appropriate, statistical uncertainty will be expressed by the 95% confidence levels. P–values of 0.05 are used for statistical significance. All statistical analysis will be performed with the R version 3.3.2.

9.2 Primary study parameter

The primary outcome is the number of ventilator–free days and alive at day 28 after ICU admission. The null hypothesis entails that ventilation with the ‘restricted PEEP’–arm is inferior by a margin of 10% to ventilation with the ‘liberal PEEP’–arm. If the 95% CI upper bound for inferiority of the ‘restricted PEEP’–arm is < 10%, the null hypothesis of inferiority is rejected. If the non–inferiority criterion is satisfied, then a secondary analysis of the primary endpoint for superiority will be tested. We will use an appropriate nonparametric analysis method to evaluate the confidence interval for the difference between the two medians of the ventilator–free days from both PEEP arms. Additionally, time to freedom from mechanical ventilation is expressed with Kaplan–Meier curves. Differences between both PEEP arms will be analyzed using the log–rank test.

9.3 Secondary study parameter(s)

Continuous normally distributed variables will be expressed by their mean and standard deviation or, when not normally distributed, as medians and their interquartile ranges. Categorical variables will be expressed as frequencies and percentages. Differences between groups in continuous variables will be analyzed with Students t–test or if continuous data is not normally distributed, the Mann–Whitney U test will be used. Categorical variables will be compared with the Chi–

squared test or Fisher's exact test, as appropriate. Time–dependent data will be expressed with Kaplan–Meier curves.

9.4 Cost–effectiveness analysis

Alongside the proposed RCT a prospective economic study will be performed. The economic evaluation primarily focuses on the possible gained benefits of ventilation with the lowest possible PEEP versus ventilation with the PEEP level currently practiced and the associated healthcare costs within 28 days (the primary outcome of the RCT).

Incremental Cost Effectiveness Ratios (ICER) will be calculated by extra costs per TISS/NAS point, a valuable score reflecting workload and resource utilization in daily ICU practice**.**[49,50] Cost calculations will be based on actual performance and resource use in routine ICU care during the study follow–up period.

9.4.1 Cost–analysis and time horizon of the analysis

Cost categories and overall costs will be compared between both ventilation strategies and where relevant, differences will be calculated, inclusive of 95% confidence intervals. Additional costs as a result of comorbid conditions will be excluded. The economic evaluation will be set–up as a cost–effectiveness analysis (CEA). The time horizon will be limited to the short–term follow–up (i.e., 28–days, 90–days). With this time horizon no discounting of costs and effects will be performed.

9.4.2 Measurements

The prospective cost evaluation will primarily focus on health care utilization (direct medical costs). The direct medical costs include the costs of all procedures and units associated with the ventilation strategies (e.g. fluids, vasopressors, sedatives, and ventilator days, ICU and hospital days). Health care utilization will be extracted from the hospital information system, hospital databases (e.g., the National Intensive Care Evalution (NICE) score, see www.stichting–nice.nl), case record forms (CRFs), financial reports, and patient files. Health service resource use and costs of both ventilation strategies will be measured from a health service and (if relevant) societal perspective. Protocol driven costs will be excluded.

9.4.3 Unit costs

Costs are defined as the volumes of used resources multiplied by calculated unit prices. For the evaluation of health care utilization standard prices published in the

current Dutch costing guidelines and market prices will be used. Standard guideline prices will be used (e.g., diagnostic interventions, hospital admissions).[51]

9.4.4. Statistical analysis of Cost–effectiveness

As most volumes of resource use follow a skewed distribution, differences between the two ventilation strategies will be statistically evaluated with bias–corrected bootstrap analysis.[52] Incremental cost–effectiveness ratio will be calculated with the registered TISS/NAS–score as performance and effect parameter. The economic analysis will be expanded with a scenario–analysis to extrapolate the consequence of implementation and actual performance of the ventilation strategy with 'restricted PEEP' in the target population. The validity of the developed scenarios will be explored in a sensitivity analysis changing cost estimates and probabilities.

9.5 Budget Impact Analysis (BIA)

A budget impact analysis (BIA) will be designed and executed according to the ISPOR guidelines.[53,54] The BIA will evaluate the nationwide economic/financial consequences of the adoption of treating non–ARDS patients at the ICU with ventilation with the lowest possible PEEP level or ventilation with the currently practiced PEEP level in the future. The analysis will be based on the decrease in ICU costs (e.g. ventilator–free days and alive at day 28) as estimated during the study. Registered data will be used, reflecting the size and characteristics of the eligible population in the Netherlands, the current and the new treatment mix, the effectiveness of ventilation with the currently practiced PEEP level and resource use and costs for the applied strategies and related side–effects. The BIA will be conducted from the perspective of the health care providers. When relevant, budget impact analysis is generated as a series of scenario analysis.

Additional sensitivity analysis will be performed on the price of the intervention and the diffusion rate from the hospital perspective.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This trial will be conducted according to the principles of the Declaration of Helsinki as stated in the current version of Fortaleza, Brazil, 2013 and in accordance with the Medical Research Involving Human Subjects Act (WMO).

10.2 Recruitment and consent

10.2.1 Deferred consent

For this trial we ask for deferred consent and we appeal to the emergency procedure for consent in medical research as stated in article 6, paragraph 4 of the WMO, as in a presently running trial of ventilation in a similar patient cohort, the 'protective ventilation in patients not fulfilling the consensus definition for moderate or severe ARDS at start of ventilation – PReVENT, a randomized controlled trial (METC 2014_075)[58], for reasons as explained below.

In patients admitted for ventilatory support to the ICU mechanical ventilation is needed urgently – consequently, mechanical ventilation starts right at ICU admission, or very short thereafter. The injurious effects of ventilation, however, could harm the lungs within hours and as such affect patient outcomes (see **Text box 3 – Ventilation has the potential to harm the lungs – even after a short period of ventilation**). For this reason, we consider it of utmost importance to set the ventilator according to the strategies of interest as soon as possible (i.e., within 1 hour after ICU admission, if ventilation started before admission), or within 1 hour after intubation and start of ventilation, if ventilation started after admission) – not doing so would largely reduce validity of this trial.

Patients admitted for ventilatory support to the ICU are, without exception, incompetent to give informed consent. Persons who may take the role of legal representative in accordance with the WGBO are: a predefined representative, husband or wife, registered partner or other life partner, a parent or child, brother or sister, and incidentally a curator appointed the judge. However, obtaining informed consent from a legal representative in this situation usually takes much time, even by an experienced research team (see **Textbox 4 – Experiences with deferred consent in critically ill patients**). Reasons include the absence of a legal representative at time of intubation and start of ventilation, and early after admission

to the ICU the legal representatives are far more concerned about the wellbeing of the patient then participation in a trial.[55,56]

For these reasons, we opt for using deferred consent, where informed consent from a legal representative must be obtained as soon as possible, but always within 48 hours after randomization. If informed consent is not obtained, or if a legal representative denies participation within the time window of 48 hours, the patient is excluded and data will no longer be used. Thenceforth the patient is ventilated according to the policy of the attending physician.

Textbox 3 - **Ventilation has the potential to harm the lungs – even after a short period of ventilation**

Ventilation can harm the lungs, even after a short period of ventilation. If a patient, in the proposed trial, is already ventilated for several hours, injurious effects of ventilation could already be in place, largely reducing validity of the trial outcomes. From experimental animal studies we know that mechanical ventilation can cause effects within hours of ventilation with a high PEEP level.[57] These findings are in line with results from clinical studies, showing ventilator-related effects after relative short periods of ventilation, e.g. after ventilation during general anesthesia for surgery.[58] A recent randomized controlled trial of patients undergoing cardiac surgery with hypoxemia, comparing a ventilation strategy including a PEEP level of 8 cm H_2O with a ventilation strategy with a PEEP level of 13 cm H_2O, showed an important effect of mechanical ventilation on the incidence of postoperative pulmonary complications [59]

Textbox 4 – Experiences with deferred consent in critically ill patients

Most critically ill patients who need ventilation cannot be approached for informed consent for a study at ICU admission. Indeed, those patients are usually in severe respiratory distress, sedated or in coma. A prospective observational study on study recruitment practices in critically ill patients performed by a respected and experienced research group in Canada showed that the time from recognizing study eligibility to obtaining informed consent by a legal representative was as high as 12 hours, even while time from recognition to the first contact with a legal representative was as short as 2 hours.[55]

The experience of ICU patients enrolled under deferred consent is mainly positive. To investigate contentment of patients that were included using deferred consent, a questionnaire was designed for – and distributed under the participants of the large NICE–SUGAR trial[56], a trial compared a strict blood glucose control strategy with one that accepts higher blood glucose levels.[57] Of the responders (79% of all participants), a large majority (96%) said to have granted consent if they would have been asked. A large majority (93%) mentioned they were happy with the decision made by the representative at the moment they were incapable of giving informed consent.[57]

This is in line with our personal experience from the PReVENT trial (METC 2014_075),[58] a currently ongoing RCT in ventilated ICU patients without ARDS in The Netherlands, a study that compares two other ventilation strategies. From the PReVENT study we learned that it is very well possible to inform legal representatives about the trial within 24 hours. However due to longer travel distances for some of the legal representatives, obtaining written informed consent was sometimes not possible within the 24 hours: in as many as 19 out of 174 patients (11%) this was a reason for exclusion of the patient. Interestingly, informed consent could have been obtained within 48 hours in all these cases.

10.2.2 Ethical aspects

We can underpin the idea of 'clinical equipoise'.[60] Ventilation strategies with lower PEEP levels (sometimes even no PEEP) and higher PEEP levels have been used over the last decades in patients without ARDS, and we actually do not know what the best PEEP level in these patients is. A recent observational study in ventilation

practice in ICU patients shows that a median PEEP level of 8 cm H_2O is used in patients without ARDS in the Netherlands, and a medium level of 6 cm H_2O is used in the European cohort.[33]

10.2.3 No deferred consent in patients who die before obtaining informed consent

In case a patient dies before informed consent could be obtained from the legal representative, we propose to use the data and inform the legal representative about the research without obtaining informed consent. This in in line with the advice from Jansen and colleagues regarding ethical validity and practical feasibility of deferred proxy consent in emergency critical care research and in line with the advice of the Central Committee on Research Involving Humans (CCMO, the Dutch national Ethics Committee) in these circumstances in the early lactate–directed therapy in the ICU.[56,61]

The CCMO judged that the situation when a patient dies before consent could be obtained is comparable with the situation in which the research project has already finished at the time deferred consent can be obtained. They concluded that the legal representative should be notified about the study, but that seeking consent was not useful anymore due to the lack of consequences. The representation of the patient by a legal representative ends when the patient dies. In the Dutch law, the consent of the patient or his/her relative primarily relates to the participation in the study and not to using the data collected in the study. [56]

10.2.4 Conclusion deferred consent

Critically ill patients in need of ventilation are, without exception, incapable to give informed consent at the moment of ICU admission. Obtaining informed consent from a legal representative takes too much time to allow timely start of the ventilation strategies to be compared in this trial. Timely start is essential due to the risk of the injurious effects on the lungs even after a short period of ventilation not following protocol and thereby reducing the validity of the trial. Both ventilation strategies to be compared in this trial have been used in the last decades, and we do not know what the best PEEP level is.

10.3 Benefits and risks assessment, group relatedness

Burden and risks of the ventilation strategies are uncertain. Ventilation with the lowest possible PEEP level could increase the risk of atelectasis and also the risk of potentially dangerous hypoxemia. Ventilation with the PEEP level currently practiced could increase the amount of overdistended lung tissue and increase hemodynamic compromise. Both ventilation strategies are currently used; there is no additional risk for patients enrolled in this study compared to current practice.

We specifically chose not to exclude incompetent patients for two reasons. First, critically ill patients needing mechanical ventilation should be considered incompetent due to their needs for continuous sedation. Second, the strategies to be compared in this study are to be used in critically ill, intubated and ventilated patients. These conditions are not present in patients who are not suffering from a critical disease. We therefore consider it impossible not to include these patients in a study comparing strategies for mechanical ventilation.

10.4 Compensation of injury

The sponsor/investigator has a liability insurance, which is in accordance with article 7 subsection 6 of the WMO. As this study compares two ventilation strategies used for standard care an exception from the requirement for insurance to cover for damage to research subjects through injury or death caused by the study is applicable.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

All patients will be addressed to the inventions with a random patient identification code. The codebook will be stored digitally and in paper. The paper version will be stored behind a lock and the digital form will be encrypted with a double password. All data will be stored for the length of the study and for 15 years afterwards. All handling of personal date will comply with the Dutch Personal Data Protection Act.

11.2 Monitoring and Quality Assurance

Queries on the database will be done by a statistician and analyzed by the monitor to signalize early aberrant patterns, trends, issues with consistency of credibility and other anomalies.

On site monitoring will compromise controlling presence and completeness of the research dossier and the informed consent forms, source data checks will be performed as described in the monitoring plan. Every participating center will be visited after the inclusion of the first ten patients and thereafter at least once every year. A monitoring plan is being developed.

11.3 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All substantial amendments will be notified to the METC and to the competent authority. Non–substantial amendments (typing errors and administrative changes) will not be notified to accredited METC and the competent authority, but will be recorded and filed by the sponsor.

11.4 Annual progress report

The investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, unexpected problems and amendments

11.5 End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the 90^{th} day after the last patients inclusion in the study. In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the

investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

11.6 Public disclosure and publication policy

The study protocol will be registered before inclusion of the first patient on Clinicaltrials.gov. The results of the study will find their way into (inter–)national scientific journals and guidelines. We will submit analyses to scientific journals in the field of intensive care medicine as well as anesthesiology, since both ICU physicians and anesthesiologists apply ventilation in the ICU setting.

12. PUBLICATION POLICY

The PROVENet policy will be followed for publication. The intention is to publish the paper by the PROVE Network investigators. This means that there will be no names of individual researchers above a publication. The Principal Investigator is mentioned as the contact person, the members of the Steering Committee, the Writing Committee, and all local investigators of participating centers are summarized at the end of a manuscript or in the appendix depending on the journal policy. In this way http://www.ncbi.nlm.nih.gov/pubmed/ can link the names of all investigators to a publication. If a journal does not accept this, another approach will be discussed within the Steering Committee, and an explanation and conclusion will be posted on the website of the project.

From each participating center in the RELAx trial one local investigator per participating center will be on the authors list for publication. When a participating center includes more than the anticipated 82 patients per center, a second local investigator will be added to the authors list for publication. In case a participating center includes more than 164 patients, a third local investigator will be added to the authors list for publication.

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APPENDIX I

Table 1. The Berlin definition for ARDS [36,37]

Timing	Within 1 week of a known clinical insult, or new/worsening respiratory symptoms		
Chest imaging*	Bilateral opacities—not fully explained by effusions, lobar/lung collapse, or nodules		
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload; need objective assessment (e.g., echocardiography) to exclude hydrostatic edema if no risk factor present		
Oxygenation	Mild	Moderate	Severe
	200 < PaO_2/ FiO_2 ≤ 300 mmHg	100 < PaO_2/ FiO_2 ≤ 200 mmHg	PaO_2/ FiO_2 ≤100 mmHg
	26.7 < PaO_2/ FiO_2 ≤ 40 kPa with PEEP ≥ 5 cm H_2O or CPAP ≥ 5 cm H_2O	13.3 < PaO_2/ FiO_2 ≤ 26.7 kPa with PEEP ≥ 5 cm H_2O	PaO_2/ FiO_2 ≤ 13.3 kPa with PEEP ≥ 5 cm H_2O

*Chest radiograph or CT scan; **If altitude higher than 1000 m, correction factor should be made as follows: PaO_2/ FiO_2 9 (barometric pressure/760)

Abbreviations: ARDS, acute respiratory distress syndrome; PaO_2, partial pressure of arterial oxygen; FiO_2, fraction of inspired oxygen; PEEP, positive end–expiratory pressure; CPAP, continuous positive airway.

DEFINITIONS

- APACHE (Acute Physiology and Chronic Health Evaluation) II: a point score ranging from 0–71, calculated from 12 measurements (age, temperature (rectal), mean arterial pressure, pH, heart rate, respiratory rate, sodium (serum), potassium (serum), creatinine, hematocrit, white blood cell count, GCS) higher scores correspond to more severe disease and higher risk of death
- MRC (Medical Research Council): grades strength in functional muscle groups in each extremity, ranging 0–5, a score of 5 corresponds to normal – healthy strength
- Pneumonia: new or progressive radiographic infiltrate plus at least two of the following: fever tympanic temperature > 38,5, leukocytosis or leucopenia and/or purulent secretions

- Pneumothorax: air in the pleural space with no vascular bed surrounding the visceral pleura on chest radiograph or other kind of imaging suitable for diagnosis severe atelectasis
- Recruitment maneuver: increase of inspiratory pressure or the level of PEEP for at least 40 seconds
- SAPS (Simplified Acute Physiology Score) II: point score ranging from 0–163, as APACHE
- Severe atelectasis: at least complete lobar atelectasis of a lung on chest radiograph or other kind of imaging suitable for diagnosis severe atelectasis
- Severe hypoxemia: SpO_2 < 88% or < PaO_2 7.3 kPa more than 5 minutes or a rise of the oxygen fraction > 60% for more than 5 minutes related to a hypoxemic event

APPENDIX II

SCHEME FOR UNASSISTED VENTILATION WITH TRACHEOSTOMY

The following suggested scheme can be used for unassisted ventilation with a tracheostomy, but should be individualized in every patient:

1. Unassisted ventilation for 30 minutes, three times per day
2. Unassisted ventilation for 1 hour, three times per day
3. Unassisted ventilation for 2 hours, three times per day
4. Unassisted ventilation for 4 hours, three times per day
5. Unassisted ventilation for 6 hours, two times per day
6. Unassisted ventilation for 18 hours
7. Unassisted ventilation for 24 hours

APPENDIX III

PATIENTS EXAMPLES FOR CLARIFICATION VENTILATION WITH 'RESTRICTED PEEP'–ARM

Patient A is intubated and ventilated due to decreased level of consciousness as a result of intoxication with presumed GHB. Patient A fulfills the inclusion criteria and is included in the RELAx study and randomized to the 'restricted PEEP'–arm. The ventilation is started with a PEEP level of 5 cm H_2O and FiO_2 of 0.4, the saturation is stable and remains SpO_2 > 94%. Following the flowchart, the oxygenation target range is reached and stable, hence the PEEP level can be 'down-titrated' with increments of 1 cm H_2O with reassessment of the saturation every 15 minutes following each adjustment of the PEEP level. The PEEP level is successfully 'down-titrated' to a PEEP level of 0 cm H_2O with a SpO_2 93%. Since the oxygenation target range is reached and stable, the attending physician is able to decrease the FiO_2 level from 0.4 to 0.21.

Patient B is a trauma patient with a flail chest, and is intubated and ventilated due to respiratory insufficiency. Patient B is a candidate for the RELAx study and is randomized to the 'restricted PEEP'–arm. Ventilation is started with a PEEP level of 5 cm H_2O, soon the oxygenation target range is reached and the PEEP level is successfully 'down-titrated' to 0 cm H_2O with a FiO_2 of 0.3 while maintaining the oxygenation target (SpO_2 > 92%). The admission is complicated by a ventilator acquired pneumonia (VAP) and purulent secretion is noticed, treatment with antibiotics is started. On the fifth day of admission, suddenly the saturation drops to SpO_2 88%. The FiO_2 is increased to 0.6 and the PEEP level was set back at 5 cm H_2O. Since lots of purulent secretion was removed earlier that day, a mucus plug is considered and the attending physician performs a recruitment maneuver successfully with improvement of oxygenation (SpO_2 93%). During reassessment, the saturation remains stable and within the oxygenation target range, therefore the PEEP level can be 'down-titrated' again.

Patient C is admitted due to a respiratory infection. Patient C is intubated due to respiratory insufficiency which developed the same day and is admitted to the ICU. Patient C is eligible for the RELAx study and is randomized to the 'restricted PEEP'–arm. Ventilation is started with a PEEP level of 5 cm H_2O and a FiO_2 of 0.5, the saturation is SpO_2 92%. Attempts for 'down-titration' of the PEEP level are unsuccessful and therefore the PEEP level and the FiO_2 remains unchanged. However, that afternoon the SpO_2 drops to 88%, the FiO_2 is increased to 0.6 and the PEEP level of 5 cm H_2O is maintained. During reassessment, the oxygenation target range is not reached and consequently adjustments are made with increasing the FiO_2 and the PEEP level further, until 10 cm H_2O and 0.8.

A chest radiograph is obtained with the appearance of bilateral infiltrates. Patient C is clinically diagnosed with ARDS, since the respiratory failure cannot be explained by cardiac failure or fluid overload. Ventilation is continued according to the existing ARDS guidelines.

APPENDIX IV

Substudy – 'RELAxECHO'

Background

Cardiac function, in particularly of the right ventricle, depends on intrathoracic pressures[1,2]. Use of positive end–expiratory pressure (PEEP) could increase right atrial pressure, pulmonary vascular resistances and right ventricular afterload[3-5] . The net effect of PEEP may be a decrease in right ventricle (RV) volume and output, with no changes in ejection fraction [3]. One small study showed a negative effect of high PEEP on right ventricular strain[6], a surrogate measure of contractility. It is uncertain whether low PEEP has an independent effect on right ventricle myocardial strain. The myocardial performance index (MPI) is regarded as an easy and reproducible echocardiographic parameter of both systolic and diastolic function. The MPI is relatively independent of changes in loading conditions in various clinical settings [8-11]. The RELAx study provides a unique opportunity to study cardiac performance and especially the performance of the right ventricle during varying levels of PEEP (between 0 and 8 cm H2O) in patients with uninjured lungs.

Aim

The aim of RELAxECHO, a substudy of the RELAx study, is to assess and compare changes in cardiac function as measured by transthoracic echocardiography (TTE) in the two study groups.

Hypothesis

We hypothesize that ventilation with liberal PEEP decreases right ventricular function after 24-48 hours of mechanical ventilation.

Endpoint

The primary endpoint of this sub study is the myocardial performance index of the right ventricle in the first 24-48 hours of mechanical ventilation.

In– and exclusion criteria

Inclusion criteria:

- Admitted to the ICU of the Academic Medical Center
- Enrolled in the RELAx study

Exclusion criteria:

- Ventilation with PEEP > 2 cm H_2O in the ‘restricted PEEP’–arm and ventilation with PEEP < 7 cm H_2O in the ‘liberal PEEP’–arm
- Refractory circulatory instability requiring > 5 µg/kg/min dopamine or dobutamine, > 1 mg/hour milrinone, or norepinephrine dose of > 0.4 µg/kg/min
- Documented poor left ventricular function (e.g. left ventricular ejection fraction ≤ 30%)

Original sample size calculation

We estimated 28 patients in each study group to achieve a power of 80%, with a two–sided significance level of 0.05, to detect a 0.06 difference in change in myocardial performance index between ventilation with restricted PEEP (defined as a PEEP ≤ 2 cm H_2O) and ventilation with liberal PEEP (defined as a PEEP ≥ 7 cm H_2O), assuming a standard deviation of 0.08. The sample size is increased by 20% to correct for dropouts (i.e., if myocardial performance index cannot be determined from the TTE due to poor echogenicity), meaning that a total of 68 patients are required. The decision about the sample size is based upon the consideration that the quantity of PEEP has an effect on right ventricular function [6]. Differences in right ventricular function are expressed in the myocardial performance index, which is a parameter known to be relatively load–independent.

Sample size re-calculation

Based on the results of a recent study in a similar patient cohort, showing a much larger decrease of 0.23 in myocardial performance index with lower tidal volume reduction, [7] the sample size was recalculated on 12 November 2019 as follows. With a still conservative effect size on MPI of the right ventricle of 0.12 (an effect size half the size of the previous study [7]), and a mean MPI of the right ventricle of 0.41 and a standard deviation of 0.13, we need 18 patients in each study group to detect a difference of 0.12 in MPI of the right ventricle with PEEP reduction with 80% power with a two–sided significance level of 0.05. The sample size is increased by 20% to correct for dropouts, meaning that a total of 44 patients (22 per group) are required.

Methods

Cardiac ultrasound is performed within 24 to 48 hours after enrollment in the RELAx study. The cardiac echocardiography will be performed by trained physicians under supervision of cardio-intensivists, will perform the echocardiography, using the GE Healthcare Vivid 9 ultrasound machine with a 2–5 MHz sector probe. Traditional

echocardiographic measures, tissue Doppler imaging (TDI) and speckle tracking echocardiography (STE) parameters will be collected online and with post-acquisition offline analysis[12]. Images of the ventricles are analyzed offline for the myocardial performance index, strain and strain rate and diastolic parameters. Ultrasound clips will be saved for further offline STE analysis and quality control. Measurements will be performed after at least 5 minutes of stable mean arterial pressure. Bidimensional and Doppler measurements will be made in accordance with current recommendations of the American Society of Echocardiography[13].

Statistical analysis

Normally distributed variables are expressed by their mean and standard deviation; non–normally distributed variables are expressed by their medians and interquartile ranges. Categorical variables will be expressed as n (%). To test groups of continuous normally distributed variables, Student's t–test will be used. Likewise if continuous data is not normally distributed the Mann–Whitney U test will be used. Categorical variables will be compared with the Chi–square test or Fisher's exact tests or when appropriate as relative risks. Statistical significance is considered to be at a p–value of 0.05. Where appropriate, statistical uncertainty will be expressed by 95% confidence levels. Analysis will be performed with R (www.r-project.org).

Informed consent

Deferred informed consent from a legal representative is obtained as soon as possibly for this sub study as part of the parent study RELAx. In case a patient is awake and adequate informed consent will be obtained from the patient.

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APPENDIX V

Substudy – 'RELAxLUS'

Background

Ventilation with low PEEP may increase the risk of atelectasis in critically ill patients receiving invasive ventilation, as has been shown before in patients undergoing intraoperative ventilation (1, 2). Lung ultrasound (LUS) is a non–invasive relatively simple bedside technique used to semi–quantify changes in lung aeration in ventilated patients (3), and very capable to detect atelectasis (4).

Aim

The aim of RELAxLUS, a substudy of the RELAx study, is to assess and compare changes in pulmonary aeration and presence of atelectases as detected by LUS in the two study groups.

Hypothesis

We hypothesize that ventilation with restricted PEEP results in a decrease in lung aeration and an increase in atelectases.

Endpoint

The primary endpoint of this sub study is the change in lung ultrasound aeration score in the first 48 hours of invasive ventilation.

In– and exclusion criteria

Inclusion criteria:

- Admitted to the ICU of the Academic Medical Center
- Enrolled in the RELAx study

Exclusion criteria:

- Evidence of cardiac failure or fluid overload, based on an objective assessment such as echocardiography in the medical record and/or on judgment of the treating physician

Methods

LUS is performed at three predefined time points: within 12 hours after enrolment in the RELAx study (this LUS examination is standard of care in patients that are expected to need invasive ventilation > 24 hours), between 24 to 48 hours after enrolment and within the first 24 hours after extubation. Experienced and trained physician will perform LUS examinations, using a 2–5 MHz convex probe. Each

hemithorax is divided into six areas: the anterior, lateral and posterior areas, each divided in upper and lower quadrants, using the parasternal line, the anterior axillary line, the posterior axillary line and the paravertebral line as borders (Figure 1). The 12 regions are examined and a semi–quantitative score is calculated to estimate lung aeration at each time point, and documented in a case report form (see Table 1). Additional sonographic signs previously described for atelectasis will be reported when present for each of the 12 lung regions examined. These include the absence or reduction in lung sliding, the presence of subpleural consolidations and presence of static air bronchograms in consolidated areas (5).

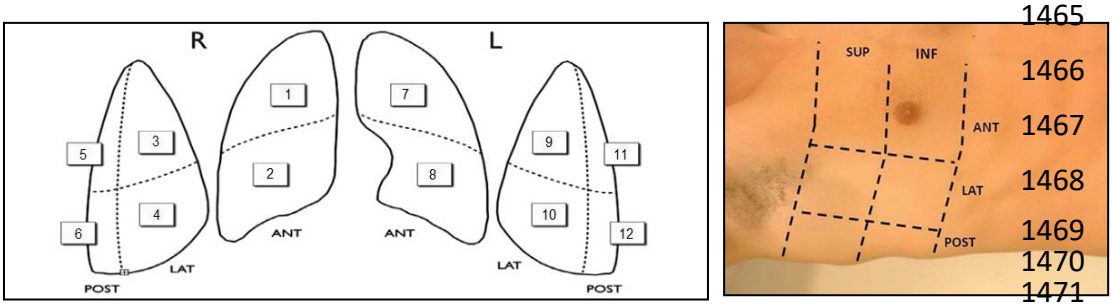

Figure 1. Six zones are scanned per hemithorax.

Table 1. LUS aeration score

Pattern	Score	View	Interpretation
A	0	Only A lines visible or isolated ≤2 B–lines	Normal lung aeration
B1	1	Multiple well-defined either regularly spaced or irregularly spaced B–lines	Moderate loss of lung aeration
B2	2	Multiple coalescent B–lines	Severe loss of lung aeration
C	3	Hypoechoic or tissue–like area	Consolidated lung tissue

Informed consent

Written informed consent is obtained for the two extra LUS examinations as part of the informed consent for the parent study (RELAx), i.e., the one between 24 and 48 hours after enrolment, and the one within the first 24 hours after extubation, as the first LUS examination is standard of care in these patients.

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APPENDIX VI

Substudy – 'RELAxBiomarkers'

Background

Mechanical ventilation has a strong potential to inflame and damage lung tissue. Plasma level of several markers of inflammation and lung damage, including tumor necrosis factor (TNF)–alpha, Interleukin (IL)–6 and IL–8, the soluble form of the Receptor for Advanced Glycation End–products (sRAGE), Surfactant Protein (SP)–D, Clara Cell protein (CC)–16 and Krebs von den Lungen 6 (KL6), have been shown to rise in response to intraoperative ventilation and depending on ventilator settings used [1]2. Plasma levels of these biomarkers also rise in response to mechanical ventilation using large tidal volumes [2]. The RELAx trial offers the unique opportunity to study the dependence of plasma levels of biomarkers of inflammation and lung damage on the level of PEEP used during the first week of mechanical ventilation in patients with uninjured lungs.

Aim

The aim of RELAxBiomarkers, a substudy of RELAx, is to describe and compare changes in plasma levels of biomarkers of inflammation and pulmonary injury.

Hypothesis

We hypothesize that ventilation with liberal PEEP, compared to ventilation with restricted PEEP, increases plasma levels of biomarkers of inflammation and pulmonary injury.

Endpoints

The endpoint of this substudy is the difference in plasma levels of biomarkers of inflammation and pulmonary injury between the two study groups.

In- and exclusion criteria

Inclusion criteria

- Admitted to the ICU of the Academic Medical Center
- Enrolled in the RELAx study

Exclusion criteria

- Receiving immunosuppressive medication

Methods

Blood sampling and handling

Left–over blood from arterial blood samples used for arterial blood gas analysis, taken as part of standard of care in the morning, will be collected within 12 to 16 hours after enrolment in the RELAx study, and thereafter till day 7 or until ICU discharge, whichever comes first.

Blood samples are centrifuged at 2,000 rpm for 15 minutes. Supernatant is collected and stored at –80^{O}C until batchwise analysis, using customized Luminex kits for measurements of biomarkers of inflammation and lung injury, including TNF–alpha, IL–6, IL–8, sRAGE, SP–D, CC–16, and KL6.

Statistical analysis

Variables are expressed in mean plus standard deviation, or medians plus interquartile ranges where appropriate. Categorical variables are expressed as proportions. Student's t and Mann–Whitney U test are used depending on distribution of data. Categorical variables will be compared with the Chi–square test or Fisher's exact tests or when appropriate as relative risks. Statistical significance is considered to be at a p–value of 0.05. Where appropriate, statistical uncertainty will be expressed by 95% confidence levels. All analysis will be performed with R (www.r-project.org).

Informed consent

Written informed consent for the use left–over blood from arterial blood samples is asked as part of the informed consent for the parent study, RELAx.

References

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