In Individualized positive end-expiratory pressure and regional gas exchange in porcine lung injury

Muders T, Luepschen H, Meier T, Reske A, Zinserling J, Kreyer S, Pikkemaat R, Maripuu E, Leonhardt S, Hedenstierna G, Putensen C, and Wrigge H

**Supplemental Digital Content 7 - *Statistical Analysis and Ethics***

This study was approved by the Animal Research Ethics Committee in Uppsala, Sweden (C*274/7)*, and performed in the Hedenstierna laboratory, Department of Clinical Physiology, Uppsala University Hospital, Uppsala, Sweden. Animal housing, care, and experiments were performed in adherence with the Guide for the Care and Use of Laboratory Animals (National Academy of Science 1996).

The lung injury model allowed rapid changes between states of lung recruitment and enabled repeated measurements in a cross-over design to reduce the number of animals (Reduction). The pigs (males and females, weight: 28.2 ± 2.4 kg; age: 12 ± 1 weeks) were purchased from a local farmer and were derived from several long-standing colonies (swedish country breeds). Animals had free access to food and water until 12 h before the beginning of experiments. Transportation to the lab was performed immediately before the beginning of experiments (8.00 AM). Well established medication schemes 1,2 were used for analgosedation and euthanasia (8.00 PM). We used non-invasive imaging techniques such as EIT and SPECT/CT to evaluate the physiological effects of the intervention (Refinement). Replacement of the pig model by studies in lower developed animals, cell cultures or simulation was not suitable (Replacement).

For this exploratory study setting no reliable pilot data or data from publications were available. Based on experiences with other animal model studies using different ventilatory settings 1,2, it was deemed reasonable to assume differences between levels in the range of 75% of the standard deviation at each level and a correlation of 0.5 between levels. Sample size considerations were referring to statistical tests between two levels of the repeated measures design with three levels. No adjustment for multiplicity was figured in due to the exploratory nature of the comparisons and target power of 80% was used. With a sample size of 15, the test of a single contrast between two PEEP settings at a 0.05 alpha level in a one way repeated measures analysis of variance with 3 levels was found to have 80.3% power to detect a contrast C of 3 (C=∑cᵢμᵢ, scale parameter D=SQRT(∑cᵢ²) of 1.41, with contrast cofficients cᵢ and level means μᵢ), assuming a standard deviation at each level of 4, a between level correlation of 0.5 and an a resulting effect size of 0.752.

All animals finished the whole study protocol. Due to invalid SPECT-raw-data one pig (#1) had to be excluded. Finally, fourteen animals were analyzed. We followed the ARRIVE guideline.

Animals were allocated to one group. Three different PEEP-settings were tested in a cross-over design in a randomized order. Block-wise randomization (blocks of 6) was performed using sealed envelopes.

Primary outcome measures were differences in PEEP levels and following amounts of gas and blood flow to different $\dot{V}$/$\dot{Q}$-compartments.

Data (expressed as mean ± standard deviation) were tested for normal distribution (Sharpiro-Wilks test) and analyzed using two-tailed testing. Differences between “baseline” and “lung injury” were analyzed using paired t-tests. One-way repeated measures analysis of variance (ANOVA) was used to identify differences in cardiorespiratory parameters. Two-way repeated measures analysis of variance (ANOVA) was used to identify differences in lung volumes and masses between the three PEEP-strategies and within-group differences in distribution of gas and blood flow to $\dot{V}$/$\dot{Q}$ compartments and along the ventro-dorsal axis. Post-hoc tests (Newman-Keuls) were performed to separate differences between PEEP-strategies and within single $\dot{V}$/$\dot{Q}$-compartments. Post-hoc tests (two-way repeated-measures ANOVA) were performed to separate differences between PEEP-strategies in regional distributions along the ventro-dosal axis.

Results from blood gas analyses, SPECT- and CT-scans were compared using linear correlation and Bland-and-Altman analysis, when appropriate.

Statistical analyses were performed using STATISTICA for Windows 6.0 (StatSoft, Inc., Tulsa, OK, USA). P<0.05 was considered to be statistically significant.

1. Wrigge H, Zinserling J, Muders T, Varelmann D, Günther U, Groeben C von der, Magnusson A, Hedenstierna G, Putensen C: Electrical impedance tomography compared with thoracic computed tomography during a slow inflation maneuver in experimental models of lung injury. Crit Care Med 2008; 36:903–9

2. Muders T, Luepschen H, Zinserling J, Greschus S, Fimmers R, Guenther U, Buchwald M, Grigutsch D, Leonhardt S, Putensen C, Wrigge H: Tidal recruitment assessed by electrical impedance tomography and computed tomography in a porcine model of lung injury\*. Crit Care Med 2012; 40:903–11