Influence of respiratory rate and end-expiratory pressure variation on cyclic alveolar recruitment in an experimental lung injury model

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Abstract

Introduction: Cyclic alveolar recruitment/derecruitment (R/D) is an important mechanism of ventilator-associated lung injury. In experimental models this process can be measured with high temporal resolution by detection of respiratory-dependent oscillations of the paO2 (ΔpaO2). A previous study showed that end-expiratory collapse can be prevented by an increased respiratory rate in saline-lavaged rabbits. The current study compares the effects of increased positive end-expiratory pressure (PEEP) versus an individually titrated respiratory rate (RRind) on intra-tidal amplitude of Δ paO2 and on average paO2 in saline-lavaged pigs.

Methods: Acute lung injury was induced by bronchoalveolar lavage in 16 anaesthetized pigs. R/D was induced and measured by a fast-responding intra-aortic probe measuring paO2. Ventilatory interventions (RRind (n = 8) versus extrinsic PEEP (n = 8)) were applied for 30 minutes to reduce Δ paO2. Haemodynamics, spirometry and Δ paO2 were monitored and the Ventilation/Perfusion distributions were assessed by multiple inert gas elimination. The main endpoints average and Δ paO2 following the interventions were analysed by Mann-Whitney-U-Test and Bonferroni’s correction. The secondary parameters were tested in an explorative manner.

Results: Both interventions reduced Δ paO2. In the RRind group, ΔpaO2 was significantly smaller (P < 0.001). The average paO2 continuously decreased following RRind and was significantly higher in the PEEP group (P < 0.001). A sustained difference of the ventilation/perfusion distribution and shunt fractions confirms these findings. The RRind application required less vasopressor administration.

Conclusions: Different recruitment kinetics were found compared to previous small animal models and these differences were primarily determined by kinetics of end-expiratory collapse. In this porcine model, respiratory rate and increased PEEP were both effective in reducing the amplitude of paO2 oscillations. In contrast to a recent study in a small animal model, however, increased respiratory rate did not maintain end-expiratory recruitment and ultimately resulted in reduced average paO2 and increased shunt fraction.

Keywords: acute lung injury, cyclic alveolar recruitment, porcine model, respiratory- dependent paO2 oscillations, varying shunt fractions

Introduction

Due to a wide variety of pulmonary and extrapulmonary aetiologies, acute lung injury (ALI) and the acute respiratory distress syndrome, as defined by the American European Consensus Conference in 1994 [1], remain major challenges in modern critical care medicine. A mortality reduction was achieved within the past 10 to 15 years and accompanied by rapid progress in assessing and understanding the underlying mechanisms. Nevertheless, mortality rates of more than 30% are still described [2,3]. Mechanical ventilation is commonly regarded as the cornerstone strategy in ALI, although various other supportive therapies are known to have a beneficial effect [4,5]. However,
the optimal ventilatory setting in ALI is still controversially discussed. Moreover, an additional injury to the pre-injured lung, defined as ventilator-associated lung injury (VALI), can result from mechanical ventilation. Systemic or pulmonary inflammation caused by mediators that are released within the lung through injurious ventilation (biotrauma) is the consequence. Lung protective ventilation strategies aiming to minimize baro- and volutrauma by limitation of inspiratory pressure and tidal volume led to improved survival outcome and are accepted as the current standard therapy [6,7]. In addition to volutrauma (stretch injury), atelectrauma (cyclic alveolar recruitment and derecruitment, R/D), is another major component of VALI. Within the injured lung, inspiratory pressure can lead to an opening of collapsed lung areas, which immediately re-collapse in expiration. This breath by breath collapse is associated with shear-stress and inflammation and, therefore, promotes the pre-existing injury of lung tissue [8]. Cyclic R/D has recently been the focus of research studies, particularly in terms of clinical detection and treatment [9,10]. Variations of the pulmonary shunt fraction caused by cyclic R/D that can induce respiratory-dependent oscillations of the arterial partial pressure of oxygen (paO2) were described by Baumgardner et al. by means of ultrafast, invasive measurement of the paO2 [11]. The occurrence and amplitudes of paO2 oscillations (ΔpaO2) can be used to quantify the extent of cyclic R/D [10]. Theoretically, positive end-expiratory pressure (PEEP) that exceeds the lower inflection point of the static pressure-volume-curve should effectively maintain end-expiratory recruitment, minimize cyclic R/D induced biotrauma and improve gas exchange. However, limitations due to over-distension of non-dependent lung areas leading to volutrauma or circulatory depression may override these benefits [12-14]. Furthermore, PEEP does not respond to the dynamic behaviour and the distinct time constants underlying R/D of atelectasis [15,16]. Other experimental strategies to maintain end-expiratory recruitment include, shortening expiration times or an increased respiratory rate (RR) [11,17]. We hypothesised that: (1) a goal-directed RR and increased PEEP can both reduce the amplitude of intratidal Δ paO2 to less than 50 mm Hg; and (2) RR and PEEP adjusted to minimize intratidal Δ paO2 result in equivalent average lung recruitment by avoiding end-expiratory collapse. Hence, this study aims to compare the effectiveness of high extrinsic PEEP and an individually titrated RR (RRind) as ventilatory interventions in a porcine ALI model designed to create a maximum of cyclic R/D. Furthermore, effects on haemodynamics, oxygenation and ventilation/perfusion (V/Q) distribution are characterised.

Materials and methods
After approval by the State and Institutional Animal Care Committee (Landesuntersuchungsamt Rheinland-Pfalz, Koblenz, Germany; approval number: 23177-07/G09-1-029), 16 juvenile pigs (German country race, weight 25 to 27 kg) were investigated in a prospective-randomised study. Two pilot experiments were conducted for set up and feasibility of the protocol.

Anaesthesia and preparation
Following sedation by an intramuscular injection of ketamine (8 mg kg⁻¹) and midazolam (0.2 mg kg⁻¹) and vascular access via an ear vein, anaesthesia was induced by intravenous (i.v.) administration of propofol (4 mg kg⁻¹), fentanyl (4 μg kg⁻¹) and a single dose of pancuronium (0.15 mg kg⁻¹) to facilitate endotracheal intubation. Anaesthesia was maintained by a continuous i.v. infusion of propofol (8-12 mg kg⁻¹ h⁻¹) and fentanyl (0.1 to 0.2 mg h⁻¹) for the entire experiment. After orotracheal intubation using a standard (internal diameter 7.5 mm) endotracheal tube, pressure-controlled ventilation (Ventilator: Servo 900C, Siemens, Erlangen, Germany) was initiated with a tidal volume of 10 to 12 mg kg⁻¹, PEEP of 5 mbar, fraction of inspired oxygen (FiO2) of 0.3 to 0.4, inspiration to expiration ratio (I:E) of 1:2 and a variable RR to maintain normocapnia. A balanced saline solution was infused continuously (5 ml kg⁻¹ h⁻¹).

The following vascular catheters were placed by surgical cut-down: an arterial line and a PiCCO® catheter (Pulsion Medical Systems, Munich, Germany) via the left and right femoral arteries, respectively; a central venous line via a femoral vein; an introducer for a pulmonary artery catheter (Edwards Lifescience, Irvine, CA, USA) via the right external jugular vein; an arterial introducer for ultrafast measurement of the paO2 via the right carotid artery. Spirometry and haemodynamic parameters were continuously recorded by a Datex S/5 unit (Datex Ohmeda GmbH, Duisburg, Germany). To ensure correct catheter positioning the typical pressure waveforms of the arterial, pulmonary arterial and central venous pressure were obtained and referenced to the mid-chest level. A Rapidlab 248 device (Bayer Healthcare, Leverkusen, Germany) was applied for arterial and mixed venous blood gas analyses. Body temperature was measured by a rectal probe and body surface warming was performed by a heating blanket system. Cardiac output (CO) was assessed by single-indicator transpulmonary thermodilution using the PiCCO® system. Three repetitive i.v. injections of 10 ml cooled (< 8°C) balanced saline solution were given for each calibration.
Ultrafast paO₂ measurement

Through the right carotid introducer, a fiberoptic probe (FOXY-AL300; Ocean Optics, Dunedin, FL, USA) measuring paO₂ based on oxygen-sensitive fluorescence quenching with a time resolution up to 10 Hz, was inserted into the ascending aorta. The probe was calibrated in vitro according to a previously reported routine [9,18]. The validity of the calibration was confirmed in vivo by repetitive blood gas analyses. For ultrafast data visualisation and storage, a dedicated acquisition software was used (OOI Sensors; Ocean Optics, Dunedin, FL, USA).

Multiple Inert Gas Elimination Technique (MIGET)

The novel micropore membrane inlet mass spectrometry (MMIMS)-MIGET was used for assessment of the V/Q distribution. An additional shunt calculation from distribution was determined according to

$$\frac{V}{Q} \cdot \frac{Q}{V} = 1.0$$, high

$$0.1 < \frac{V}{Q} < 10$$, normal

$$\frac{V}{Q} > 100$$.

Lung injury model and baseline measurements

Baseline measurements were conducted in a healthy state and in ALI. The FiO₂ was set and maintained at 1.0 before the baseline. ALI was induced via repetitive bronchoalveolar lavages: the endotracheal tube was clamped in inspiration, 30 ml kg⁻¹ of warmed balanced saline solution were instilled by gravity and afterwards immediately removed. Bronchoalveolar lavages were repeated to achieve a paO₂/FiO₂ ratio ≤ 300 mmHg at a PEEP of 5 mbar for 30 minutes. A fluid optimisation routine of 50 to 100 ml of hydroxyethyl starch was added, if the mean arterial pressure (MAP) persisted < 60 mmHg after ALI induction.

Experimental protocol

Following baseline measurements in ALI the animals were randomised into two intervention groups: 1) increase of the PEEP to 10 to 15 mbar (PEEP group) and 2) individually titrated respiratory rate (RRind group).

The respirator settings were set up to peak inspiratory pressure (Ppeak) 40 mbar, zero PEEP, RR 5 to 8 minutes⁻¹ and I:E of 1:4 in pressure-controlled mode to provoke maximal cyclic R/D (time point R/D I). The inability to cause cyclic R/D with the dedicated ventilator settings was defined as obligatory criterion for exclusion. ΔpaO₂ values with a stable amplitude ≥ 50 mmHg were accepted as the pathophysiological correlate of considerable R/D in order to clearly exceed the reported variations that occur in healthy, anaesthetized animals [10,11,21]. Thereafter, the randomised intervention (Figure 1) was initiated to terminate cyclic R/D under continuous ultrafast paO₂ measurement. In the PEEP group, extrinsic PEEP was immediately raised up to at least 10 mbar. Further increases were allowed up to a maximum of 15 mbar if Δ paO₂ was > 20 mmHg and if the MAP was > 60 mmHg. Intrinsic PEEP was also measured dynamically as the difference between total and extrinsic set PEEP in both groups (as automated by a S/5 unit, Datex Ohmeda GmbH, Duisburg, Germany). The RR was titrated individually until a Δ paO₂ < 20 mmHg under real-time conditions was achieved. The peak and end-inspiratory airway pressures (Ppeak, Pendinsp) were held constant to the pre-intervention value in both groups. If haemodynamic instability, defined by a MAP < 60 mmHg, occurred after initiation of the interventions, i.e. epinephrine was administered continuously. The Δ and average paO₂ values were reassessed 10, 20 and 30 minutes following the intervention. Spirometry and haemodynamic data were stored. Thereafter, the initial ventilator settings were set again to restore cyclic R/D (time point R/D II) and evaluate the influence of both interventions on the amount of recruitable lung tissue. MIMMS-MIGET measurements were performed during baseline and after 30 minutes of intervention. After completing the protocol the animals were euthanized in general anaesthesia by lethal injection of propofol (200 mg) and potassium chloride (40 mmol).

Statistical methods

For a description of the continuous variables’ distribution, the median and interquartile range (IQR) are given. Additional descriptive data are reported as mean and standard deviation (± SD). The intergroup difference of the main endpoints Δ and average paO₂ following the respective intervention was analysed using the mean of the
measurements over the different time points for every pig. These mean values were compared using Mann-Whitney-U-Tests. To account for multiple testing a simple Bonferroni correction was used for the two main endpoints. The multiple significance level was set at 5%. Haemodynamic data and MMIMS-MIGET derived $V/Q$ after 30 minutes were assessed as secondary endpoints in an explorative manner. The resulting $P$-values are descriptive only. All statistical analyses were performed using the statistical software R version 2.13.1.

**Results**

A total of 16 animals were included in the study. One animal died during ALI induction and was replaced by an additional animal. In the baseline measurements before provocation of R/D, acute lung injury (paO$_2$/FiO$_2$ < 300 mmHg) was documented in both groups. The applied respirator settings and blood gas analyses are summarised in Table 1.

**Cyclic recruitment and oxygenation**

Cyclic R/D measured by respiratory-dependent oscillations of the paO$_2$ was provoked in every animal. A median $\Delta$ paO$_2$ of 78 (IQR = 22) mmHg in the PEEP group and 72 (IQR = 26) mmHg in the RR$_{ind}$ group was observed. The corresponding median paO$_2$ values were 288 (IQR = 68) mmHg (PEEP group) and 372 (IQR = 94) mmHg (RR$_{ind}$ group). These differences were not statistically significant ($\Delta$paO$_2$: $P = 0.462$; paO$_2$: $P = 0.130$). As a result of the defined protocol for adjusting RR and PEEP, the RR$_{ind}$ intervention consisted of a titration to a RR of 15 ± 1 minute$^{-1}$, while in the PEEP group the PEEP increased up to 13 ± 2 mbar. In both groups an immediate reduction of the $\Delta$ paO$_2$ was achieved (Figure 2). The $\Delta$ paO$_2$ reduction was more
pronounced in the RRind group while in the PEEP group a higher Δ paO2 persisted over 30 minutes (P_adjusted < 0.001 after Bonferroni correction). The application of high PEEP induced an increase in the average paO2. In the RRind group the reduction of Δ paO2 was in contrast accompanied by a large decrease in average paO2 over 30 minutes (Figure 3; P_adjusted < 0.001 after Bonferroni correction). At the end of every experiment the original settings to provoke R/D were repeated to restore R/D (R/D II). In the PEEP group the readjustment to the initial respiratory setting (Table 1) induced a higher Δ paO2 (101 (IQR = 59) mmHg) than recorded before the PEEP increase. In the RRind group the Δ paO2 was 58 (IQR = 53) mmHg, while in four animals the Δ paO2 was < 50 mmHg despite a P_peak increase up to 45 ± 7 mbar. Hence, the PEEP intervention led a higher amount of recruitable lung tissue, while the RRind induced an increase of non-recruitable, fixed atelectasis.

Analysis of the ventilation/perfusion distribution

The perfusion-based V/Q analysis showed healthy conditions (normal V/Q: 96 ± 5% in the PEEP group; 90 ± 14% in the RRind group). A sustained impairment occurred following ALI induction before induction of Δ paO2 in both groups (normal V/Q: 65 ± 11% versus 74 ± 14%). After 30 minutes of intervention (Figure 4) the PEEP application led to pulmonary recruitment corresponding to the reported paO2 values: shunt fraction of 3% (IQR = 4), low V/Q 0% (IQR = 0.2), normal V/Q 96% (IQR = 4) and high V/Q 1% (IQR = 1). Compared to the RRind group (shunt 15% (IQR = 6), low V/Q 24% (IQR = 9), normal V/Q 52% (IQR = 15), high V/Q 3% (IQR = 6)), a considerably improved overall lung function defined by the MMIMS-MIGET was witnessed. Venous admixture was calculated from blood gas data at baseline and after initiation of the respective interventions, but not during presence of high respiratory-dependent oscillations of the paO2 due to high fluctuations.

Table 1 Ventilatory parameters and blood gas analyses (mean ± SD)

| Parameters | PEEP group | R/D I | Intervention Initial | Intervention 10 to 30 minutes | R/D II | RRind group | R/D I | Intervention Initial | Intervention 10 to 30 minutes | R/D II |
|------------|------------|------|---------------------|-----------------------------|-------|-------------|------|---------------------|-----------------------------|-------|
| **Ventilation** | | | | | | | | | | |
| P_peak (mbar) | 25 ± 4 | 40 ± 4 | 40 ± 4 | 40 ± 4 | 40 ± 5 | 20 ± 3 | 38 ± 5 | 37 ± 3 | 38 ± 3 | 45 ± 7 |
| P_end (mbar) | 23 ± 5 | 35 ± 4 | 35 ± 4 | 35 ± 4 | 37 ± 7 | 19 ± 3 | 34 ± 4 | 33 ± 3 | 34 ± 3 | 40 ± 9 |
| P_mean (mbar) | 11 ± 1 | 14 ± 3 | 21 ± 3 | 22 ± 3 | 14 ± 3 | 10 ± 1 | 13 ± 3 | 13 ± 3 | 13 ± 3 | 15 ± 4 |
| RR (min⁻¹) | 28 ± 5 | 6 ± 1 | 6 ± 1 | 6 ± 1 | 6 ± 1 | 27 ± 3 | 6 ± 1 | 15 ± 2 | 17 ± 3 | 6 ± 1 |
| PEEP (mbar) | 5 ± 1 | 0 | 13 ± 1 | 13 ± 2 | 0.3 ± 0.2 | 5 ± 1 | 0 | 0.1 ± 0.1 | 0.1 ± 0.2 | 0 |
| V_t (ml kg⁻¹) | 10 ± 1 | 35 ± 8 | 24 ± 5 | 24 ± 6 | 41 ± 9 | 10 ± 1 | 36 ± 7 | 32 ± 7 | 32 ± 7 | 28 ± 6 |
| FiO₂ | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| T_ins (s) | 0.7 ± 0.2 | 2.0 ± 0.4 | 2.0 ± 0.4 | 1.9 ± 0.3 | 2.0 ± 0.4 | 0.8 ± 0.1 | 2.0 ± 0.4 | 0.9 ± 0.1 | 0.8 ± 0.1 | 2.1 ± 0.4 |
| T_exp (s) | 1.5 ± 0.3 | 8.1 ± 1.8 | 7.9 ± 1.7 | 7.6 ± 1.2 | 8.2 ± 1.3 | 1.5 ± 0.3 | 7.9 ± 1.1 | 3.4 ± 0.3 | 3.0 ± 0.5 | 8.3 ± 1.4 |
| **Blood gas analysis** | | | | | | | | | | |
| ph | 7.3 ± 0.1 | 7.3 ± 0.1 | 7.4 ± 0.1 | 7.5 ± 0.1 |
| paO₂ (mmHg) | 199 ± 69 | 539 ± 72 | 219 ± 85 | 163 ± 71 |
| paO₂-Foxy (mmHg) | 187 ± 48 | 307 ± 90 | 500 ± 86 | 353 ± 131 | 223 ± 58 | 372 ± 111 | 381 ± 131 | 174 ± 62 | 209 ± 139 |
| paCO₂ (mmHg) | 62 ± 12 | 57 ± 9 | 55 ± 3 | 32 ± 7 |
| Hb (mg dl⁻¹) | 8 ± 1 | 9 ± 1 | 9 ± 2 | 9 ± 2 |
| BE (mmol l⁻¹) | 2 ± 4 | 2 ± 3 | 3 ± 6 | 4 ± 4 |
| Qs/Qt (%) | 26 ± 6 | 26 ± 5 | 24 ± 5 | 25 ± 6 |

P_peak, mean airway pressure; PEEP_total, PEEP extrinsic + PEEP intrinsic; T_ins, inspiration time; T_exp, expiration time; paO₂-Foxy, paO₂ measured by the ultrafast invasive probe (Foxy-AL 300); Qs/Qt, (%), calculated pulmonary shunt fraction. No blood gases were sampled during presence of high respiratory-dependent oscillations of the paO₂ due to high fluctuations.

**Haemodynamics**

The haemodynamic parameters are summarised in Table 2. To maintain stable testing conditions despite...
the challenging ventilator setting in this R/D model, a decrease of the MAP < 60 mm Hg was treated by continuous i.v. administration of epinephrine. The vasopressor requirement to achieve the defined criterion was higher in the PEEP group \( (P < 0.05) \). No intergroup differences in MAP and CO (each \( P > 0.05 \)) were detected over 30 minutes. The mixed venous oxygen saturation \( (SvO_2) \) decreased over time in the RRind when compared to the PEEP group \( (P < 0.05) \).
Figure 4 Ventilation/Perfusion distribution after PEEP or RR_{ind} intervention. MMIMS-MIGET derived $V/Q$ after 30 minutes of intervention maintained lung recruitment in the PEEP group and impaired pulmonary function in the RR_{ind} group. Intergroup differences: shunt, low and normal $V/Q$ each $P \leq 0.001$, high $V/Q^* = 0.012$ (Mann-Whitney-U-Tests).

Table 2 Haemodynamic data and vasopressor support (median (IQR))

| Parameter                  | PEEP group | RR_{ind} group |
|----------------------------|------------|----------------|
|                            | Baseline   | ALI            | Initial                        | Intervention 10 to 30 minutes | Baseline ALI | R/D I | R/D II | Baseline ALI | R/D I | R/D II | Initial                        | Intervention 10 to 30 minutes |
| MAP (mmHg)                 | 91 (29)    | 73 (18)        | 61 (24)                      | 66 (4)                       | 74 (12)       | 99 (26) | 84 (24) | 78 (34)       | 74 (24) | 61 (4)* |
| CO (l min$^{-1}$)          | 3.7 (1.1)  | 3.7 (1.1)      | 2.9 (0.6)                    | 3.3 (0.7)                    | 3.5 (0.8)     | 3.1 (1.0) |
| SvO$_2$ (%)                | 83 (14)    | 78 (8)*        | 81 (8)                       | 66 (12)*                     |               |         |         |
| Epinephrine ($\mu g$ kg$^{-1}$ min$^{-1}$) | 0          | 0               | 0.2 (0.1)*                  | 0.2 (0.1)*                  | 0            | 0 (0.1)* |

* indicate intergroup difference ($P < 0.05$, Mann-Whitney-U-Test).
In contrast to a prior study [17] and despite the favourable effect on Δ\(\text{paO}_2\), the RR\(_{\text{ind}}\) intervention clearly failed to maintain or increase oxygenation like the PEEP application. These findings are reflected in sustained impairment of the V/Q in the RR\(_{\text{ind}}\) group. The MMIMS-MIGET showed a persistent average shunt fraction in both groups (Figure 4), which was consistent with the conventional shunt calculation (Table 1). Neither MMIMS-MIGET shunt nor conventional calculation, however, offers a dynamic assessment of the pulmonary shunt that is capable of following fast, respiratory-dependent variations. The persistence and intergroup differences of Δ\(\text{paO}_2\) are, therefore, not directly related to the average shunt values. The significantly lower amount of atelectasis in the PEEP group (shunt fraction: 3% (IQR = 4)) is still exposed to high peak pressures and long exhalation times and may, therefore, be recruited cyclically. In the high range of \(\text{paO}_2\), even very small changes in shunt fraction around a mean value of 3% are capable of producing substantial Δ\(\text{paO}_2\) (online supplement of [11]). The persisting Δ\(\text{paO}_2\) of 23 (IQR = 26) mmHg in the PEEP group is, therefore, not inconsistent with the small average shunt of about 3%. In contrast, following the RR\(_{\text{ind}}\) intervention the average \(\text{paO}_2\) is much lower and the average shunt fraction is much larger, but the small oscillations of \(\text{paO}_2\) suggest that very little of the atelectatic lung is recruited cyclically. Therefore, there appears to be less cyclic R/D in the RR\(_{\text{ind}}\) group, compared to the PEEP group.

The optimal level of PEEP in ALI is still controversial and presumably depends on the extent and severity of the underlying injury [13]. Referring to the “baby lung” concept, a forced reopening of all collapsed lung areas may lead to further injury and hyperinflation of the lung parenchyma [24,25]. Optimal parameters to titrate the RR remain unclear since the guidance by Δ\(\text{paO}_2\) alone was effective only in diminution of Δ\(\text{paO}_2\), but resulted in a markedly decreased average \(\text{paO}_2\) and lung recruitment. Although the RR\(_{\text{ind}}\) approach reduces the exhalation time per breath cycle, our results suggest that in this model R/D is limited through RR\(_{\text{ind}}\), not by preventing end-expiratory collapse, but rather by limiting inspiratory recruitment. The RR may play a role as an intervention for cyclic R/D only if gas exchange is maintained by an adequate PEEP.

The slow approach to a steady state average \(\text{paO}_2\) after RR\(_{\text{ind}}\) (over 30 minutes in Figure 3) also supports the assumption that R/D in saline-lavaged pigs is a fairly slow process. Cyclic R/D may not have been completed within a single inspiration or exhalation in this experiment. The initial provocation of cyclic R/D offers comparable findings (Figure 1) in that immediately after R/D
induction there was often a pronounced slow drift in the average paO2.

**Influence of RR and PEEP on haemodynamics**

Especially in a state of compromised haemodynamics, respiratory interventions should avoid negative haemodynamic effects. Syring *et al.* [17] noted a greater CO and SvO2 when the RR was used to prevent end-expiratory collapse. Consistent with this concept the animals in the RRind group required less vasopressor administration ($P < 0.05$). The haemodynamic alterations were regarded as only secondary in the present study. The primary focus was set on maintaining stable conditions for the assessment of respiratory-dependent paO2 variations. It should be noted that the protocol for vasopressor support was based on MAP. The decreases in MAP in the high PEEP group could be associated with left ventricular afterload reduction rather than compromised CO. The SvO2 declined over 30 minutes in the RRind setting despite comparable MAP and CO. However, this may be related to the impaired pulmonary gas exchange and oxygenation rather than an effect of reduced CO.

**Detection of R/D**

Cyclic R/D is not detectable by currently available routine clinical monitoring. Dynamic computed tomography, the current gold standard, offers the highest temporal and spatial resolution to monitor dynamic processes like R/D [26]. However, radiation exposure and the need for patient transport limit the practicability in the critically ill. The indirect method focusing on Δ paO2 and varying shunt fractions by an indwelling, invasive probe is an experimental technology. The concept of detecting R/D indirectly by Δ paO2 may lead to translation to the clinics as related approaches might be developed in the near future [9,27,28].

**Limitations of the study**

The lavage model imitates certain features of clinical ALI, particularly surfactant dysfunction and depletion, but, of course, is not identical with ALI. It is widely used to examine the extent and characteristics of atelectasis and alveolar recruitment [29]. Additionally, our experimental model was designed to produce a mild degree of injury with a small amount of fixed shunt and a large amount of recruitable atelectasis. More severe degrees of injury will require further studies. Finally, our observations were collected over a relatively short period of time, compared to the clinical course of ALI. Although some experimental studies indicated a more severe damage and higher inflammatory response in recruiting and derecruiting lung areas [30,31], the long-term extent and impact on human ALI is yet to be determined.

Two experimental technologies were applied in the present study. The MMIMS is a novel and alternate method of MIGET [32,33]. Only the detection of the inert gases by mass spectrometry differs from the conventional gas chromatographic method. The MMIMS technology is still being evaluated in ongoing studies. In a prior study, the MMIMS-MIGET derived shunt fraction was shown to be highly correlated to the conventionally calculated shunt [19]. The ultrafast paO2 measurement was previously applied and described in detail by several experimental studies [11,17,18,22,30,34]. But a rapidly responding paO2 probe is not yet available for clinical use. One limitation, in the clinical setting, for the use of Δ paO2 as an index of varying shunt fraction is that the oscillation amplitude is blunted at low haemoglobin saturations. Beyond a paO2 of 90 mmHg a complete saturation of the haemoglobin is maintained [35], which allows for direct comparison of average and Δ paO2 values.

The peak inspiratory pressure was held on a constant, high level during interventions in both groups, since the influence of the inspiratory pressure on alveolar recruitment is well known [36-38]. Due to the standardisation of the peak pressure following the intervention, however, the tidal volume differed between the two groups, while in the PEEP group also the mean airway pressure increased (Table 1).

**Conclusions**

The effects of extrinsic PEEP and a titrated RR on varying shunt fractions through cyclic R/D were characterised in a porcine lavage model. Different kinetics of alveolar recruitment were seen compared to previous small animal models.

Respiratory-dependent oscillations of the paO2 were reduced to a higher degree by high respiratory rates than by the feasible PEEP in the present model. The RR titration, however, resulted in a significantly lower average oxygenation and lung recruitment, compared to the use of high PEEP.

**Key messages**

- Cyclic alveolar recruitment measured by respiratory-dependent oscillations of the paO2 regularly occurs in saline-lavaged pigs during non-protective ventilation.
- As single interventions, increased RR and PEEP both significantly reduce respiratory-dependent oscillations of the paO2.
- PEEP maintains end-expiratory recruitment despite persisting amounts of cyclic recruitment, while RR as a single intervention does not maintain sufficient oxygenation and lung recruitment.
Maximal cyclic R/D in this large animal model is induced by prolonged exhalation times, in contrast to the maximal cyclic R/D by prolonged inspiration reported in a small animal model.

Abbreviations

ALI: acute lung injury; BE: base excess (mmol l⁻¹); CO: cardiac output (l min⁻¹); FiO₂: fraction of inspired oxygen; Hb: haemoglobin (g dl⁻¹); IE: Inspiration to Expiration ratio; IQR: interquartile range; i.v.: intravenous; MAP: mean arterial pressure (mmHg); MMWS-MIGET: micropore membrane inlet mass spectrometry - multiple inert gas elimination technique; PaO₂: arterial partial pressure of oxygen (mmHg); PaO₂-Foxy: PaO₂ measured by the FOXY AL; PaO₂-FoxY: PaO₂ measured by the FOXY AL-300 probe (mmHg); PaO₂-D: respiratory-dependent oscillations of PaO₂; PeEP: positive end-expiratory pressure (mbar); P×end-mean: end-inspiratory pressure (mbar); P×mean: mean airway pressure (mbar) P×peak inspiratory pressure (mbar); Q×/Q: calculated pulmonary shunt fraction (%); R/D: cyclic alveolar recruitment and derecruitment; RR: respiratory rate (min⁻¹); RRind: individually titrated RR (min⁻¹); SD: standard deviation; SO₂: mixed venous oxygen saturation; Tinsp: inspiration time; Texp: expiration time; V/Q: ventilation/perfusion distribution; VALI: ventilator-associated lung injury.

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Authors’ contributions

EKH coordinated and supervised the experiments. EKH, SB, AB, BD and KUK carried out the experiments. EKH, BD and SB performed the data analysis. AE conducted the statistical analysis. EKH drafted the manuscript. KM, MD and JEB participated in the study design, supervision of the laboratory and revision of the manuscript. All authors edited and approved the final manuscript.

Competing interests

JEB is the owner of Oscillogy LLC, which commercially distributes the MMWS-MIGET system. JEB participated in set up and design of this study, but had no influence on data acquisition and analysis. None of the other authors declares a conflict of interest.

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