INTRODUCTION. Mechanically ventilated (MV) patients may present airway inflammation and increased secretion production. However, it is not known whether cellular profiles in bronchial samples may be useful to evaluate the clinical condition of patients and bronchial sampling for such purposes is not yet standardized. [1]
OBJECTIVES. Our aim was to evaluate homogenized and non-homogenized samples obtained bronchoscopically from different parts of the bronchial tree, in order to access the optimum material for cell counting and protein evaluation.
METHODS. Patients admitted in the Intensive Care Unit of the University Hospital of Larissa, Greece, were enrolled in the study if they were intubated and mechanically ventilated. 3 types of bronchial samples were collected via bronchoscopy (PENTAX FB-15X):
a) tracheo-bronchial secretions macroscopically visible at bronchoscopy (VS)
b) tracheo-bronchial aspirates from surfaces without macroscopically visible secretions (IS)
c) mini bronchial lavage after the instillation of 5 ml of sterile normal saline (miniBL) on surfaces without macroscopically visible secretions and
d) bronchial lavage (BL) after the instillation of 50 ml of sterile normal saline in subsegmental bronchi.
Samples were homogenized in a Heidolph Silent Crusher S. Homogenized and non-homogenized samples were used for cell count after Trypan Blue staining and for protein extraction. Total protein was measured using the Bradford method after treatment with Lysis buffer (20 mM Tris-Cl pH 8.0, 150 mM NaCl, 1% Triton X-100, 1 mM dithiothreitol, 100 mg/mL-1 PMSF). Cell extracts were cleared by centrifugation (1000g, 20min, 4°C) and optical density was measured photometrically in samples with or without cells.
RESULTS. There were no significant differences in both cell numbers and total protein concentration between homogenized and non-homogenized samples. Handling time- time to extract protein and create smears - was 35 min less compared to non-homogenized samples. Total cell number in homogenized samples of IS, VS and miniBL were statistically significantly lower than in BL [677.2*103±144.7*103, 2249*103±508.4*103, 964.2*103±221*103and 2492*103±792.7*103 respectively, ($p< 0.05$)]. Total protein measurements in IS, VS were statistically significantly higher than in BL [12.21±1.99 μg/ml, 17.61±2.17 μg/ml and 8.41±1.81 μg/ml, respectively, ($p< 0.05$)]; there was no statistically significant difference between samples with or without cells.
CONCLUSIONS. Homogenization of bronchial samples in MV patients does not seem to affect cell numbers and total protein concentration. Homogenized samples were easier to handle and therefore could represent a biomaterial useful for protein extraction and examination.

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Respiratory PaO_2 oscillations and their relationship to dynamic atelectasis measured by CT during tidal ventilation in a surfactant depletion model of ARDS

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INTRODUCTION. It has been hypothesised that respiratory oscillations in PaO_2 (O_2Os) in the injured lung are caused by cyclical recruitment of atelectasis (CA)[1,2]. These phenomena have been observed independently using rapid PaO_2 measurement[3,4] and CT[5,6] respectively. Here we explore the hypothesised relationship between O_2Os and CA by contemporaneous measurement of PaO_2 and tidal lung density changes in a porcine surfactant-depletion model of lung injury.
OBJECTIVES. We hypothesised that the amplitude of O_2Os increases with increasing CA during tidal ventilation in a porcine, surfactant depletion model of Acute Respiratory Distress Syndrome (ARDS). The aim of this study was to establish the role of CA as a determinant of O_2Os.
METHODS. This study of n=5 domestic pigs (mean weight (±SD) = 29.6 (±1.7) kg) at the Hedenstierna Laboratory, Uppsala University, Sweden was approved by the local ethics committee and adhered to ARRIVE guidelines. Animals under general anaesthesia were studied after saline lavage during tidal ventilation with VCV & PCV at V_T 10 ml kg^{-1}, RR 12 min^{-1} and variable I:E ratios. Contemporaneous measurements of atelectasis and PaO_2 were recorded using single, juxtadiaphragmatic slice dCT (temporal resolution of 250ms) and a rapid intra-arterial, fluorescence-quenching PaO_2 sensor.
RESULTS. A total of n = 148 sections of 30 s O_2O data were analysed. Fig. 1(A) demonstrates there is no direct relationship between the change in atelectasis and the respiratory PaO_2 oscillation amplitude (R^2's = 0.06 - 0.18) across the group. Mean PaO_2 and respiratory PaO_2 oscillation amplitude for each ventilator condition are shown in Tab.1 & Fig.1(B).
CONCLUSIONS. This is the first study to contemporaneously use a dCT measurement of CA and dynamic measurement of PaO_2 in the injured lung. It demonstrates that the presence of CA is not the sole, main determinant of respiratory PaO_2 oscillation amplitude in a saline-lavage model of lung injury in pigs, and supports further investigations in the dynamic, often overlooked, role of pulmonary perfusion within the complex context of pulmonary responses to mechanical ventilation.

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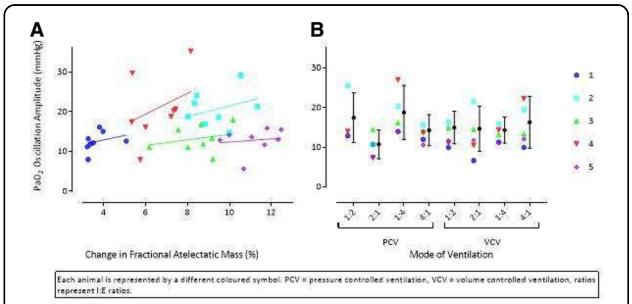

Fig. 1 (abstract 0767). (A) O2O amplitude vs change in atelectasis, (B) mean O2O amplitude by mode of ventilation

Table 1 (abstract 0767). Mean PaO2 & O2O amplitude during different ventilatory conditions

I:E Ratio	PCV		VCV	
	Mean PaO_2	O_2O Amplitude	Mean PaO_2	O_2O Amplitude
1:2	189 (36)	17 (6)	198 (34)	15 (4)
2:1	148 (50)	11 (4)	195 (41)	14 (6)
1:4	204 (19)	19 (7)	197 (27)	14 (3)
4:1	170 (29)	14 (4)	198 (24)	16 (7)

Values shown are mean (±SD) mmHg. PCV = pressure controlled ventilation, VCV = volume controlled ventilation

0768

Development of an ex vivo invasive mechanical ventilation model for preclinical aerosol studies

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INTRODUCTION. Nebulization is a widely used technique for delivering drugs to patients under mechanical ventilation (MV) in intensive care units (ICU). However, the effectiveness of this drug delivery and optimal conditions for implementation still remain poorly documented. Further clinical trials for MV patients are needed but in vivo human studies are scarce because of ethical restrictions due to imaging technique in nuclear medicine. Consequently, few in vivo trials are feasible and anatomical models remain controversial (1). Thus we need an easy to manage, cost-effective, reproducible and physiologically validated model.
OBJECTIVES. This study aim to validate the MV of an ex vivo chimeric lung model previously developed in our laboratory (2) for deposition pattern of inhaled particles.
METHODS. The intra-thoracic part of the respiratory tract was obtained from porcine slaughterhouses and arranged in a sealed enclosure. Data about wounds potentially leading to abnormal results were collected. Tracheal intubation respected physiological angulation. MV was achieved with an Evita 4 ventilator (Dräger, Germany). Ventilation parameters were set based on a recent epidemiological study of MV patients (3). They were as follows: volume controlled, heated humidifier, fraction of inspired oxygen: 21%, tidal volume: 540 mL, positive end expiratory pressure: 9 mbar, respiratory rate: 20 cycles per minute with an inspiratory / expiratory ratio of 1/2, inspiratory flow: 35 liters per minute (3, 4).
The first part of the study was dedicated to physiological measurements using a pneumotachograph and a differential pressure sensor.
The second part consisted in 81m Krypton scintigraphies to assess the homogeneity of ventilation. A manual count of total relative uptake corrected for background signal attenuation was made for each lung.
RESULTS. Twenty-three respiratory tracts were set for MV. Physiological measurements showed good coherence with human data. Average values and their standard deviations (SD) are as follows: tidal volume, 479 mL SD 52 mL; minute volume, 9.6 L/min SD 1.1 L/min; leakage volume, 1.2 L/min SD 1.1 L/min; resistance, 3.1 cmH2O/L/sec SD 0.9 cmH2O/L/sec; compliance, 339.6 mL/cmH2O SD 195 mL/cmH2O. The 12 scintigraphies realized showed homogenous ventilation (Fig. 1) having an average of 52.3% and 47.7% of respectively right and left lung counts (SD, 3.7%) meaning great similarity with human studies (5, 6).
CONCLUSIONS. This preclinical model provides an easy to use, reproducible and cost-effective tool to later achieve larger aerosol studies under mechanical ventilation.

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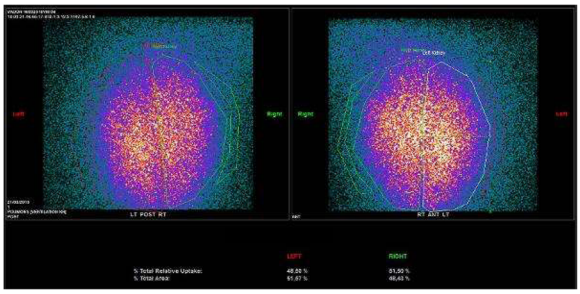

Fig. 1 (abstract 0768). 81m Krypton planar scintigraphies of porcine lung invasive ventilation with the regions of interest

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Not only pendelluft: multifold local inspiratory air-redistribution events continuously occur in collapsible lungs. An experimental study

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INTRODUCTION. Macroscopic air-redistribution events related to strong inspiratory efforts causes unpredictable overstretch in heterogeneous lungs during spontaneous breathing (SB). The micromechanics of air-redistribution in heterogeneous lungs has not been investigated and quantified before.