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# Pathophysiological and Histomorphological Effects of One-Lung Ventilation in the Porcine Lung

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**Abstract**
Kozian, A. 2009. Pathophysiological and Histomorphological Effects of One-Lung Ventilation in the Porcine Lung. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 493. 54 pp. Uppsala. ISBN 978-91-554-7647-2.

Thoracic surgical procedures require partial or complete airway separation and the opportunity to exclude one lung from ventilation (one-lung ventilation, OLV). OLV is commonly associated with profound pathophysiological changes that may affect the postoperative outcome. It is injurious in terms of increased mechanical stress including alveolar cell stretch and overdistension, shear forces secondary to repeated tidal collapse and reopening of alveolar units and compression of alveolar vessels. Ventilation and perfusion distribution may thus be affected during and after OLV. The present studies investigated the influence of OLV on ventilation and perfusion distribution, on the gas/tissue distribution and on the lung histomorphology in a pig model of thoracic surgery.

Anaesthetised and mechanically ventilated piglets were examined. The ventilation and perfusion distribution within the lungs was assessed by single photon emission computed tomography. Computed tomography was used to establish the effects of OLV on dependent lung gas/tissue distribution. The pulmonary histopathology of pigs undergoing OLV and thoracic surgery was compared with that of two-lung ventilation (TLV) and spontaneous breathing.

OLV induced hyperperfusion and significant V/Q mismatch in the ventilated lung persistent in the postoperative course. It increased cyclic tidal recruitment that was associated with a persistent increase of gas content in the ventilated lung. OLV and thoracic surgery as well resulted in alveolar damage. In the present model of OLV and thoracic surgery, alveolar recruitment manoeuvre (ARM) and protective ventilation approach using low tidal volume preserved the ventilated lung density distribution and did not aggravate cyclic recruitment of alveoli in the ventilated lung.

In conclusion, the present model established significant alveolar damage in response to OLV and thoracic surgery. Lung injury could be related to the profound pathophysiological consequences of OLV including hyperperfusion, ventilation/perfusion mismatch and increased tidal recruitment of lung tissue in the dependent, ventilated lung. These mechanisms may contribute to the increased susceptibility for respiratory complications in patients undergoing thoracic surgery. A protective approach including sufficient ARM, application of PEEP, and the use of lower tidal volumes may prevent the ventilated lung from deleterious consequences of OLV.

*Keywords:* Alveolar Recruitment Manoeuvre, One-Lung Ventilation, Lung Protective Ventilation, Tidal Volume, Computed Tomography, Tidal Recruitment, Animal Model, Open Thoracic Surgery, Ventilation/ Perfusion Distribution, Single Photon Emission Computed Tomography, Diffuse Alveolar Damage

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*To my daughters Nicola & Melina*

# List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Kozian, A., Schilling, T., Röcken, C., Fredén, F., Maripuu, E., Strang, C., Hachenberg, T., Hedenstierna, G. (2008) One-Lung Ventilation Induces Hyperperfusion and Alveolar Damage in the Ventilated Lung – an Experimental Study. *Br J Anaesth,* 100(4):549-59

II Kozian, A., Schilling, T., Schütze, H., Heres, F., Hachenberg, T., Hedenstierna, G. (2009) Lung Computed Tomography Density Distribution in a Porcine Model of One-Lung Ventilation. *Br J Anaest,*102(4):551-60

III Kozian, A., Schilling, T., Röcken, C., Breitling, C., Hachenberg, T., Hedenstierna, G. (2009) Increased Alveolar Damage after Mechanical Ventilation in a Porcine Model of Thoracic Surgery. *J Cardiothorac Vasc Anesth,* in print

IV Kozian, A., Schilling, T., Schütze, H., Hachenberg, T., Hedenstierna, G. (2009) Alveolar Recruitment Manoeuvre and Low Tidal Volume Ventilation Protect the Dependent Lung during One-Lung Ventilation. *Manuscript*

# CONTENTS

# ABBREVIATIONS

| | |
|---|---|
| ALI | Acute Lung Injury |
| ARDS | Acute Respiratory Distress Syndrome |
| ARM | Alveolar Recruitment Manoeuvre |
| $C_{dyn}$ | Dynamic Compliance |
| $C_{stat}$ | Static Compliance |
| CD45 | CD (Cluster of Differentiation) Surface Antigen 45 |
| CI | Cardiac Index |
| CT | Computed Tomography |
| CVP | Central Venous Pressure |
| DAD | Diffuse Alveolar Damage |
| $DO_2$ | Oxygen Delivery |
| ECG | Electrocardiogram |
| $ETCO_2$ | End-Tidal Carbon Dioxide |
| $FiO_2$ | Fraction of Inspired Oxygen |
| H&E | Haematoxylin & Eosin |
| HR | Heart Rate |
| HU | Hounsfield Units |
| IPPV | Intermittent Positive Pressure Ventilation |
| $^{81m}Kr$ | Krypton Isotope (Ventilation) |
| LCA | Leukocyte Common Antigen |
| MAA | Macro Aggregated Albumin |
| MAC | Minimal Alveolar Concentration |
| MAP | Mean Arterial Pressure |
| MPAP | Mean Pulmonary Arterial Pressure |
| MV | Minute Ventilation |
| OLV | One-Lung Ventilation |
| PAC | Pulmonary Artery Catheter |
| $PaO_2$ | Partial Pressure of Oxygen in arterial Blood |
| $PaCO_2$ | Partial Pressure of Carbon Dioxide in arterial Blood |
| PAOP | Pulmonary Artery Occlusion Pressure |
| Paw | Airway Pressure |
| PEEP | Positive End-Expiratory Pressure |
| Qs/Qt | Venous Admixture / Shunt |
| ROI | Region of Interest |
| RR | Respiratory Rate |
| $SaO_2$ | Arterial Oxygen Saturation |
| $SpO_2$ | Peripheral arterial Oxygen Saturation |

| | |
|---|---|
| $SvO_2$ | Mixed venous Oxygen Saturation |
| SPECT | Single Photon Emission Computed Tomography |
| TBS | TRIS buffered Saline |
| $^{99m}Tc$ | Technetium Isotope (Perfusion) |
| TIVA | Total Intravenous Anaesthesia |
| TLV | Two-Lung Ventilation |
| $V_T$ | Tidal Volume |
| VILI | Ventilator Induced Lung Injury |
| V/Q | Ventilation / Perfusion |
| $VO_2$ | Oxygen Consumption |

# INTRODUCTION

## Morbidity and Mortality after Pulmonary Resections

Lung resection is associated with a mortality of 2.1%, mainly determined by pulmonary complications [1-3]. Postoperative acute lung injury is the major cause of pulmonary morbidity and mortality [4, 5] but the underlying mechanisms have not yet been fully clarified.

## Thoracic Surgery

Surgical lung trauma is a potent trigger of postoperative alveolar damage. The operated, nondependent lung suffers from temporary collapse, instrumentation and manipulation. Thus, the surgical lung damage depends on the invasiveness [6], extent and location of the thoracic surgical procedure [7, 8]. In addition, hypoperfusion induced by hypoxic pulmonary vasoconstriction [9], dysfunction of the surfactant system [10] and oxidative stress resulting from re-expansion [11], ischemia and reperfusion [12] may increase alveolar injury during and after thoracic surgery.

## One-Lung Ventilation

The anaesthetic management of patients for thoracic surgical procedures is challenging. Thoracic surgical procedures often require certain techniques including partial or complete exclusion of lung tissue from tidal ventilation. The ventilation of only a single lung (one-lung ventilation, OLV) however may be associated with life-threatening gas exchange impairment [13, 14]. In fact, OLV may be associated with a decrease of arterial oxygen saturation below 90% in ~10% of the patients [15, 16]. Nonetheless, hypoxaemia during thoracic surgery is not mainly caused by the reduced alveolar surface but much more by venous admixture resulting from residual perfusion of the nonventilated lung and from atelectasis and poorly aerated regions in the ventilated lung. Atelectasis formation has been demonstrated to be associated with decreased arterial oxygen tension by >50% and increased

intrapulmonary shunt by >11% during OLV [17]. The traditional approach for mechanical ventilation during OLV [18] therefore include the use of relatively high tidal volumes in the range of 10-12ml·kg$^{-1}$ with zero PEEP in the dependent, ventilated lung, to maintain hypoxic pulmonary vasoconstriction and thus to overcome hypoxaemia during OLV. The delivery of the whole tidal volume to only one lung however results in increased mechanical stress to the ventilated pulmonary tissue.

Postoperative lung computed tomography (CT) scans showed an enhanced lung density in comparison with preoperative images in thoracic surgical patients who developed ALI/ARDS after lung resection [19]. Postoperatively, regions of consolidated lung tissue were detected almost exclusively in the non-operated, ventilated lung suggesting that OLV may play a key role in the development of pulmonary complications.

As a consequence, OLV is considered to be a major pathogenetic factor in the development of clinically relevant pulmonary complications after thoracic surgery.

## Mechanisms of Lung Injury

Several OLV-related risk factors for postoperative respiratory failure have been recognised including ventilation/perfusion mismatch, increased transpulmonary pressures (i.e. pulmonary capillary pressures), cyclic collapse and recruitment of lung tissue [20] and ventilation with tidal volumes as used during normal TLV with subsequently increased airway pressures [21, 22].

Airway pressures >30cm$H_2O$ and tidal volumes of 8-12ml·kg$^{-1}$ during OLV [23] may promote overdistension and induce alveolar damage. However, a protective ventilation approach to prevent lung injury during OLV by reduction of tidal volumes and application of PEEP did not completely inhibit thromboxane $B_2$ formation in isolated rabbit lungs [20] or even enhanced the alveolar proinflammatory response in rats [24]. Likewise, OLV with tidal volumes of 5ml·kg$^{-1}$ only partially decreased the expression of proinflammatory IL8, TNFα and neutrophil infiltration in patients undergoing thoracic surgery and OLV [25].

High tidal volume ventilation may therefore not be the only variable affecting alveolar integrity during and after OLV.

Collapsed lung tissue or atelectasis is the major source of deterioration of arterial oxygenation during OLV [26]. In particular, increased cyclic opening and collapse of alveolar units and small airways have been recognised to initiate shear stress and promote a proinflammatory immune response within the lungs [24, 27]. However, mechanical lung injury by cyclic collapse and re-opening of alveoli is not solely advocated to the use of high tidal volumes.

Low tidal volume ventilation may in fact injure small peripheral airways by cyclic opening and closing resulting in changes of lung mechanics [28].

Further mechanisms contributing to the deleterious effects of OLV include changes in pulmonary blood flow characteristics and persistent atelectasis formation resulting in increased shunt perfusion.

The increase of pulmonary capillary pressure during OLV [25] may harm the pulmonary blood-gas barrier and may cause fluid extravasations into the interstitial space and the alveoli resulting in a pulmonary oedema.

Consequently, perioperative ALI is of multifactorial origin. Mechanical stress by hyperinflation, hyperperfusion and cyclic recruitment combined with proinflammatory or biochemical factors are thought to be contributive to ALI. One can postulate a 'multiple hit' theory including surgery related factors, one-lung ventilation, underlying diseases and co-morbidity, prior therapies and other unidentified events for thoracic surgery patients that may increase susceptibility to ALI [29].

## Lung Protection during Thoracic Anaesthesia

The management of patients undergoing thoracic surgery may offer opportunities for anaesthetic intervention. Besides the well recognised advantages of a restrictive fluid management and sufficient pain therapy during and after thoracic surgery [2], protective ventilation approaches including low tidal volumes, the use of volatile anaesthetics and alternative ventilation approaches are progressively utilised.

Alveolar recruitment manoeuvres (ARM) are sufficient to improve arterial oxygenation by the transformation of consolidated lung regions into normally aerated compartments and by the improvement of ventilation and perfusion matching in both lungs during conventional TLV [30] or within the ventilated lung during OLV [31, 32]. However, the ARM combined with PEEP may be valuable not only to treat hypoxaemia during OLV; more importantly, as part of a protective ventilation strategy, it may decrease cyclic alveolar collapse and facilitate reopening of alveolar units.

Clinical data revealed that only low tidal volume ventilation of the dependent lung decreases the risk of postpneumonectomy respiratory failure [33, 34]. Consequently, the reduction of tidal volume is an effective method to improve outcome, but is not sufficient to completely abolish a proinflammatory response to OLV [25, 35].

Injurious OLV has been demonstrated to increase the recruitment of granulocytes and the expression of pro-inflammatory mediators in the alveoli of the ventilated lung [25]. The immune response was attenuated by volatile anaesthetics (desflurane) as indicated by decreased alveolar expression of pro-inflammatory cytokines [36]. TIVA with propofol resulted in higher

alveolar cytokine concentrations and in increased alveolar granulocyte recruitment. Recent experimental data revealed that the use of the volatile anaesthetic desflurane not only attenuated the release of alveolar mediators but also decreased the expression of systemic pro-inflammatory cytokines. Like other halogenated anaesthetics, desflurane may have protective effects on mechanical forces during OLV. Furthermore, it may modulate granulocyte recruitment and neutrophil activation suggesting protective effects on OLV-induced lung injury.

Anaesthesia techniques using alternative ventilation approaches [37] or spontaneous breathing [38] may further offer new aspects to prevent or reduce mechanical stress to lung tissue during OLV.

The experimental studies included in this thesis were thus initiated to establish a standardised porcine model of thoracic surgery that allows investigations of OLV effects on pulmonary pathophysiology and histopathology. The model may offer opportunities to examine the efficiency of lung protective ventilation approaches or different anaesthesia techniques.

# AIMS of the STUDY

The overall objective of the studies included into this thesis was to evaluate the reasons for impaired pulmonary function and increased alveolar damage especially in the ventilated lung during and after one-lung ventilation.

The specific aims of these studies were:

I. To evaluate the effects of one-lung ventilation on ventilation-perfusion distribution by single-photon emission computed tomography and on lung histology in a pig model of thoracic surgery.

II. To investigate the effects of one-lung ventilation and experimental thoracic surgery on regional distribution of overaerated, normally aerated, poorly aerated and atelectatic lung compartments and to localise lung regions afflicted with tidal recruitment or hyperinflation in pigs.

III. To evaluate the effects of one-lung ventilation and surgical manipulation on histology and leukocyte recruitment in porcine lung tissue in comparison with histological alterations of standard two-lung ventilation or spontaneous breathing.

IV. To visualise the effects of alveolar recruitment manoeuvres followed by single lung ventilation with different tidal volumes on radiological lung density to detect lung regions afflicted with cyclic tidal collapse and reopening.

The knowledge obtained in these studies may contribute to achieve a better understanding of the respiratory pathophysiology and pulmonary histopathology.

# METHODS

## Animals

The studies were performed in accordance with the recommendations of the Swedish National Board for laboratory animals. The animal use had been approved by the Animal Care and Use Committee of the Uppsala University (Sweden).

Forty-nine healthy 2-3 month old piglets of the Hampshire, Yorkshire and Swedish country breeds with a body weight 24.4±2.4kg from a local breeder were used. All animals fasted over night and had free access to water.

## Anaesthesia

An identical anaesthesia protocol was chosen in all animals (**study I – IV**).

The pigs were anaesthetised by an intramuscular injection of xylazine (2.2mg·kg$^{-1}$, Rompun®; Bayer, Leverkusen, Germany), tiletamine/zolazepam (6mg·kg$^{-1}$, Zoletil®; Virbac, Carros, France) and atropine (0.04mg·kg$^{-1}$, NM Pharma, Stockholm, Sweden).

After the loss of corneal reflex response, the pigs were placed supine and 18G catheters (Becton Dickinson, Heidelberg, Germany) were inserted in both ear veins.

After intubation, anaesthesia was maintained by continuous infusions of fentanyl (5µg·kg$^{-1}$·h$^{-1}$, Leptanal®; Janssen-Cilag AB, Sweden), pancuronium (0.3mg·kg$^{-1}$·h$^{-1}$, Pavulon®; Organon, Oss, Netherlands), ketamine (25mg·kg$^{-1}$·h$^{-1}$, Ketaminol vet.®; Intervet, Boxmeer, Netherlands) and propofol (3mg·kg$^{-1}$·h$^{-1}$, Diprivan®; Astra, Södertälje, Sweden).

Isotonic saline solution (3-4ml·kg$^{-1}$·h$^{-1}$, Fresenius Kabi AB; Halden, Norway) was administered to maintain urine output at >1ml·kg$^{-1}$·h$^{-1}$ and to keep arterial blood pressure and haemoglobin concentration constant.

Body temperature was monitored and was kept constant by thermoconvection.

At the end of the experiment the pigs were killed by an intravenous bolus injection of 150meq potassium chloride.

In **study III**: Two groups were added: a group (n=3) of spontaneous breathing pigs that were killed immediately after loss of consciousness and reflex responses to obtain reference values on pulmonary histology of untreated animals, and a group (n=6) receiving balanced anaesthesia by administration of 6.2±0.2Vol% desflurane (~1MAC) instead of propofol to maintain anaesthesia.

# Instrumentation

In all pigs (**study I - IV**) a flow-directed pulmonary artery catheter (PAC, 7.0French, Swan-Ganz thermodilution catheter, Baxter, Irvine, USA) and a single lumen central venous catheter (4.0French, Becton-Dickinson Critical Care Systems, Singapore) were inserted via the left external jugular vein. The balloon tip of the PAC was located in wedge position for cardiac output measurements and mixed venous blood sampling.

All pigs received a left carotid arterial catheter for continuous arterial blood pressure measurements and arterial blood sampling (20G; Becton-Dickinson Critical Care Systems, Singapore).

Finally, a suprapubic urinary catheter (Sympakath®; Ruesch AG, St.Gallen, Switzerland) was placed to monitor urine output.

# Ventilation Management

## Two-Lung Ventilation

The trachea was orally intubated with an ID 7.0mm cuffed endotracheal tube (Mallinckrodt, Athlone, Ireland).

After intubation, animals were mechanically ventilated with intermittent positive pressure ventilation (IPPV) provided by a ventilator (Servo I; Maquet Critical Care, Solna, Sweden, **study I, II and IV**). The tidal volume was set at $10ml \cdot kg^{-1}$, the $FiO_2$ at 0.40 and PEEP at $5cmH_2O$. Respiratory frequency was adjusted to achieve a normal arterial $pCO_2$ of 40-45mmHg in all animals. After tracheotomy, the tube was replaced by an ID 8.5mm endotracheal tube (Mallinckrodt, Athlone, Ireland) and TLV was re-established as before.

In **study III**: The animals undergoing mechanical ventilation were ventilated by an anaesthesia ventilator (Maquet KION, Solna, Sweden) with an identical setup as in study I, II and IV: $V_T=10ml \cdot kg^{-1}$, $FiO_2$ of 0.40, PEEP of $5cmH_2O$, and respiratory frequencies adjusted to maintain normocapnia.

## One-Lung Ventilation

To achieve lung separation, a bronchial blocker (9.0French Arndt-Endobronchial Blocker Set, COOK®, Bjaeverskov, Denmark) was placed bronchoscopically guided in the left main bronchus (EF-B 14L, Xion medical, Berlin, Germany). Sufficient bronchus blockade was confirmed by short blocker inflation.

The ventilation of the nondependent left lung was discontinued by bronchoscopically controlled inflation of the bronchus blocker and OLV of the dependent right lung was started.

In **study I, II and III**: Ventilation settings remained unchanged throughout the entire study period: $V_T$=10ml·kg$^{-1}$, respiratory frequency was adjusted to a $paCO_2$ of about 40mmHg; $FiO_2$ was set to 0.4 to achieve a $paO_2 \geq 100$mmHg. PEEP remained unchanged at 5cm$H_2O$.

In **study IV**: The tidal volume was reduced to $V_T$=5ml·kg$^{-1}$ in one group during OLV; respiratory frequency was adjusted accordingly to maintain normocapnia ($paCO_2$ ~ 40mmHg); $FiO_2$ (0.4) and PEEP (5cm$H_2O$) were kept constant.

## Alveolar Recruitment Manoeuvre

Alveolar recruitment manoeuvres (ARM) were applied to the whole lung in a standardised manner using the ventilator integrated functions: both lungs were recruited by one vital capacity manoeuvre with a constant airway pressure of 40cm$H_2O$ for 10 seconds.

# Thoracic Surgery

OLV-animals were placed in the right lateral position. A left sided lateral thoracotomy of about 10cm was performed. After preparation of the fatty layer, the fascia and the muscular layers were identified and separated. The thorax was opened between two ribs cranial of the diaphragm and kept open by a retractor. The pleural layer was identified, cut, and the lung visualised. Successful left lung collapse was verified by direct inspection. A surgical procedure was simulated by handling the collapsed left lung. The chest wound margins were loosely adapted after simulated thoracic surgery and OLV.

# Study Protocols

## Study I

Eleven anaesthetised and ventilated pigs ($V_T$=10ml·kg$^{-1}$, $FiO_2$=0.40, PEEP=5cm$H_2O$) were studied. After lung separation, OLV and thoracotomy were performed in 7 pigs (OLV group). During OLV and in the two-lung ventilation control group (n=4), ventilation settings remained unchanged throughout the study. The impact of OLV and thoracic surgery on V/Q distribution was assessed by SPECT with $^{81m}$Kr for ventilation and $^{99m}$Tc-labelled macro-aggregated albumin for perfusion and compared with V/Q distribution during conventional TLV at corresponding time points (figure 1). Cardiopulmonary and ventilation data were obtained at analogous time points [T1-T4]. Finally, lung tissue samples were harvested and examined for alveolar damage. SPECT findings were related to lung histopathology after OLV or TLV.

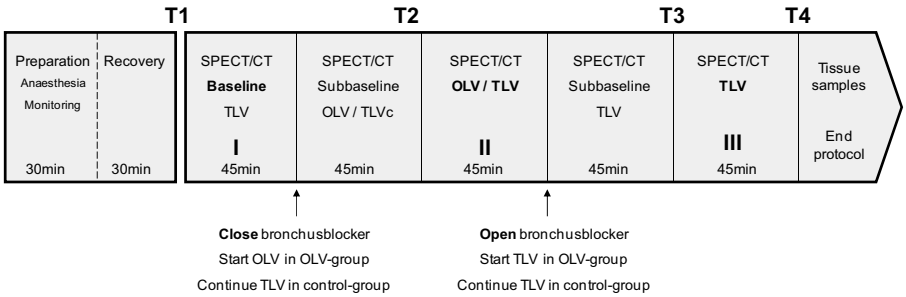


Figure 1. Experimental workflow in study I

## Study II

Nine anaesthetised and mechanically ventilated ($V_T$=10ml·kg$^{-1}$, $FiO_2$=0.40, PEEP=5cm$H_2O$) pigs were studied. After lung separation by an endobronchial blocker, lateral thoracotomy and OLV were performed in six pigs. Three animals served as controls. Static end-expiratory and end-inspiratory CT scans were done before, during and after OLV and at corresponding times in TLV controls (figure 2). CT images were analysed by defined regions of interest (ROI) and summarised voxels were classified by defined lung density intervals (atelectasis, poorly aerated, normally aerated, overaerated). Lung regions afflicted with tidal recruitment and overaeration were localised by morphometric analysis using spiral computed tomography (CT). Tidal recruitment was defined as the increase in the volume of gas entering

atelectatic and poorly aerated lung regions. Cardiopulmonary and ventilation data were obtained at time points T1-T3.

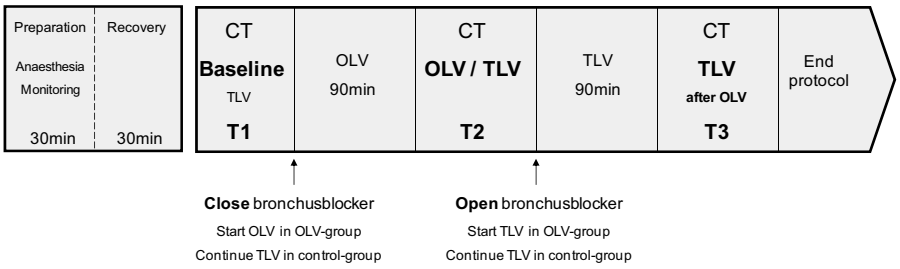


Figure 2. Experimental workflow in study II

## Study III

The extent and distribution of lung injury was analysed by post mortem histological analysis of lung tissue in an established porcine model of OLV and thoracic surgery (figure 3). Lung injury after OLV (OLV-desflurane, OLV-popofol) was described by an established scoring system (Diffuse Alveolar Damage - DAD score) and compared with DAD scores of spontaneously breathing animals (n=3) and those with conventional TLV (n=6). Further, the impact of one standard intravenous (propofol, n=6) and one standard inhalational (desflurane, n=6) anaesthetic in clinical dosage on leukocyte recruitment was investigated by immunohistochemical staining of cells bearing the CD45 surface antigen in lung tissue samples.

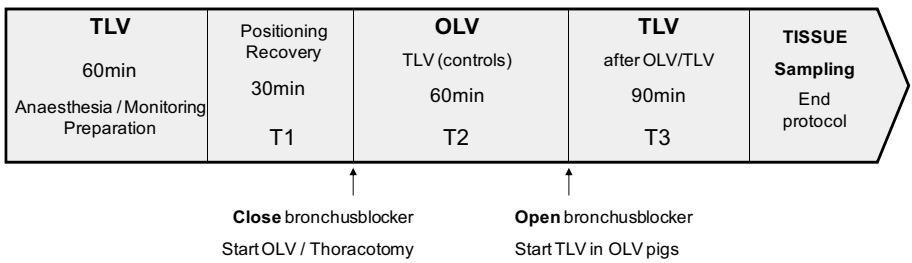


Figure 3. Experimental workflow in study III

## Study IV

This experimental study evaluated the impact of alveolar recruitment manoeuvres (ARM) and OLV with different tidal volumes ($V_T$) on haemodynamics, ventilation mechanics, gas exchange and regional pulmonary gas/tissue distribution within the lungs.

The radiological lung density was analysed by end-expiratory and end-inspiratory CT scans that were acquired during TLV before and after ARM of 40cm$H_2O$ for 10s applied to the whole lung. Pigs were then randomised into groups receiving different $V_T$ (5ml·kg$^{-1}$, n=4; 10ml·kg$^{-1}$, n=4) during OLV. Voxels were categorised by defined radiological density intervals (atelectasis, poorly aerated, normally aerated, overaerated). Tidal recruitment was defined as the increase in the volume of gas entering atelectatic and poorly aerated lung regions. Cardiopulmonary and ventilation data were acquired at time points T1-T4. The study protocol is given in the experimental workflow (figure 4).

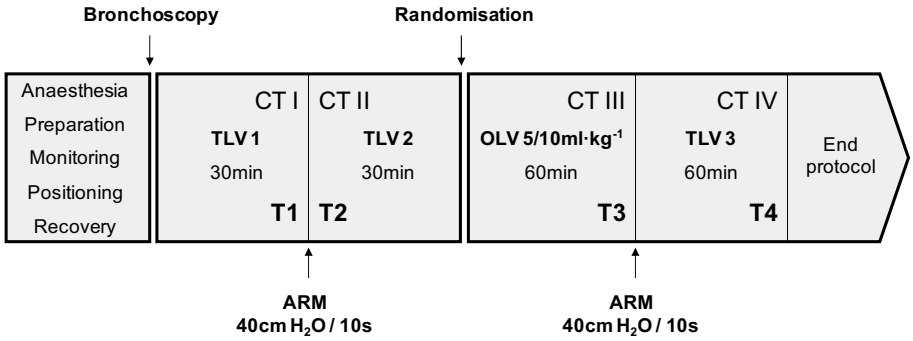


Figure 4. Experimental workflow in study IV

# Measurements

## Haemodynamics / Ventilation / Gas Exchange

The following cardio-respiratory variables were recorded in all animals: Cardiac output, heart rate (HR), mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), central venous pressure (CVP) and pulmonary artery occlusion pressure (PAOP) as well as arterial and mixed venous blood gases. Airway pressures (Paw peak, Paw plateau, Paw mean, PEEP), minute ventilation (MV) and static ($C_{stat}$) and dynamic ($C_{dyn}$) lung compliances were measured using the ventilator integrated functions.

Blood gas analysis was performed immediately after arterial and mixed venous blood sampling. Gas exchange variables were measured with standard blood gas electrodes (ABL 500; Radiometer, Copenhagen, Denmark).

All data were monitored continuously and recorded at the defined measurement points (T1-T4). Systemic vascular resistance (SVR), pulmonary vascular resistance (PVR), pulmonary capillary pressure, oxygen delivery ($DO_2$), oxygen consumption ($VO_2$) and venous admixture were calculated according to standard formulas.

## Computed Tomography

In **study II and IV**, Computed tomography (CT) images were acquired by a Somatom Sensation 16 CT Scanner (Siemens Medical Systems; Erlangen, Germany). All scans were done in a standardised manner: expiratory hold and subsequent inspiratory hold manoeuvres using the ventilator integrated functions without ventilator circuit disconnection. By pressing the inspiratory hold button of the ventilator, expiratory valves closed automatically after application of the defined tidal volume. The Servo I ventilator held the applied volume until the button was released after CT.

Transverse scans were performed with the following settings: 120kV, 100mA, rotation time 0.5s, collimation 16x1.5mm, pitch 1.05, slice thickness of 5mm and a scan time of approximately 7s. Images were reconstructed with an increment of 5mm, yielding 43-52 slices from each scan depending on lung dimensions, which were determined by an initial frontal chest tomogram.

CT images were transferred to a personal computer (MAXDATA Notebook ECO 4000 A) connected to a separate screen (EIZO FlexScan S1911, resolution: 1280 x 1024) for further analysis.

Files were recorded as GE proprietary interfile format and subsequently converted to Analyze™ format (1995, Biomedical Imaging Resource, Mayo Foundation) by header file parsing. The binary image data were not altered in this process. Image scaling units (Hounsfield units, HU) were preserved. Regions of Interest (ROI) were defined in each CT image using the MRICO software (V 1.40, 2005, C. Rorden).

In all slices from expiratory and inspiratory CT scans, the entire left and right lungs were chosen as a ROI by drawing the external boundaries of the lungs at the inside the ribcage and the internal boundaries along the mediastinal organs. ROIs were manually defined slice-wise in the lung window (-500 to 1500 HU) and corrected in the mediastinal window (50 to 350 HU) in cases of low contrast. The trachea, main bronchi and large blood vessels were excluded from the ROI; voxels outside the ROI were ignored.

The ROIs were applied onto the CT images using MATLAB (V 7.0, MathWorks Inc.) scripts, thus reading the gray values for each voxel. By counting the voxels in given intensity intervals, the relative size of four partitions (nonaerated [HU from +100 to -100], poorly aerated [-100 to -500], normally aerated [-500 to -900] and overaerated [-900 to -1000]) was

calculated for each slice along the longitudinal axis, either relative to the whole lung volume or to the actual slice. The results were additionally plotted as HU distributions for the whole lung to display the overall change.

Lung dimensions were normalised to a common length of 100 points before calculating distributions of lung density, while leaving the value magnitude and shape unchanged. Thus, all spatial values are given according to their relative position along the spatial axis in percent. The data were selectively grouped for acquisition and subject.

## Single Photon Emission Computed Tomography

**Study I:** The animals were placed in the gamma camera in the right lateral position. The pulmonary blood distribution was assessed by intravenous injection of $^{99m}$Technetium-labelled macro-aggregated albumin ($^{99m}$Tc-MAA, Pulmocis; CISbiointernational, Gif sur Yvette, France), ventilation was studied by inhalation of Krypton, produced by a Rubidium generator on site (Mallinkrodt; Netherlands). Single photon emission computed tomography (SPECT)-measurements were performed before, during and after OLV.

A background SPECT scan was done before each SPECT (except the first SPECT), measuring the radioactivity of the first isotope injection to allow background radioactivity calculation. A dynamic CT-scan (covering the same volume as the SPECT, duration 12min) was done immediately after each SPECT. The CT scan was a low resolution scan primarily used for attenuation correction.

Images were acquired by a SPECT/CT dual-head gamma camera (Millennium; General Electric Systems, Milwaukee, WI) with an all-purpose low-energy collimator.

Acquisition was performed in two separate energy windows, one at 140±10keV for $^{99m}$Tc and on at 190±10keV for $^{81m}$Kr. SPECT acquisition was made in 60 projections (30 per head) and stored in a 128 by 128 matrix, resulting in a pixel size of 4.4mm. The acquisition time was 60 seconds per projection. This rather long time resulted from the comparatively low activity of $^{81m}$Kr. The overall scan time was approximately 42min.

Data were reconstructed on an eNTEGRA workstation (Millennium; General Electric Systems, Milwaukee, WI). The reconstruction was done with an iterative model (OSEM, 4 iterations and 8 subsets) and a Hann filter (cut-off 0.85) for the post reconstruction filtering. The reconstructed transversal slices where corrected for radiation spill-over using a HERMES workstation (Nuclear Diagnostics, Stockholm, Sweden).

For each reconstructed slice, in apex-base and anterior-posterior directions, the contents were analysed by special software. Before calculating the activity distribution, a background subtraction of 10% of the global maximum was performed in all slices.

After evaluation of the lung borders assessed by CT, the left and the right lungs were chosen as regions of interests (ROI) by drawing the external boundaries of the lungs along inside the ribs and the internal boundaries along the mediastinal organs. The lungs were divided into 41 equally thick slices in the inferior (dependent, right lung) to superior (nondependent, left lung) direction, to assess the vertical VA/Q distribution. Similarly, the lungs were divided into 49 equally thick slices from the base to the apex for analysis of ventilation/perfusion distribution in that plane.

## Ventilation-Perfusion Ratio in SPECT

**Study I:** For calculation of V/Q ratios, the proprietary interfile format files were converted to Analyze™ format (1995, Biomedical Imaging Resource, Mayo Foundation) by header file parsing without changing the binary image data. Regions of interest (ROI) of the upper and lower lungs were manually defined in the CT images using the MRICO software (V 1.40, 2005). These ROIs were applied to the SPECT data images using SPM functions (Wellcome Department of Cognitive Neuroscience, University College, London, UK) in MATLAB (V 7.0, MathWorks Inc.), thus reading the $^{81m}$Krypton and $^{99m}$Technetium counter values for each voxel in each lung. Voxels outside the ROI were ignored. For estimation of absolute ventilation and perfusion, counts per voxel were summarised separately for each lung and normalised to the individual maximum activity. For V/Q relations, logarithmic V/Q ratios were calculated by: V/Q-voxel = $\mathrm{Log}_{10}$ ($^{81m}$Kr activity/$^{99m}$Tc activity). The results were plotted as V/Q distributions over the whole lung.

## Tissue Sampling

**Study III:** The entire lungs were excised via median sternotomy immediately after the pigs were killed. Blocks of lung tissue (approximately 1x1x0.5cm) were harvested from both of the lungs largest diameter at representative locations: peripheral (subpleural), intermediate and central (parahilar) regions. Samples from each location were immediately fixed in 4% neutral-buffered p-formaldehyde solution (Formalin; Apoteket AB, Göteborg, Sweden) and stored at 20°C for at least 72h and finally embedded in paraffin.

In **study I**: For technical reasons, bilateral thoracotomy was performed and lung tissue samples from the level of the lungs largest diameter were harvested from the peripheral right and left lower lobe for histological staining. The tissue samples were fixed in an identical manner.

## Staining procedures

Paraffin-embedded lung tissue samples were sectioned (2-3µm slices) and stained with haematoxylin and eosin (H&E) for light-microscopic analysis. The sections were randomly selected by an assisting technician blinded to the experimental protocol. The slides were evaluated by a blinded pathologist. The extent of histomorphological changes was scored by the diffuse alveolar damage (DAD) score [39].

A different set of tissue sections (2µm) was used for immunohistochemical staining to identify $CD45^+$-cells. The samples were de-waxed in xylene, rehydrated by descending ethanol concentrations (96%, 70%) and washed in purified water. Following a washing step with TRIS-buffered saline (TBS) for 10min, the slices were placed in 0.025M citrate buffer (pH 6.0). The antigen was unmasked by heating in a conventional microwave oven (4×5min, 600W). After washing with TBS, all samples were incubated with 150µl of a mouse-α-CD45 monoclonal antibody (MAC323, ABD Serotec, Oxford, UK; diluted 1:20 with TBS+1%BSA) at room temperature and washed again three times in TBS (5min). Afterwards, the samples were incubated with a biotinylated goat- α–mouse antibody for 60min and washed again. The slides were incubated (30min) with alkaline phosphatase (Streptavidin-AP®, DAKOCytomation, Glostrop, Denmark) and washed (3×5min).

Fuchsin+Chromogen® and Fuchsin+Activating agent® were mixed and the substrate solution was immediately used after the endogenous alkaline phosphatase was blocked by Levamisole®. After an incubation of 15 to 20min the slides were washed and stored in purified water over night at 4°C. Finally, all sections were counterstained with haematoxylin and cover slipped.

## Leukocyte Common Antigen ($CD45^+$ - Cell Counting)

**Study III:** $CD45^+$-cells were counted by light microscopy (Model CHK; Olympus, Taiwan) using a magnification of ×400. In the three different sections of each lung ten separate non-overlapping fields of view were analysed. The $CD45^+$-cell numbers of each region were averaged.

## Diffuse Alveolar Damage Score

**Study I and III:** The damage of the alveolar compartment was assessed in the H&E-stained sections by light microscopy (Model CHK; Olympus, Taiwan) using magnifications of ×40, ×100, and ×400, respectively. The alveolar injury was characterised by the following features [40, 41]: alveolar

oedema, interstitial oedema, microhaemorrhage, neutrophil infiltration, microatelectasis and alveolar overdistension. Four isolated non-overlapping fields of view of the different samples were analysed separately. The values of all sectors per lung (n=12) were averaged.

The severity of the DAD features was characterised as follows: 0=normal appearance, 1=slight effect, 2=intermediate effect and 3=severe effect. The extent of alveolar damage in each sector was described as follows: 0=no damage, 1=up to 25%, 2=25–50%, 3=50–75%, 4=75% to almost complete and 5=complete. Each property was given by its severity multiplied by the extent.

The DAD score was calculated by summarising the products of severity and extent of each DAD quality [42].

## Statistical Analysis

Statistical analysis was done using the Statistical Package for the Social Sciences (v. 14, SPSS Inc., Chicago, IL, USA). A $p$ value $<0.05$ was considered to be significant for all statistical procedures.

Data were tested for normal distribution with the Shapiro-Wilks W test. Normally distributed data are presented as mean±standard deviation (cardiopulmonary, ventilation and gas exchange variables, **study I - IV**) or as mean±standard error of the mean (SPECT data, **study I**). These data were analysed by a repeated measures one-way analysis of variance (ANOVA) with post-hoc Bonferroni correction. CT data were slice-wise analysed by two-sample t-tests and repeated measures ANOVA (**study II and IV**).

Data are expressed as median and interquartile range (P25-75) in the case of non-normal distribution (DAD score and $CD45^+$-cell numbers). The alveolar damage scores and $CD45^+$-cell numbers were compared using the Mann-Whitney U test (**study I and III**).

# RESULTS

## Ventilation and Perfusion (SPECT)

In **study I**, SPECT revealed that ventilation was primarily distributed to the nondependent lung during baseline TLV. Perfusion distribution between the two lungs was similar, with 50.7% to the upper lung and 49.3% delivered to the lower lung during baseline TLV (figure 5).

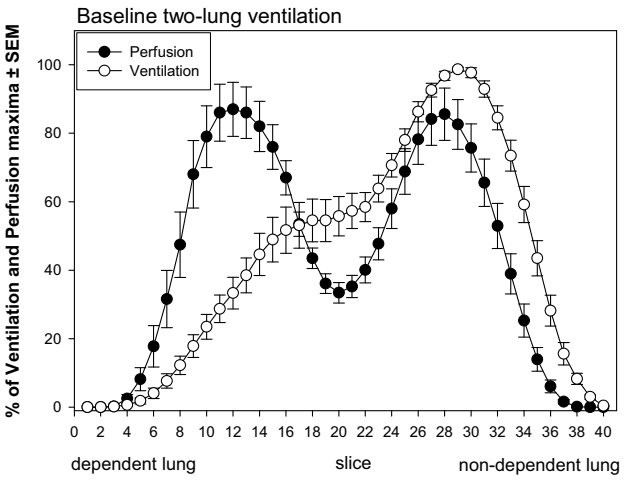


*Figure 5.* Ventilation and perfusion [mean±SEM] derived from SPECT scans in transversal planes during baseline TLV. Perfusion ($^{99m}$Tc-MAA activity) and ventilation ($^{81m}$Kr-activity) is given in percent of individual maximal ventilation and perfusion.

During OLV, only the dependent right lung was ventilated. The entire tidal volume was delivered to the right lung, which resulted in an upward shift of the mediastinum and the accessory lobe of the right lung became more apparent. Only a small percentage (<10% of whole perfusion) was circulated to the nonventilated lung during OLV (figure 6).

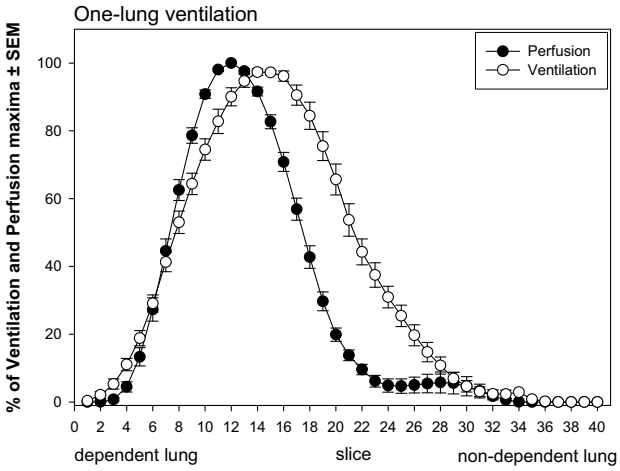


*Figure 6.* Ventilation and perfusion [mean±SEM] derived from SPECT scans in transversal planes during OLV. Perfusion ($^{99m}$Tc-MAA activity) and ventilation ($^{81m}$Kr-activity) is given in percent of individual maximal ventilation and perfusion.

After resuming TLV, the ventilation distribution returned to similar baseline TLV values. Perfusion was increased to 61.3% in the dependent lung and decreased to 38.7% in the nondependent lung (figure 7, $p<0.01$).

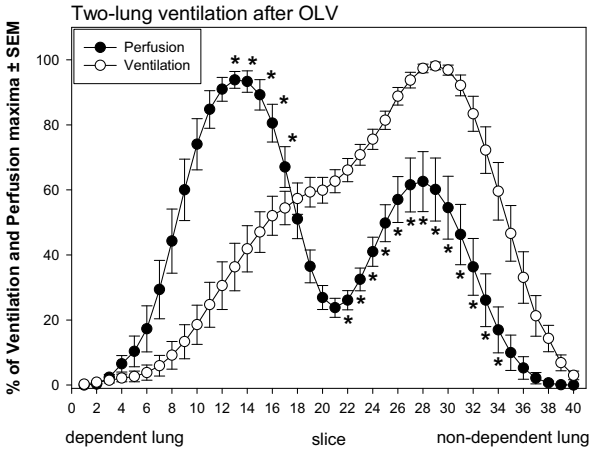


*Figure 7.* Ventilation and perfusion [mean±SEM] derived from SPECT scans in transversal planes during TLV after OLV. Perfusion ($^{99m}$Tc-MAA activity) and ventilation ($^{81m}$Kr-activity) is given in percent of individual maximal ventilation and perfusion (*= $p< 0.05$: TLV *vs.* baseline).

Despite the normalisation of haemodynamic (cardiac output, intrapulmonary shunt) and ventilation parameters, the persistent increase of perfusion in the

dependent lung resulted in a considerable V/Q mismatch of the lungs after OLV (figure 8).

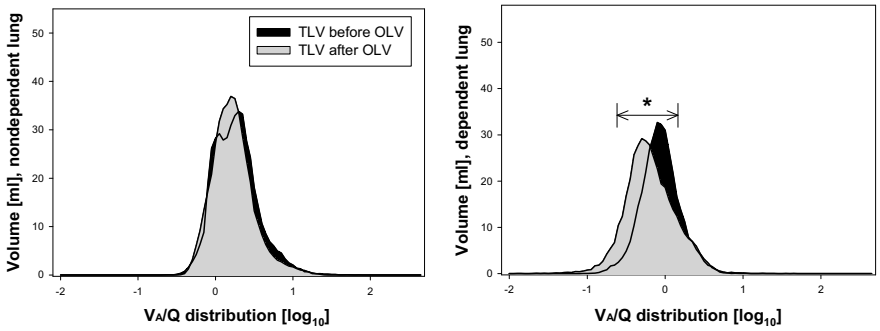


*Figure 8.* Distributions of V/Q ratios [mean] in nondependent and dependent lungs at TLV before and after OLV. V/Q ratios were calculated in transversal planes. (*=p<0.05: baseline TLV *vs.* TLV after OLV)

# Lung Density Distribution - Cyclic Recruitment (CT)

In **study II**, both normally and poorly aerated lung regions dominated in apical lung zones. Almost all atelectasis was localised in the dependent, juxtadiaphragmatic and dorsal lung regions.

## Dependent lungs of OLV pigs

Before OLV, end-expiratory density distribution was dominated by poorly aerated compartments, a considerable amount of atelectasis and a small fraction of normally aerated areas. At end-inspiration, atelectasis and poorly aerated regions were decreased whereas normally aerated regions were increased reflecting the tidal recruitment (figure 9, table 1).

OLV delivered the whole tidal volume to the dependent lung. It resulted in a reduction of atelectasis and in an increase of both poorly and normally aerated compartments.

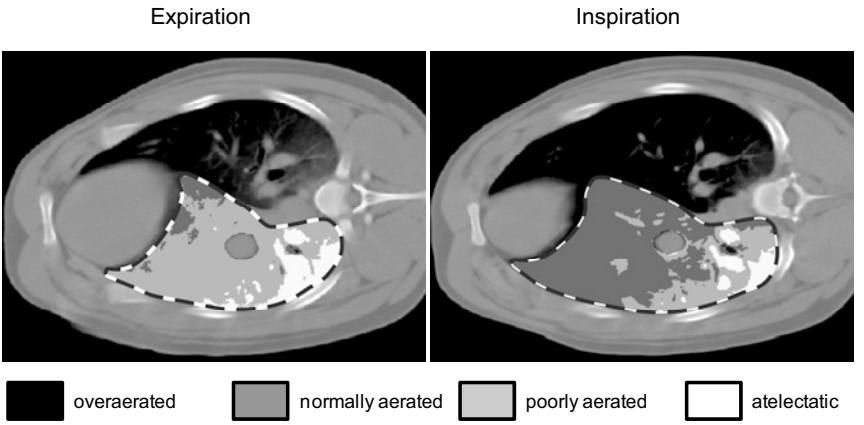


*Figure 9.* Juxtadiaphragmatic lung CT images of a representative animal (OLV pig No. 3) during TLV before OLV ($V_T$=10ml·kg$^{-1}$). In each image, ROI is depicted by (---); overaerated (-1000 to -900HU), normally aerated (-900 to -500HU), poorly aerated (-500 to -100 HU), and atelectatic (-100 to +100HU) lung regions are coded by gray scale.

*Table 1.* Volumes of lung compartments [ml] at expiratory and inspiratory hold manoeuvres in the dependent lungs of OLV pigs. Data during TLV before OLV, during OLV, and TLV after OLV are displayed as means (SD). *=p<0.05 *vs.* CT I

| | CT I [TLV before OLV] | CT II [during OLV] | CT III [TLV after OLV] |
|---|---|---|---|
| Expiration | | | |
| Lung volume | 313(27) | 413(42)* | 404(37)* |
| Atelectasis | 92(13) | 41(12)* | 38(10)* |
| Poorly aerated | 206(15) | 294(19)* | 280(24)* |
| Normally aerated | 14(7) | 78(35)* | 86(31)* |
| Overaerated | 1(0) | 1(0) | 1(0) |
| Inspiration | | | |
| Lung volume | 417(34) | 692(74)* | 571(79)* |
| Atelectasis | 60(15) | 16(4)* | 19(6)* |
| Poorly aerated | 184(13) | 124(33)* | 171(37) |
| Normally aerated | 172(45) | 551(96)* | 380(98)* |
| Overaerated | 1(0) | 1(1) | 1(0) |

During inspiration there was a further decrease in atelectasis so that cyclic recruitment was even larger during OLV than during preceding TLV. Overaerated lung compartments covered only an insignificant fraction.

After resuming TLV, lung density distribution was dominated by normally/poorly aerated regions. The volume of atelectasis was decreased but 5% remained in the dependent lungs at end-expiration (figure 10).

The total volume of the dependent lungs increased during OLV, both at end-expiration and at end-inspiration. After restoration of TLV, dependent lung volumes remained increased.

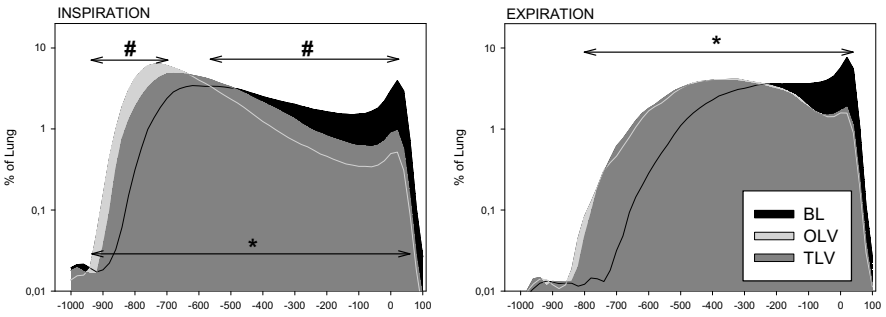


*Figure 10.* Distribution histogram of lung voxels HU -1000 to +100 in the dependent lungs of OLV pigs [mean percent of lung volume end-expiratory and end-inspiratory] at baseline TLV (BL), during OLV and at TLV after OLV (*=p< 0.05: OLV *vs.* baseline TLV; #=p< 0.05: TLV after OLV *vs.* OLV).

## Nondependent lungs of OLV pigs

Normally and poorly aerated regions were evenly distributed at end-expiration during baseline TLV. Atelectasis was minor and overaeration did not occur.

After resuming TLV (after OLV), the amount of normal and poor aeration and atelectasis was the same as before the period of lung collapse.

# Diffuse Alveolar Damage (DAD)

In **study I**, post mortem analysis of tissue samples demonstrated that the calculated DAD score was significantly higher in the ventilated lung. Alveolar and interstitial oedema, haemorrhage, neutrophil infiltration, and microatelectasis were increased in the right, dependent, ventilated lung in comparison to the left, nondependent lung. Alveolar overdistension was not different between both lungs.

In **study III**, DAD scores of spontaneously breathing (SB) pigs included minimal fractions of alveolar and interstitial oedema, neutrophil infiltration and alveolar overdistension. Signs of microhaemorrhage and microatelectasis were not found. Summarised DAD and individual DAD characteristics were equally distributed within both lungs. There were no

differences between subpleural, intermediate and parahilar lung tissue samples of the left and the right lungs.

Standard TLV elevated DAD scores in both lungs characterised by increased neutrophil infiltration, microatelectasis and microhaemorrhage in the nondependent lung, and interstitial oedema, neutrophil recruitment and microatelectasis in the dependent lung. Only minor signs of alveolar overdistension and alveolar oedema were seen and were not different to the SB group. In all samples, the DAD characteristics were homogeneously distributed within the lungs.

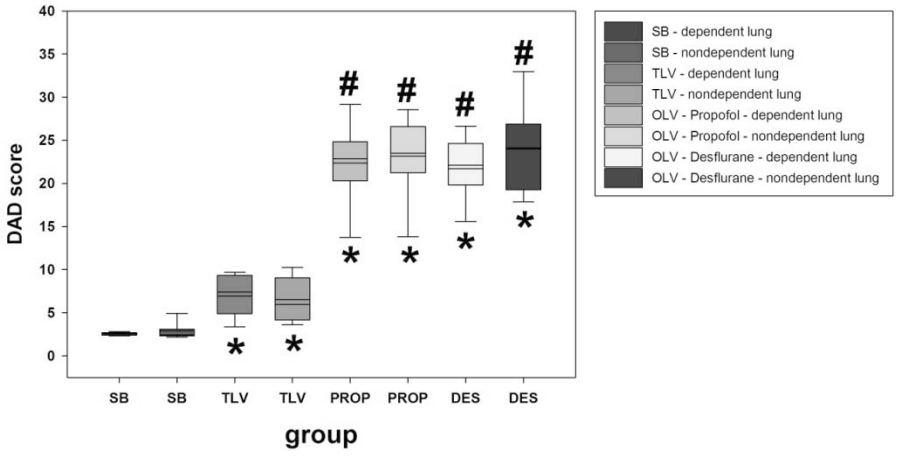


*Figure 11.* Diffuse Alveolar Damage scores in the dependent and nondependent lung in pigs after spontaneous breathing (SB), conventional two-lung ventilation (TLV) and after thoracic surgery and one-lung ventilation (OLV) with inhalational anaesthesia with desflurane (DES) or TIVA with propofol (PROP).*=$p<0.05$: TLV, OLV *vs.* spontaneous breathing pigs; #= $p<0.05$: OLV *vs.* TLV.

OLV and open thoracic surgery resulted in higher DAD scores in the dependent and nondependent lungs in comparison with pigs that underwent standard TLV or SB (figure 11).

Significant alveolar and interstitial oedema, neutrophil infiltration, alveolar overdistension, microhaemorrhage and microatelectasis were distributed homogeneously from subpleural to parahilar lung regions with no differences between tissue samples obtained from dependent or nondependent lungs (table 2).

# Alveolar Recruitment of Leukocytes

In **study III**, the mean $CD45^+$-cell number of SB-pigs was 5.0cells/view in all lung samples without differences between subpleural and parahilar lung regions.

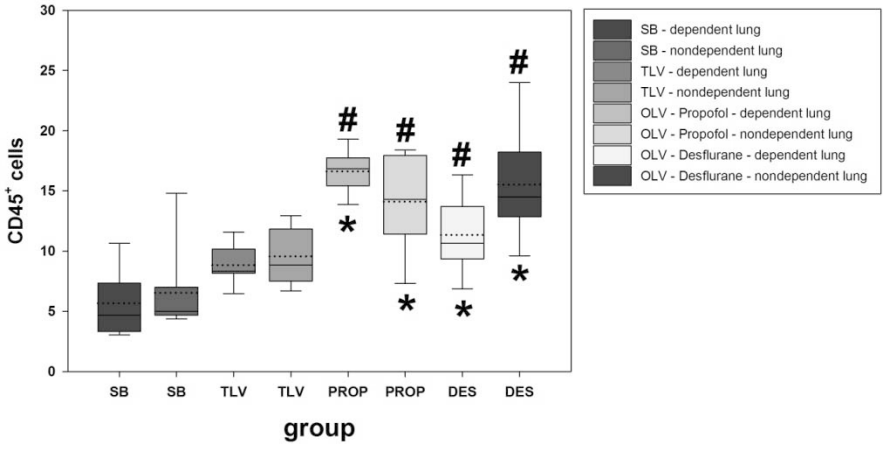


*Figure 12.* $CD45^+$-cell numbers in the dependent and nondependent lung in pigs after spontaneous breathing (SB), conventional two-lung ventilation (TLV) and after thoracic surgery and one-lung ventilation with inhalational anaesthesia with desflurane (DES) or TIVA with propofol (PROP). *=$p<0.05$: TLV, OLV *vs.* spontaneous breathing; #= $p<0.05$: OLV *vs.* TLV.

After standard TLV, the presence of $CD45^+$-cells was not different in comparison with SB pigs.

After OLV, the recruitment and infiltration of $CD45^+$-cells were diffusely distributed within both lungs and the number of $CD45^+$-cells *per* view was increased in comparison with TLV or SB (figure 12).

There were no regional differences or differences between the ventilated or the manipulated lung. The infiltration of $CD45^+$-cells was similar in the lungs of OLV pigs that received propofol or desflurane anaesthesia.

*Table 2.* Diffuse alveolar damage (DAD) parameters of lung tissue samples. Data are displayed as median and interquartile range [$P_{25}$-$P_{75}$].
*=p<0.05: TLV, OLV *vs.* spontaneous breathing (SB), #=p<0.05: TLV *vs.* OLV

| Group | Sample | AO | IO | MH | LI | MA | OD |
|---|---|---|---|---|---|---|---|
| SB | left | 0.3 [0.3-0.4] | 0.7 [0.6-0.9] | 0.1 [0.1-0.2] | 0.8 [0.7-0.9] | 0.1 [0.0-0.2] | 0.6 [0.1-0.8] |
| | right | 0.2 [0.1-0.4] | 0.8 [0.6-0.9] | 0.1 [0.1-0.2] | 0.9 [0.7-0.9] | 0.1 [0.0-0.2] | 0.5 [0.4-0.5] |
| TLV | left | 0.3 [0.2-2.6] | 0.6 [0.2-1.0] | 1.6* [0.2-3.2] | 1.5* [1.3-1.7] | 0.5* [0.3-0.6] | 0.8 [0.6-1.2] |
| | right | 0.3 [0.2-0.4] | 2.0* [1.3-2.8] | 0.2 [0.1-0.4] | 2.7* [1.8-3.2] | 1.0* [0.7-2.0] | 0.6 [0.5-1.0] |
| OLV | left | 4.1*# [3.7-4.6] | 4.4*# [3.9-5.0] | 4.5*# [3.9-4.8] | 5.0*# [4.8-5.5] | 3.1*# [1.9-3.7] | 2.3*# [1.4-2.5] |
| | right | 3.8*# [2.8-4.1] | 4.1*# [3.8-4.5] | 4.3*# [3.8-4.8] | 5.0*# [4.5-5.5] | 2.1* [1.5-2.6] | 2.8*# [2.3-3.5] |

left = nondependent, nonventilated, manipulated lung, right = dependent, ventilated lung of OLV pigs
AO=alveolar oedema, IO=interstitial oedema, MH=microhaemorrhage, LI=leukocyte infiltration, MA=microatelectasis, OD=overdistension

# Alveolar Recruitment Manoeuvre

S**tudy IV**: During TLV before ARM, the total expiratory dependent lung volume (mean 318ml) consisted only of a small fraction of normally aerated compartments (15±9ml) in all animals. The dependent lung had mainly poorly aerated (213±13ml) and atelectatic (90±16ml) lung regions at this time point. Atelectasis was preferentially localised in the most dependent, caudal and paradiaphragmatic lung regions. Apical lung zones exclusively contained normally and poorly aerated lung compartments.

Inspiration resulted in an increase of the total lung volume (mean 426ml). It was associated with decreased atelectatic (56±10ml) and poorly aerated compartments (183±10ml) in favour of normally aerated regions (187±29ml) reflecting a considerable tidal recruitment.

Haemodynamic, ventilation and gas exchange variables reflected normal values for a pig that underwent mechanical TLV.

The alveolar recruitment manoeuvre, which consisted of a constant inspiratory peak airway pressure of 40cm$H_2O$ applied to the whole lung, was performed for 10sec. It increased the total lung volume by 34-38% (expiration/inspiration) and shifted the global lung density spectrum towards regions with high gas content following the recruitment of consolidated lung compartments. Atelectasis was decreased from 28% to 11% but normally

aerated lung compartments increased from 5% to 20% at end-expiration. The amount of poorly aerated lung volume remained unchanged.

Inspiration further increased normally aerated regions. Poorly aerated regions were decreased in favour of normal aeration without evidence of overaeration (figure 13, 14, table 3).

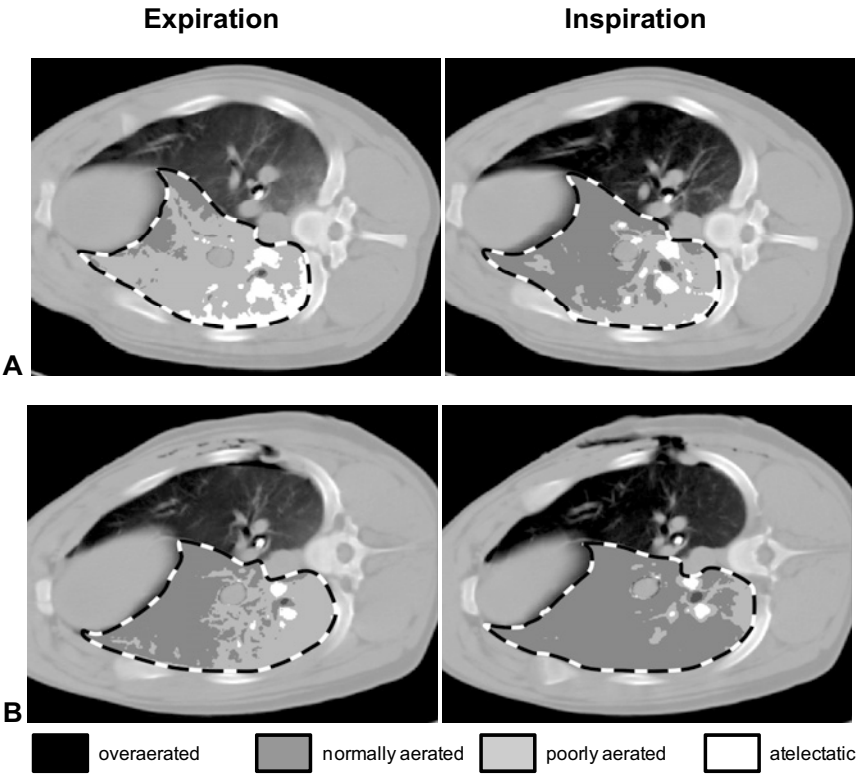


*Figure 13.* Paradiaphragmatic lung CT images of scans during TLV ($V_T$=10ml·kg$^{-1}$) before (A) and TLV after ARM (B). In each image, ROI is depicted by (---); overaerated (-1000 to -900HU), normally aerated (-900 to -500HU), poorly aerated (-500 to -100 HU), and atelectatic (-100 to +100HU) lung regions are coded by gray scale.

ARM resulted in increased arterial oxygenation (to 138%) and static (by 21%) and dynamic lung compliance (by 17%), but was not followed by different ventilation mechanics or haemodynamic parameters. HR (91±6), MAP (77±8), CVP (6±1), and CI (3.8±0.6) remained constant compared with pre-ARM levels.

*Table 3.* Dependent lungs: Volumes of lung compartments [ml] and relative fractions [%] at expiratory and inspiratory hold during TLV before and after ARM. Data are displayed as means (SD) within groups ventilated with a tidal volume of 5ml·kg$^{-1}$ or 10ml·kg$^{-1}$ during OLV. *=p<0.05: TLV after ARM *vs.* TLV before ARM

| | Expiration | | Inspiration | |
|---|---|---|---|---|
| | before | after | before | after |
| Lung volume | 318 (34) | 379 (41)* | 426 (35) | 526 (69)* |
| Atelectasis | 90 (16) | 41 (12)* | 56 (10) | 26 (9)* |
| Poorly aerated | 213 (13) | 262 (41) | 183 (10) | 144 (33)* |
| Normally aerated | 15 (9) | 57 (18)* | 187 (29) | 355 (49)* |
| Overaerated | 0 (0) | 0 (0) | 1 (0) | 1 (0) |

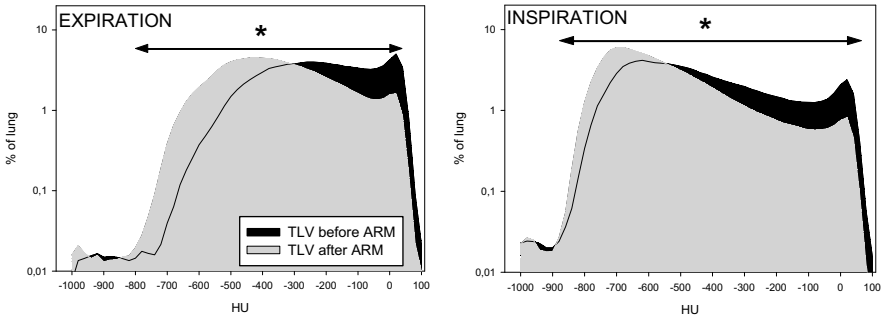


*Figure 14.* Distribution histogram of lung voxels in the dependent lungs of pigs (mean percent of lung volume end-expiratory and end-inspiratory) during TLV before and after application of an alveolar recruitment manoeuvre (ARM). *= p< 0.05: TLV after ARM *vs.* TLV before ARM

# Tidal Volume affects Cyclic Recruitment

In **study IV**, the broncoscopically controlled inflation of the bronchus blocker separated both lungs resulting in a collapse of the nondependent lung and in the delivery of a defined tidal volume of 5ml·kg$^{-1}$ or 10ml·kg$^{-1}$ to the dependent lung only during OLV.

OLV with 10ml·kg$^{-1}$ induced a shift of the inspiratory density spectrum towards compartments with higher gas content. This shift was induced by the reduction of consolidated lung compartments (poorly aerated and

atelectasis) and an increase of normally aerated lung regions reflecting high tidal recruitment. Overaeration covered only an insignificant fraction.

In contrast, ventilation with $V_T$=5ml·kg$^{-1}$ did not affect the radiological density spectrum and the lung density distributions within the four compartments during expiration and inspiration. Both lung density spectrum and distribution remained completely unaffected in comparison with pre-OLV values (figure 15).

Only OLV with $V_T$=10ml·kg$^{-1}$ but not with $V_T$=5ml·kg$^{-1}$ increased peak, plateau and mean airway pressures in comparison with ventilation data prior to OLV. Intrapulmonary shunt increased in all animals, whereas $paO_2$ decreased and $pvCO_2$ increased only during OLV with $V_T$=5ml·kg$^{-1}$.

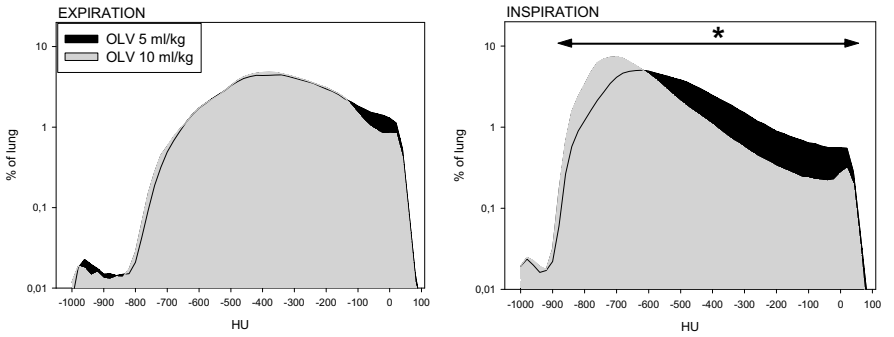


*Figure 15.* Distribution histogram of lung voxels in the dependent lungs of pigs (mean percent of lung volume end-expiratory and end-inspiratory) during OLV with $V_T$=5ml·kg$^{-1}$ or $V_T$=10ml·kg$^{-1}$ (*= $p < 0.05$: $V_T$=5ml·kg$^{-1}$ *vs.* $V_T$=10ml·kg$^{-1}$).

After resuming TLV, the global lung density was redistributed to pre-OLV conditions in all animals without differences between the groups.

However, inspiratory total lung volumes were reduced after OLV with $V_T$=5ml·kg$^{-1}$ to 91%, but not after OLV with $V_T$=10ml·kg$^{-1}$ (97%) compared with TLV after ARM. Poorly aerated regions increased and normally aerated regions decreased in both groups in comparison with TLV after ARM.

Airway pressures, lung compliance and arterial oxygenation returned to the initial values after restoration of TLV without differences between both groups. Haemodynamic data were within normal ranges and remained stable throughout the study period in all animals.

# DISCUSSION

## Summary of the Results

Standard TLV and even more OLV induced lung injury. The resulting DAD scores were equally distributed without differences in the dependent, ventilated and nondependent, temporarily collapsed lungs. Likewise, OLV increased the recruitment of $CD45^{+}$-leukocytes without difference between intravenous (propofol) or volatile (desflurane) anaesthesia.

Mechanisms that have been identified include a substantial V/Q mismatch secondary to hyperperfusion in the ventilated lung and corresponding hypoperfusion of the manipulated lung, as well as cyclic recruitment in the dependent lungs during mechanical ventilation in lateral position that was aggravated by isolated ventilation of the dependent lung. OLV resulted in increased dependent lung volumes and in a persistent shift of the lung CT density spectrum towards compartments with higher gas content.

ARM resulted in a shift of the density spectrum towards compartments with higher gas content and increased total lung volume and was associated with improved arterial oxygenation and lung compliance without changes in haemodynamics during conventional TLV.

Isolated ventilation of the dependent lung with a tidal volume of $5ml \cdot kg^{-1}$ was sufficient to protect the dependent lung tissue from increased cyclic collapse and reopening after ARM. In contrast, OLV with a tidal volume of $10ml \cdot kg^{-1}$ re-increased cyclic recruitment and was accompanied by elevated airway pressures but preserved arterial oxygenation.

## Pulmonary Blood Flow Distribution

The pulmonary vasculature is the dominant factor in determining perfusion distribution although gravity influences V/Q matching [43-46]. Recent experiments using aerosol deposition revealed similar ventilation heterogeneities closely related to perfusion [47, 48]. These studies also confirmed that positioning has only a minor effect on pulmonary blood flow [43, 49, 50].

SPECT data during TLV before OLV revealed an almost even distribution of perfusion over both lungs in pigs [I]. Ventilation was primarily distributed to the nondependent lung, similar to preferential

ventilation of upper lung regions and perfusion of dependent regions in anaesthetised humans in supine position [51]. Perfusion distribution is only slightly affected by gravitation [52]; additionally, even PEEP or lateral decubitus position had no influence on pulmonary blood flow [53, 54].

During OLV, only a minimal fraction of pulmonary blood flow passed through the nonventilated lung, reflecting the efficient hypoxic pulmonary vasoconstriction in pigs [55]. This may explain the insignificant increase of intrapulmonary shunt in OLV pigs.

After OLV, vasoconstriction in the upper lung induced by microatelectasis and hypoxic exposure [9] may result in a continuous decrease of blood flow. Previously collapsed lung tissue is more prone to formation of atelectasis caused by alterations of surfactant [10] even after complete re-expansion by repetitive vital capacity manoeuvres [56].

In addition, OLV may induce a proinflammatory response preferably in the ventilated lung [36] indicated by an increased neutrophil infiltration in the dependent lung of OLV pigs. Mechanical stretch alters alveolar type II cell mediator release toward a proinflammatory pattern [57]. Most immune mediators act as vasodilators. Therefore, cytokine-induced direct vasodilatation may have resulted in a shift of perfusion to the dependent lung in the OLV group.

OLV-induced lung tissue damage can thus be attributed to hyperperfusion and hyperinflation of the ventilated lung. The increased pulmonary capillary pressures during OLV may cause stress failure of the pulmonary blood-gas barrier with extravasations of fluid into the interstitial space (interstitial oedema) and the alveoli (alveolar oedema) as well as rupture of small blood vessels (microhaemorrhage). This effect was possibly amplified by hyperinflation of the ventilated lung during OLV. Increased airway pressures by relatively high tidal volumes produced overdistension of alveoli and stretching of lung capillaries. In fact, barotrauma of the lung has been associated with ultrastructural changes in the alveolo-capillary membrane, loss of plasma and red blood cells into interstitial and alveolar space [58].

## Cyclic Recruitment

Lung consolidation preferentially in dependent regions is observed rapidly after induction of anaesthesia and muscle paralysis in animals and humans [59-61]. Several factors may influence the lung density distribution along the vertical axis. These include regional differences of pleural and transpulmonary pressures with cephalocaudal and vertical gradients, shape and weight of lung tissue as well as gravity dependent changes of pulmonary blood volume and compression of the lungs from adjacent anatomical structures (intraabdominal organs [62], exertion and weight of the heart [63]).

Influenced by shape and weight of lung tissue, the tidal volume is preferentially distributed to nondependent regions [64]. Lateral body position additionally decreases functional residual capacity in dependent regions and shifts the ventilation maxima to nondependent areas. Consequently, a displacement of atelectasis from dorsal to most dependent regions was observed [65].

The volume of atelectasis and poorly aerated lung tissue is increased during TLV before OLV. This can be advocated to bronchoscopic manipulations of the airways open to the atmosphere while inserting and inflating the bronchus blockers in OLV pigs [II, IV]. However, the procedure is obligatory to avoid cuff herniation or blocker insufficiency.

OLV reduced consolidated lung compartments, increased gas content and dependent lung volume [II]. During OLV, atelectatic tissue was changed into poorly aerated and poorly aerated into normally aerated lung tissue resulting in a shift of the spectrum towards lower density and in significant tidal recruitment within the different density compartments [66].

Although dependent lungs were ventilated with the entire tidal volume during OLV, a significant overaeration was not observed. Overaeration is a radiological phenomenon describing a defined lung density of -1000 to -900 HU. Overstretching in contrast implies a mechanical force applied to the lung tissue that may occur at the boundaries of atelectatic, poorly and normally aerated tissue [67] and may have been underestimated by CT.

After collapse, the density distribution of the nondependent lungs returned to that before OLV despite complete lung collapse and repetitive vital capacity manoeuvres. In contrast, the dependent lungs retained an increased lung volume with higher gas content. The fraction of normally aerated lung regions increased according to redistribution of ventilation towards dependent areas. Possible causes may be decreased alveolar recoil forces or air trapping. These mechanisms however could not be discriminated by computed tomography.

Cyclic recruitment of alveolar units results in shear stress with extensively elevated transmural pressures to the lung parenchyma [68]. Shearing and stretching may have profound consequences on lung function and mediator release, and have been detected as key factors in initiating an inflammatory response [27, 69, 70].

## Diffuse Alveolar Damage

Histopathological analysis of the lung is used to estimate the alveolar damage resulting from mechanical ventilation [71-73]. Accordingly, the Diffuse Alveolar Damage (DAD) score was established to evaluate the alveolar integrity [39]. DAD includes a sequence of pathologic lung features

that reflect the harmful consequences of mechanical ventilation or surgical manipulation [41]. Consequently, the extent of specific morphological findings (diffuse alveolar injury, pulmonary oedema, alveolar haemorrhage) correlates with increased mortality following acute respiratory failure [74].

The DAD scoring system was adapted by several investigators to include a weighting system [75, 76]. The score used in the studies [I, III] was closely related to that and may therefore allow quantitative analysis of ventilation-induced histopathological changes.

Mechanical ventilation with high [77] or low tidal volumes [78] may induce alveolar pro-inflammatory cytokine release and pulmonary tissue damage. The highest DAD values in sheep that underwent high-pressure ventilation were found in the most dependent lung regions [79]. In contrast, study III revealed diffusely distributed damage of lung parenchyma. Although the pigs underwent lateral positioning, DAD was not influenced by location in parahilar, intermediate and subpleural regions or in the direction of gravity. However, atelectasis formation was described predominantly in dependent lung regions [65, 80]; thus there was no close link between atelectasis and regional DAD scores. As a consequence, not the presence of atelectasis itself, but the cyclic collapse and re-opening of alveoli may be considered the major pathogenic factor of alveolar damage.

Mechanical forces during OLV exceed the strains of standard TLV and may be considered as a “second hit” to the lung tissue [81]. Delivery of the entire tidal volume to the dependent lung was accompanied by increased inspiratory airway pressures; consequently, OLV-induced lung injury was three times higher than alveolar damage after TLV. The injury involved the entire dependent lung without differences in tissue samples from subpleural, intermediate and parahilar locations.

Diffuse distribution of lung injury does also not reflect OLV-induced changes of regional perfusion [I] or OLV induced cyclic tidal recruitment in the most dependent and basal regions of the dependent lung [II]. Cyclic collapse and reopening however, is not limited solely to atelectatic or poorly aerated lung regions [66] but may also occur at the boundaries of atelectatic, poorly and normally aerated tissue [67].

Experimental thoracic surgery induced lung injury in the nondependent lungs of OLV pigs, thus DAD was increased by surgical manipulation [I, III]. Epithelial damage and leukocyte influx in the nondependent lung may vary with surgical technique, intensity and duration of manipulation and an unconsidered component of re-expansion pulmonary oedema after lung collapse [82].

## Leukocyte Recruitment and Anaesthetic Drugs

The Leukocyte Common Antigen CD45 is principally expressed by white blood cells (granulocytes, lymphocytes, monocytes) and plays a key role in regulation of the inflammatory process [83]. The sequestration of leukocytes into the alveoli is a very early sign in the pulmonary response to stress and precedes histopathological consequences of injurious ventilation [84]. Likewise, the degree of leukocyte influx into the lungs of ARDS patients reflects the extent of lung injury and correlates to increased pulmonary permeability [85, 86]. However, TLV did not increase leukocyte recruitment despite induction of significant alveolar histological changes [III]. The results of study III suggest that mechanical ventilation itself may induce alveolar damage but otherwise healthy lungs may tolerate moderate stress applied to the alveoli and do not necessarily develop clinically relevant proinflammatory responses [87].

OLV increased early leukocyte sequestration in pigs parallel to the observation of increased alveolar cell recruitment by OLV in patients undergoing thoracic surgery [25]. Like other halogenated anaesthetics, desflurane may increase dynamic compliance by decreased peak inspiratory pressure and respiratory resistance at 1MAC concentrations [88] suggesting protective effects on mechanical forces during OLV. Accordingly, desflurane administration decreased granulocyte cytokine release and resulted in different alveolar leukocyte subsets but did not change the total number of intraalveolar cells [36]. The present data [III] confirmed this result. The total number of $CD45^+$-cells was unaffected by propofol and desflurane anaesthesia. However, the short postoperative observation period in the present experiment may reflect only the early leukocyte influx. It could thus not be excluded that the use of different anaesthetics has changed the leukocyte activation pattern. Moreover, there were no differences in ventilatory mechanics between OLV groups. This may have prevented immunomodulatory effects of anaesthetics.

## Alveolar Recruitment Manoeuvre and PEEP

ARM combined with PEEP improves the pulmonary gas exchange by restoration of physiological ventilation/perfusion distribution and prevention of atelectasis formation [31, 89]. Mild to moderate PEEP levels are sufficient to keep recruited alveoli open in healthy subjects without deterioration of haemodynamics or hypoxic pulmonary vasoconstriction [90, 91]. An external PEEP of 5cm$H_2O$ did not increase the total PEEP in patients during OLV [92]. In pigs, PEEP levels of 5-10cm$H_2O$ were associated with improved oxygenation and continuous lung volume recruitment but

15cm$H_2O$ PEEP resulted in overdistention and increased shunt compared with the other PEEP levels [93]. Appropriate PEEP however is found only after a recruitment manoeuvre. A maximum amount of effectively expanded alveoli is yielded by the highest compliance with the lowest dead space fraction [94].

The reduction of $FiO_2$ to 0.4 will protect the lungs from re-collapse for a prolonged period [56]. Furthermore, evidence exists that the lowest possible $FiO_2$ should be delivered to the thoracic patient to prevent oxidative damage and postoperative ALI [95].

However, the ARM and PEEP are valuable not only to treat hypoxemia during mechanical ventilation. More importantly, as part of a protective ventilation strategy, it may decrease cyclic alveolar collapse and reopening of alveolar units. Data of study IV demonstrate that consolidated lung compartments in the dependent lungs resulting from induction of anaesthesia and bronchoscopic airway manipulations (bronchus blocker positioning) could be significantly reduced by the application of 40cm$H_2O$ to the whole lung for 10s and PEEP of 5cm$H_2O$ during TLV. The restoration of physiological gas/tissue relationship included reduced consolidated lung compartments and thus provides improved initial conditions for following OLV.

## Effects of Tidal Volume Reduction

Atelectasis formation and intrapulmonary shunt increase followed by gas exchange impairment are major problems during OLV and thoracic surgery. Shunt is caused by both temporary nondependent lung collapse and partial collapse of the dependent, ventilated lung resulting from atelectasis and poor aeration of lung compartments.

Despite the consequences of an extensively decreased lung volume, traditionally relatively high tidal volumes in the range of 10ml·$kg^{-1}$, $FiO_2$ of 1.0 and zero end-expiratory pressure during single lung ventilation are still common in clinical practice. The objective is to maintain arterial oxygenation and carbon dioxide elimination by the stabilisation of alveoli in the ventilated lung [18, 96].

However, OLV with high tidal volume and successively elevated airway pressures may seriously harm alveolar integrity [23]. Experimental data on OLV with a tidal volume of 10ml·$kg^{-1}$ revealed that especially dependent and basal regions of the ventilated lung are subjected to increased cyclic collapse and reopening of lung tissue, particularly of atelectatic and poorly aerated compartments [II, IV]. Thus, shearing and stretching with extensively elevated transmural pressures have been identified to be the most important factors in initiating lung injury [24, 27]. Although consolidated lung

compartments could be reduced by ARM and PEEP in pigs, OLV with $V_T$=10ml·kg$^{-1}$ was still associated with a considerable cyclic recruitment within the dependent lungs [IV]. Consequently, this traditional approach needs to be questioned.

There is a growing body of evidence that the use of protective ventilation approaches including the reduction of tidal volume may decrease lung injury after OLV [34]. In patients undergoing OLV, the proinflammatory response and incidence of acute lung failure is reduced with the use of lower tidal volumes [33]. However, a mechanism responsible for these protective effects has not yet been identified.

It could be demonstrated that based on the redistribution of gas and tissue after ARM and PEEP the reduction of tidal volume ($V_T$=5ml·kg$^{-1}$) during OLV was sufficient to minimise tidal recruitment by the preservation of density distributions in the dependent lung and may thus exert protective effects on lung tissue [IV]. Moreover, OLV with $V_T$=5ml·kg$^{-1}$ was not associated with increased atelectasis formation in accordance to a recent CT study on TLV [97].

## Limitations of the Model

The study protocol was closely related to a typical thoracic surgical procedure. However, major limitations of the animal model include the short postoperative follow up, major physiological differences of humans and pigs, including their limited capacity to develop shunt caused by a very efficient hypoxic pulmonary vasoconstriction and the fixed ventilation setup with tidal volumes as used during TLV.

High tidal volumes are still recommended in current anaesthesia textbooks to avoid atelectasis, to minimise intrapulmonary shunt and to maintain sufficient gas exchange [18]. Protective OLV ventilation strategies are increasingly used during clinical practice as evidence is growing around the deleterious effects of injurious ventilation [33].

Histological analysis included only the initial rapid alveolar response caused by the short postoperative follow up. The later developments of clinically relevant pulmonary changes which may affect outcome have not been included into the study protocol. The cytokine triggered alveolar immune response is persistent in the postoperative period, thus the effects of volatile anaesthetics may have been underestimated.

A disadvantage of the model is the inability to discriminate between overaeration and overstretching of lung tissue by CT. Overaeration is a radiological phenomenon that may be overestimated in the development of

ventilator induced lung injury. Overstretching however implies mechanical forces applied to the lung tissue that cannot be discriminated for technical reasons.

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In summary, the experimental model of thoracic surgery was sufficient to demonstrate the injurious effects of one-lung ventilation on alveolar integrity. Two mechanisms of ALI could be identified including hyperperfusion and hyperinflation of the ventilated lung and increased cyclic recruitment of dependent lung tissue with a shift of the resting expiratory position towards increased aeration.

Total lung recruitment and PEEP reduced consolidated lung compartments during TLV. OLV with reduced tidal volume and PEEP preserved the radiological lung density spectrum, suggesting a reduced tidal recruitment and protective effects on lung tissue.

The present animal model provides the opportunity to investigate whether different anaesthesia techniques or alternative ventilation approaches may additionally reduce mechanical stress to lung tissue during OLV.

# CONCLUSIONS

I. One-lung ventilation (OLV) in pigs induced a persistent hyperperfusion in the dependent lungs without differences in ventilation distribution. Hyperperfusion resulted in a significant ventilation/perfusion mismatch. The increased pulmonary blood flow in the dependent lung may aggravate stresses to the alveolo-capillary unit and may thus contribute to pulmonary complications after thoracic surgery.

II. The increased cyclic recruitment during OLV in pigs was associated with persistent increased aeration of the dependent lung. Cyclic recruitment has been identified as a key factor of acute lung injury.

III. OLV results in diffusely distributed diffuse alveolar damage (DAD) and increased leukocyte recruitment in the ventilated lung. The OLV-induced lung injury was reproduced in different studies using an identical ventilation setup.

The surgical lung shows increased DAD scores and leukocyte recruitment. The expression of lung injury varies with the degree of invasiveness.

IV. Alveolar recruitment manoeuvres and PEEP improved arterial oxygenation and lung compliance and reduced consolidated lung compartments during conventional two-lung ventilation. OLV with reduced tidal volume did not aggravate cyclic closure and re-opening of alveoli in the dependent lung. This may have protective effects from mechanical stress on lung tissue and may thus reduce lung injury after OLV.

V. The overall conclusion will thus be that the standardised porcine model of thoracic surgery allows investigations of pathophysiological and histopathological effects of OLV and offers thus possibilities to examine the efficiency of lung protective ventilation approaches.

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