Draft a protocol for a RCT for assessing the effectiveness of Alveolar Recruitment Maneuver During Cesarean Section in Improvement of Lung Compliance

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Πρόγραμμα Μεταπτυχιακών Σπουδών

ΜΕΘΟΔΟΛΟΓΙΑ ΒΙΟΪΑΤΡΙΚΗΣ ΕΡΕΥΝΑΣ, ΒΙΟΣΤΑΤΙΣΤΙΚΗ ΚΑΙ ΚΛΙΝΙΚΗ ΒΙΟΠΛΗΡΟΦΟΡΙΚΗ

ΠΑΝΕΠΙΣΤΗΜΙΟ ΛΑΡΙΣΣΑΣ – ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ

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Title page

Study Title

A phase II Randomized Control Trial to assess the effectiveness of Alveolar Recruitment Maneuver During Cesarean Section in Improvement of Lung Compliance

Recruitment maneuver (RM) and Positive End Expiratory Pressure (PEEP)

RM is the application of a sustained increase in positive pressure to the breathing airway in order to re-open collapsed alveoli or, in other words, the periodic delivery of passive breaths at high pressure and volume, which may improve alveolar recruitment and oxygenation.

PEEP is defined as an elevation of transpulmonary pressures at the end of expiration. PEEP contributes to the re-opening of collapsed alveoli and opposes alveolar collapse thus improving V/Q matching (ventilation/perfusion ratio).

Indication studied

The investigators will test the hypothesis that RM during cesarean section, in women under general anesthesia improves lung compliance and gas exchange. The investigators will apply RM and leave PEEP at 8cmH2O and ventilation with low tidal volume in pregnant women who decided to have a cesarean section under general anesthesia. The investigators believe and will try to prove that RM improves lung compliance.

Brief Description

This will be an Interventional , Prevention, Parallel Assignment, Double Blind (patient, caregiver, outcomes Assessor), Randomized; Safety/Efficacy Study. We will use two parallel groups of pregnant women. In the first group we will use RM, PEEP and low tidal volume during mechanical ventilation (RM Group) while in the second group (Control Group) we will use the standard method (not RM, not PEEP and "standard" tidal volume). The estimated duration of the study will be 6 months.

Patient Enrollment, Start and Completion date

Enrollment: 90   patients

Study Start Date: April 2015

Primary Completion Date: September 2015

Study Completion Date: September 2015

Protocol Identification

ClinicalTrials.gov Identifier: NCT01826968

Study ID Number: Lung Recruitment in Cesareans GHPyrgosRECProt [RECProt010113]

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Sponsor

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Ethics

The Institutional Review Board will review and approve the study. The study will be performed in compliance with good clinical practice (GCP):

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

STRUCTURE AND CONTENT OF CLINICAL STUDY REPORTS

Patient Information and Consent

Written informed consent will be obtained obtained from all patients at allocation.

Abbreviations

RM: Recruitment Maneuver

FRC: functional residual capacity

Com_{dyn}: Dynamic compliance

dv/dp: difference in alveolar volume/difference in inspiratory pressure

P_{peak}: Peak airway inspiratory pressure

PEEP: Positive end expiratory pressure

V/Q: ventilation/perfusion ratio

VILI: ventilation induced lung injury

GA: General anesthesia

ALI: Acute lung injury

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

MAP: Mean arterial pressure

BPM: Beats per minute (heart rate)

$ETCO_2$: End tidal CO_2

ITT: Intension to Treat

LOCF: last observation carried forward

PCV: Pressure Control Ventilation

VCV: Volume Control Ventilation

Abstract

Introduction: In pregnant women the supine position, during cesarean section, reduces functional residual capacity (FRC) and worsens lung compliance. In a prospective randomized double-blind clinical trial the investigators will test the hypothesis that alveolar recruitment maneuver (RM) during cesarean section, and after delivery, in women under general anesthesia improves lung compliance. The primary end point of the study will be the improvement in dynamic lung compliance (dv/dp) while the improvement in gas exchange (increase in PO_2, PO_2/FiO_2, PH and SpO_2 and decrease in PCO_2 and end-tidal CO_2) and difference in P_{peak} will be the secondary endpoints of the study.

Materials and Methods: Women undergoing cesarean section will prospectively assigned to one of two groups. In the RM group, one minute after delivery, the investigators will apply RM: The ventilator will be switched to pressure control mode (from volume control) and the inspiratory time will be increased to 50%. The Positive End Expiratory Pressure (PEEP) will be increased up to $15cmH_2O$ and the Positive End Inspiratory Pressure (Ppeak) progressively will be increased up to $45cmH_2O$. The whole RM will last 2 minutes. Then, volume control ventilation will be used again and PEEP step wised will be decreased to $8cmH_2O$. Women in the RM group will be ventilated with low tidal volume after the RM (6 ml/kg). In the control group, the investigators will not use lung RM at all. Women in this group will be ventilated with higher tidal volume (8 ml/kg), which is a common tidal volume in everyday clinical practice. In both groups, lung compliance will be measured (dynamic compliance) as dv/dp (ml/cmH_2O). Measures will be assessed before RM but after delivery, 3 minutes after delivery and after recruitment, 10 and 20 minutes after recruitment. At all time points Ppeak, blood pressure, heart beats per minutes, oxygen saturation, end-tidal CO_2 and blood gas will also be measured too. In order to detect clinically meaningful differences, a power analysis will be conducted between the two study groups with regards to dynamic lung compliance (dv/dp), which is the primary outcome of the study. Women will be assigned to one of the two groups using computer-generated blocks.

Results and Discussion: Data will be presented as mean value (+/- SD) while $p < 0.05$ will be considered significant. Mixed Analysis of Variance will be used for comparisons between and within group. There will be no significant difference in lung compliance between groups before RM ($p > 0.05$). Two-sample t-test to compare the two groups at each time point will be used as supplementary analysis, while chi-square test for categorical variables and two-sample t-test for continuous variables for patient and procedural characteristics differences between the two groups, will be used. In the RM group, according to similar studies, the investigators are expecting to see a significant increase in lung compliance after RM compared to control group were RM was not applied. In the RM group the investigators estimate that dynamic lung compliance will be significantly higher at all time points (3 minutes, 10 and 20 minutes) compared to baseline compliance or compliance before RM too. In the control group there should be no significant difference in dynamic lung compliance at the different time points. There should be no significant demographic differences between groups while the investigators estimate that there will be no statistical or/and clinical significant differences in SpO_2, blood gas, end-tidal CO2, Ppeak, and women hemodynamic.

Introduction

General anesthesia (GA) can cause changes in respiratory mechanics and lung function, which persist for days after surgery. These changes occurs immediately after induction of anesthesia but aggravated with mechanical ventilation[1-5]. The reduction in lung compliance and the formation of atelectasis after the induction of GA is a significant cause of abnormalities in regional ventilation and gas exchange[4]. Two different types of atelectasis, compression atelectasis and resorption atelectasis have an impact on patient's condition. The reduced activity of the diaphragm and the weight of lung or abdominal organs in the supine position promote the formation of compression atelectasis.

Atelectasis formation

Compression atelectasis form rapidly, whereas resorption atelectasis arise slowly and can be linked directly to small tidal volumes in combination with an FiO2 of 1.0[3]. This second kind of atelectasis appears in regions with a low ventilation/perfusion ratio (V/Q). Perfusion enables transportation of oxygen from the alveolus into the blood much faster than oxygen enters the alveolus from the upper airways. Due to oxygen's rapid diffusing capacity and the pressure difference across the membrane, gas is 'sucked out' of the alveolus until it collapses. The pressure in the collapsed alveolus is almost zero, and re-opening of this reabsorption 'sticky' atelectasis requires a positive pressure of 30 – 35 cmH2O. Compression atelectasis (in which gas remains in the occluded acinar compartment) can be re-opened more easily ('loose' atelectasis) with pressures between 10 and 12cmH2O. The onsets of compression atelectasis dependents on patient's position, for example during sleep or bed rest. In healthy patients, the areas of atelectasis and V/Q mismatch are reduced and minimized by physiological mechanisms (mainly changes in perfusion, Q). Co- morbidities that enhance the shunt fraction such as oedematous lungs [for example in acute respiratory distress syndrome (ARDS) or cardiac failure] have a markedly disturbed V/Q and lung function in these patients is worsened further as soon as anaesthesia is inducted[3].

Atelectasis during Anesthesia

Atelectasis is a major cause of impaired oxygenation during anaesthesia. The supine position alone reduces the functional residual capacity (FRC) by approximately 0.8 – 1.0 l. The FRC falls by another 0.4 – 0.5 l after induction of anaesthesia. Anesthetic induction results in a loss of inspiratory muscle tone and additionally, the loss of the dorsal flattening moves the diaphragm cephalad. The change in chest wall rigidity on one hand and the upwards shift of the diaphragm on the other decrease the transpulmonary pressure (the difference between alveolar and pleural pressure) and promote alveolar collapse and compression atelectasis. The formation of atelectasis occurs in up to 90% of patients, but it is observed particularly in obese patients in whom the regions of the lung that are atelectatic are increased by 10% compared to those of healthy patients, and can lead to clinical difficulty. Compression atelectasis can develop with spontaneous ventilation, resulting in a high shunt fraction independent of the mode of GA (intravenous or inhalational agent). The secondary reduced oxygenation due to formation of atelectasis under GA does not lead to intraoperative or postoperative morbidity in most patients, who will be extubated immediately at the end of surgery. Also mechanisms such as coughing or sneezing reopen their atelectasis. Nevertheless, some atelectasis is still present in healthy patients for 24 h after surgery, despite the area affected and, therefore, the clinical relevance may be small. However, these poorly aerated areas may become a site of infection[6]. In obese patients, 7.6% of the lung volume remains atelectatic after extubation, and this increases to 9.7% over the first 24 h after surgery. In these patients, the use of long-acting neuromuscular blocking agents should be avoided as they have been found to be a risk factor in developing hypoxemia and muscle weakness in the recovery room due to their inhibition of diaphragmatic muscle recovery after anaesthesia[7]. Our "healthy population"–pregnant women mimics obese patients.

Ventilation Induced Lung Injury

There is probably a strong link between ventilation induced lung injury (VILI) and the repetitive

shear stress applied to the lung parenchyma, the repetitive opening and closing of the atelectatic lung areas which finally destroys the lung tissue[1,4]. Alveolar Recruitment Maneuver (RM) and positive end-expiratory pressure (PEEP) could ameliorate these atelectatic lung areas and improve lung mechanics and oxygenation[3,4,8,9]. Repetitive recruitment and de-recruitment of collapsed lung units disrupt endothelial and epithelial cells, leading to increased permeability of the alveolo-capillary membrane and increased production of IL-b, IL-6, IL-8, and other inflammatory cytokines. Even short- term mechanical ventilation may act as a proinflammatory stimulus in non-injured lungs, and is dependent on the ventilator settings used[1].

Cesarean Section and General Anesthesia

Although cesarean section is usually performed under regional anesthesia, GA is always a possibility mainly in emergency situations. Pregnant women have healthy lungs but, on the other hand, pregnancy induces pathophysiological changes, which in combination with the induction of GA make these women prone to perioperative pulmonary complications. During pregnancy there is an increase in intraabdominal pressure, which causes cranial shift of the diaphragm and a reduction in FRC. The above changes become more pronounced in the supine position and predispose pregnant women to airway closure mainly in the dependent lung regions. Mechanical ventilation strategies, which could improve lung compliance and oxygenation in pregnant women, have never been investigated. In previous studies RM in combination with PEEP improved oxygenation and lung compliance after induction of anesthesia in healthy weight and obese patients[10-16]. The feasibility of the above ventilation strategy has been tested in laparoscopic and in major abdominal surgery too[2,17,18]. The above concept resembles the lung-protective ventilation strategy (low tidal volumes, PEEP and RM), which is considered the best practice in critical care patients and which reduce mortality among patients with acute respiratory distress syndrome[19]. Total compliance (CT) is the relationship between changes in pressure (dp) and changes in volume (dv) of the lungs and thorax, as expressed by the equation: CT (ml/cm H2O) = dv/dp, where CT is a function of lung compliance (CL) and chest wall compliance (CCW): $CT = 1/(1/CL+1/CCW)$. In clinical practice, only CT is measured dynamically or statically, depending on whether a peak or plateau inspiratory dp is used for the CT calculation. During a positive pressure inspiration, transthoracic pressure increases to a peak value and then decreases to a lower plateau value. Transthoracic pressure decreases to a plateau value after the peak value because over time, gas is redistributed from stiff alveoli into more compliant alveoli. Less pressure is required to contain the same amount of gas, which explains why the pressure decreases. Dynamic compliance (Com_{dyn}) is the volume change divided by the peak inspiratory transthoracic pressure, and static compliance is the volume change divided by the plateau inspiratory transthoracic pressure. Therefore, static CT tends to be slightly greater than dynamic CT, although the measurements are quite similar in patients without pulmonary diseases.

Study objectives

Overall, we will test the hypothesis that the application of RM after delivery (during cesarean section) and then the maintenance of low tidal volume and PEEP throughout the operation, improves lung Com_{dyn} and perioperative oxygenation. The aim of the study is to prove that the above anesthetic technique is effective in preventing lung atelectasis, which is a major cause of abnormalities in lung mechanics, lung ventilation and gas exchange in women under cesarean section.

For safety reasons a **preliminary data analysis** has already been performed, before the beginning of the study. At all time points after intervention there is a significant difference in Comdyn between the two groups (p<0.001) while Ppeak was significantly lower in the RM group too (p=0.021). PO2/FiO2 and PO2 were also significantly higher in the RM group vs the control group (p<0.001). For more details see the preliminary data results section too.

Investigational Plan

Study Population and control group

This will be a prospective randomized double-blind clinical trial. After approval of the Institution Ethics Committee (General Hospital of Pyrgos Scientific and Ethic Committee), this trial will conducted at Pyrgos General Hospital between April 2015 and September 2015. After having written informed consent for the participation to the study and in order to obtain GA, **ninety pregnant women** (the number originates from the power analysis - see below) with Anesthesiologists Physical status Classification scores (ASA) 1-2 will be scheduled for cesarean section. The **control group** will be managed with the usual anesthetic technique as mentioned before (no recruitment maneuver, no PEEP, usual tidal volumes). This is a no treatment concurrent control trial.

Inclusion criteria

Pregnant women > 18 years old with BMI < 40 will be eligible for the study. They should accept to have GA instead of regional anesthesia. Women with >130 beats/minute and concomitant pulmonary or/and cardiac pathologies will be excluded from the study (see next section too).

Exclusion criteria

Exclusion criteria will be age<18 years old, patient refusal, body mass index (BMI) more than 40 kg/m^2, active asthma (requiring bronchodilator therapy), pulmonary hypertension, severe pulmonary disease, intracranial hypertension, low arterial blood pressure (hemodynamically unstable patient), heart rate >130 beats/minute, cardiac dysfunction (left ventricular ejection fraction <40%) and emergency surgery.

Removal of Patients from therapy

The major cause for removing patients from therapy will be the serious hemodynamic instability and mainly persistent hypotension (mean arterial blood pressure < 50 mmHg). The majority of our population is a "healthy population" and the hemodynamic impact of the RM is not suspected to be serious. However possible underlying co-morbidities could amplify a minor to median drop in blood pressure during the RM[3,20,21]. Of course except of the RM, other reason could have a serious hemodynamic impact in pregnant women during cesarean section (inferior vena cava syndrome, blood loose etc.). Tachycardia or bradycardia could be other possible reasons for patient's removal. In many cases pregnant women suffers from tachycardia, which is due to the increased stress. We will decided to remove patients from therapy only if tachycardia (>120 beats/minute) persists after delivery. In pregnant women bradycardia (<50 beats/minute) is extremely seldom. A possible asthma attack during the operation, and after induction of anesthesia, would be another reason for removing patients from therapy.

Randomization

Randomization will be performed using computer-generated blocks (available at: http://www.randomization.com) and each pregnant woman assigned to participate in the study will have a sequentially numbered sealed envelope (from 1 to 90) containing a randomization code (A or B). Only the main investigator (DA) will be responsible to wit what A or B means (for example A=RM and B= control). Patients will be randomized to one of two ventilator management groups (RM, control) with 45 patients per group. The envelopes will be concealed until after consent will be obtained. All cesarean sections will be performed by the same team of surgeons and by the same anesthesiologists (DA, AM). In appendix 1 there is an example of manually performed permuted blocks randomization with 90 patients, which could also be used.

Treatments

Anesthetic technique (prior and post-manipulation therapy)

The anesthetic technique will be the same for both groups of patients and is standardized as follows: Anesthesia will be induced with propofol (1.5-2 mg/kg), fentanyl (1 μg/kg) and rocuronium (1.5 mg/kg) and will be maintained with sevoflurane (minimal alveolar concentration 0.6-0.8) and fentanyl (400-450 μg). The minimum alveolar concentration of sevoflurane will be titrated from the attending anesthesiologist according to the arterial blood pressure (± 15-20 mmHg baseline) and beats per minute (± 10-15 baseline). Rocuronium for muscle paralysis will be used once, during induction of anesthesia to facilitate tracheal intubation (cuffed tube no 7-7.5-Portex, Inc., London) and without subsequent bolus doses, while at the end of the operation sugammadex (200 mg) will be used for neuromuscular block reversal. Intraoperatively ondasentron 4 mg, omeprazole 40 mg, paracetamol 1000 mg and Parecoxib 40 mg will be used to all the patients. The time between the end of preoxygenation and tracheal intubation must be standard (40-60 seconds) and will be recorded for all the patients. The Perioperative use of crystalloids and colloids, blood products and vasopressors will be recorded while fluid management will be left to the discretion of the attending anesthesiologist (Ringers Lactated). Hypotension (mean arterial pressure <60 mmHg) will be treated with ephedrine bolus 5 mg i.v and if insists bolus doses of hydroxyethylstarch (HES 130/0.4, Voluven; Fresenious Kabi, Germany) up to 1000 ml will be given. The standard monitors of the American Society of Anesthesiologists will be used while the radial artery will be cannulated for invasive blood pressure monitoring and blood gas measurements.

Ventilatory Management (time and method of RM)

Before induction of anesthesia preoxygenation (100% oxygen via face mask) for 2 minutes will be conducted to all the patients. After tracheal intubation, pregnant women in both groups will be mechanically ventilated (Datex-Ohmeda, Aestiva/5, Madison, WI, USA) with volume-controlled mode and with tidal volume at 8-ml/kg ideal body weights. Inspiratory to expiratory time ratio will be set to 1:2 while 40% oxygen fraction (balanced with air) will be used. The respiratory rate will be adjusted to maintain an end-tidal CO_2 between 30-35 mmHg and zero end-expiratory pressure will be applied (ZEEP). Immediately after delivery the control group of women will continue with exactly the same ventilator management while in the RM group lung recruitment will be achieved as follows:

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The ventilator will be switched to pressure-controlled mode and the inspiratory time will be increased to 50% (inspiratory /expiratory ratio will be set to 1:1). The Peak inspiratory pressure (P_{peak}) will firstly be set to 20 cmH$_2$O (for 3 breaths) and then the investigators progressively will increase the positive end-expiratory pressure (PEEP) to have a P_{peak} up to 45-50 cmH$_2$O. The PEEP sequential will increase in four steps from 0 to 5 cmH$_2$O (for 3 breaths), from 5 to 10 cmH$_2$O (for 5 breaths), from 10 to 15 cmH$_2$O (for 7 breaths) and from 15 to 20 cmH$_2$O (for 10 breaths). The whole RM will last about 2 minutes. After the RM the investigators will use volume control ventilation again with the baseline settings mentioned above, tidal volume 6ml/kg and PEEP step-wise decrease to 8 cmH$_2$O, which must be maintained until the end of the operation. Lung recruitment in the RM group will not be repeated, as cesarean section is a short lasting operation. After completion of the operation and neuromuscular block reversal women in both groups will breath spontaneously before tracheal extubation and PEEP will be eliminated in the RM group. Also see flowchart below:

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Study Design – Ventilatory Management Flowchart

RM Group

| Baseline (usual ventilation management) | →3minutes→ | **RM** Measurements PEEP and low tidal volume | →10 minutes→ | Measurements | →20 minutes→ | Measurements | →stop mechanical ventilation→ | Postoperative measurements |

Control Group

| Baseline (usual ventilation management) | →3minutes→ | **No RM** Measurements | →10 minutes→ | Measurements | →20 minutess→ | Measurements | →stop mechanical ventilation→ | Postoperative measurements |

Blinding procedure

This will be a double blind clinical study. The patients will not know the kind of the mechanical ventilation that they will receive. The only person who will be un-blind will be the primary investigator (DA). After the RM (or not), a second anesthesiologist (AM) will come into the operating room and records the monitor measurements. The same person will also receive blood for blood gas analysis according to the specified time points. Every different patient is just a number (1,6,3...9, 43, 78. etc.) for the investigator. The measurements will be recorded in special files for each separate patient (see appendix 3,4,5, and 6) and then in a SPSS data file (SPSS version 22) and will be analyzed by two different persons (the primary investigator who can find the treatment of each patient and a second statistician who is blind). The results should be matched. Of course for this kind of study a single blind procedure (only for the investigator who record the measurements) would be enough, as the placebo effect does not exist in anesthetized patients. We decided to conduct a double blind study just for the postoperative period records.

Measurements

Primary end-point

The improvement of ***Com$_{dyn}$*** will be the ***primary end-point of the study***. In the RM group Com$_{dyn}$ will be recorded both before (baseline) and after the RM and the application of PEEP (3 minutes) and then at 10 and 20 minutes, while it will also be measured at the same time points in the control group of women. Com$_{dyn}$ (ml/cmH2O) will be measured automatically by the monitor spirometry (Datex-Ohmeda Capnomac Ultima, P&V spirometry) according to the following formula: dv/dp where maximum tidal volume will be used for dv and the difference Ppeak-PEEP will be used for dp.

Secondary measurements

Ppeak is the maximum end-inspiratory airway pressure and will be measured too. The same monitor will be also used to measure the following parameters: ETCO2, alveolar minute ventilation (tidal

volume multiplied by ventilatory frequency) and expiratory tidal volume. In both groups of patients Com$_{dyn}$ will be measured after delivery, but before RM (baseline measurement), and then 3, 10 and 20 minutes after the RM. At the same time points arterial blood pressure (systolic, diastolic and mean), heart rate, SpO2, blood gas, ETCO2, Ppeak, expiratory tidal volume and minute volume will be measured too. SpO$_2$ and blood gas will be also measured postoperatively (10 minutes after extubation). Tables 2 and 3 present the basic patient's measurements. Data analysis example comes from preliminary data analysis before the beginning of the study.

Follow up measurements

Patients will be followed up for postoperative respiratory failure, which is defined as the need for non-invasive ventilation and/or mechanical ventilation after extubation. In cases of respiratory failure, the possible pulmonary complications are also being defined: pneumonia, atelectasis, pulmonary embolism, pneumothorax, acute lung injury (ALI), asthma, non-cardiogenic pulmonary oedema and severe hypercapnia. The routine postoperative oxygen use will also be noted and will be applied for half an hour postoperatively.

Statistical analysis

Before the beginning of the study, and for **determination of sample size**, we will conduct **power analysis**, in an attempt to estimate the sample size required so that the study would have adequate power to detect clinically meaningful differences between the two study groups with regards to lung compliance (dv/dp), which is the primary outcome of the study. For this analysis, we used the G*Power Software that is freely available online at http://www.gpower.hhu.de/en.html from the Psychology Department of the University of Dusseldorf, in Germany.

Our power analysis is based on previous studies but is even more generous[17,22]. The required sample size calculation was based on the following assumptions: Clinically meaningful difference between the two groups is 20% of the mean value, and SD of the observed lung compliance is 25% of the mean value (rather generous), therefore size effect d= 20% /25% = 0.8, with two-sided testing, with alpha = 0.05 and b = 0.1 (therefore, power = 1-b = 0.9). Under these assumptions, G*Power indicated that we will need minimum 34 patients per group for this study to have adequate power. To confirm accuracy of this calculation, we will also conduct sample size estimation using the Statistica v 7.0 Statistical Software package (StatSoft Inc, Tulsa, Oklahome, USA), in order to find out if this confirmatory analysis will produce identical results. Finally we will confirm our results using the suitable mathematic formula (see appendix 2).

In order to allow for possible protocol violations, errors, patient attrition or other study shortcomings, we will enroll 45 patients in each arm of the study (about 10 women more in each group of patients). As pregnant women are a very "sensitive" population and we are afraid of much protocol violation (removal women from protocol mainly due to hemodynamic instability) we will chose to randomize 45 women per group, which is a generous number.

We will check if data are normally distributed (Kolmogorov Smirnoff and Shapiro Wilks test). Ventilatory and haemodynamic data and intraoperative and postoperative variables (all continuous variables) will be presented as mean values (+/- SD). Data will be compared between and within groups using Mixed Analysis of Variance (Mixed Anova-General Linear Model) if are normally distributed or with Kruskal Wallis test in case that we do not have normally distributed data.

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Bonferoni correction will be used for multiple comparisons correction. In order to check and supplement our Mixed Analysis of Variance we will also use two-sample t-test to compare the two groups at each time point (for ventilatory and haemodynamic variables). Patient and procedural characteristics will be compared between groups using chi-square test for categorical variables and independent-sample t-test for continuous variables. Two tailed P-values <0.05 will be considered statistically significant. For data analysis SPSS statistical software version 22 will be used.

Patients Records

For each patient we will have four data files:

1. Demographic intra- and post-operative patient data (appendix 3)
2. Mechanical ventilation data (appendix 4)
3. Haemodynamic data (appendix 5)
4. Blood gas data (appendix 6)

Data Presentation Plan

Number of eligible women and number of women excluded before randomization (lets say X):

Possible reasons for exclusion:

X1 women could decline participation and choose regional anesthesia, X2 could have emergency surgery, X3 will have active asthma, X4 will have BMI >40, X4 will be < 18 years old, X5 will suffer arterial hypertension etc. If we add: X1+X2+X3+X4+X5+. . . Xi=X

Finally we will include 45 patients in each group.

Number of women that will complete the study = F (For example 40 women in the RM group and 41 women in the Control group)

Give reasons for not complete the study: Heart rate >110bpm, Low arterial blood pressure, hypertension, asthmatic attack during the surgical procedure etc.

Flow chart:


```
                    ┌─────────────────────┐
                    │  Number of Eligible │
                    │       women         │
                    │   (pts screened)    │
                    └─────────────────────┘
┌─────────────────────────┐
│ Number of women excluded│
│          (X)            │       ┌─────────────────────┐
│   X1=reason, X2=reason, │       │  Women Randomized   │
│     X3=reason, etc      │       │       (n=90)        │
└─────────────────────────┘       └─────────────────────┘

┌─────────────────────────┐   ┌─────────────────────────┐
│   45 in the RM Group    │   │  45 in the Control Group│
│  Finally completed the  │   │  Finally completed the  │
│    study :40 women      │   │    study : 41 women     │
└─────────────────────────┘   └─────────────────────────┘
      ┌─────────────────┐         ┌─────────────────┐
      │ Give reasons for│         │ Give reasons for│
      │ not complete the│         │ not complete the│
      │study for 5 women│         │study for 4 women│
      └─────────────────┘         └─────────────────┘
```
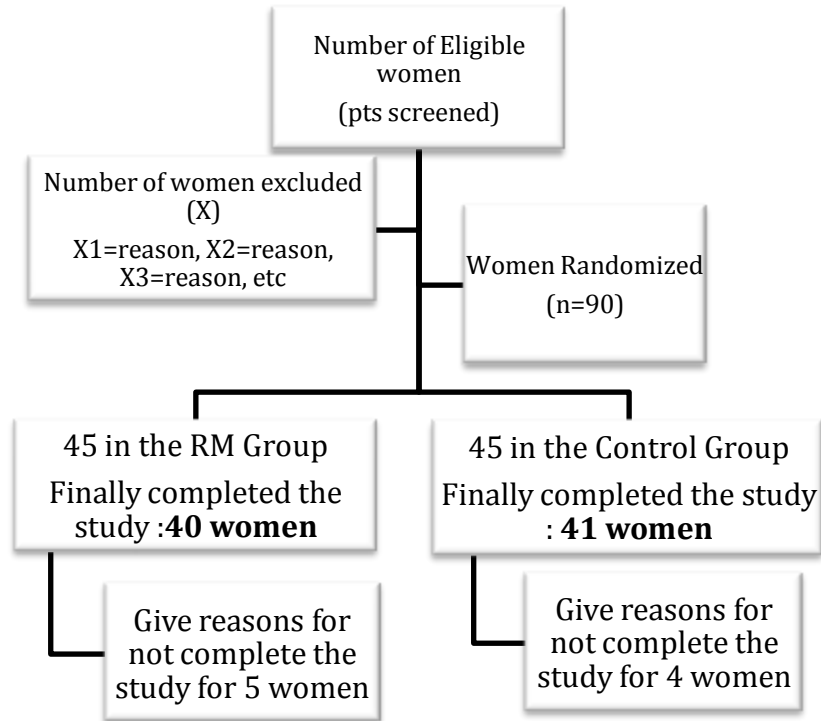
If all, or almost all of the patients will complete the study (we will have the most of the measurements in each time point) we will use a per protocol analysis. If we have many missing data we will use an intention to treat analysis of data (ITT). According to ITT analysis of data I will use all the data of all the randomized patients regardless of whether they completed the trial or not. For the missing values I will use the "last observation carried forward" (LOCF) approach

Patient characteristics should be similar between the two groups (table 1). If not we will have to mention the differences and this would be a serious limitation of our study.

Intraoperative characteristics (time to delivery, anesthesia duration, estimated blood lose, administration of crystalloids and/or colloids, administration of blood products and intraoperative use of ephedrine should be similar between the two groups (table 1).

The length of hospital stay and the possible differences between the two groups should be mentioned (table 1). The number of patients that will suffer postoperative pulmonary complications (mainly pneumonia, diagnosed with chest radiogram and clinical evaluation) and possible differences between the two groups should also be mentioned. Pneumothorax would be a serious adverse event, which could have a strong relationship with the RM manipulation of the patients and should be recorded. See adverse event section below.

Table 1: Patient characteristics presented as mean (SD) or n=number of patients. Intra- and post-operative characteristics presented as mean (SD).

Patient Characteristics	RM Group n=45	Control Group n=45
Age (yrs.) [mean (SD)]	A1	B1
BMI (kg/m^2) [mean (SD)]	A2	B2
Smoking status (n)	A3	B3
Intraoperative Characteristics		
Duration of anesthesia (min)	A4	B4
Time to delivery (min)	A5	B5
Crystalloids (ml)	A6	B6
Colloids (ml)	A7	B7
Blood (units)*	A8	B8
Ephedrine dose (mg) +	A9	B9
Postoperative Characteristics		
Length of hospital stay (days)	A10	B10
Pulmonary complications (n)	A11	B11
Pneumothorax (n)	A12	B12
Pneumonia (n)	A13	B13
Other (n)	A14	B14

Efficacy and Safety issues:

The efficacy of the RM to improve lung compliance and gas exchange will be the main efficacy issues of the study. The investigators believe that the Com_{dyn} (dv/dp) will be statistical significantly improved in the RM group compared to the control group (statistical significant difference between groups). A 20% increase in mean Com_{dyn} in the RM group compared to the control group would be clinically significant. This change in lung compliance perhaps would not have any significant impact for the majority of the patients of our study (young and healthy patients). However, underlying co-morbidities may amplify changes in lung and cardiovascular physiology and anaesthesia may serve as a 'second-hit' that worsens patient outcome. For this reason a RM and the application of protective lung ventilation (low tidal volumes and PEEP) perhaps should be the suitable anesthetic practice for all the patients.

The investigators also believe that there would be statistical significant difference within groups too. In the RM group possibly there would be a difference in lung compliance (increase) between baseline and all the other time points (3, 10 and 20 minutes after the RM). Again a 20% increase in Com_{dyn} would be clinically significant, at least for some patients.

The investigators believe that the recruitment manoeuvre will improve the intraoperative and/or postoperative PO_2, the PO_2/FiO_2 and the SpO_2. Again for the population of our study (all women have a baseline $PO_2/FiO_2>400$) this improvement will not have a significant impact. All the data will be presented in tables 2 and 3 *(see* **next example with preliminary data analysis).**

Because of the higher inspiratory pressures during RM and PEEP used, the $P_{plateau}$ and P_{peak} are anticipated to be significantly higher in the RM group, compared with those in the control group, and this would be a possible serious safety issue, as high alveolar pressures could result in pneumothorax or/and barotrauma. As the study involves young and healthy population, the investigators believe that the above safety issues will not be a serious problem of the study. A second safety issue will be the hemodynamic instability during RM. Patients with serious hemodynamic instability will be removed from the study, as already mentioned.

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End of treatment

The treatment (RM) will start after delivery (3 minutes) and will last until the end of the surgery. Specifically, in the RM group the RM will be performed 3 minutes after delivery but PEEP and low tidal volume (which are part of the whole RM technique) will be continued until the end of the operation.

End of Study

The patients will be monitored throughout the operation and in the postoperative period too (see also the measurements section-primary end point, secondary measurements and follow up). The follow up will last for all the length of hospital stay. The length of hospital stay will be recorded while all the adverse events that will be occurred during this period they will be recorded too. For this kind of population the hospital stay is usually short (<5 days).

Adverse events:

Hemodynamic instability during the RM will be the most important adverse event of the study. During the RM all women should be closely monitored for the occurrence of hypotension or the administration of a fluid bolus or vasopressor medication to support haemodynamic variables. Of course during the whole course of anesthesia the need for vasopressor should be monitored. Possible differences between the two groups should be mentioned for all the above parameters (table 1).

Postoperative lung complications (pneumonia, atelectasis, pulmonary embolism, pneumothorax, acute lung injury (ALI), asthma, non-cardiogenic pulmonary oedema and severe hypercapnia) should also be recorded. As we will mainly include young and healthy population serious lung complications and differences between groups are not anticipated.

Preliminary Data Results

For safety issues we decided to perform a preliminary data analysis before the beginning of the study. We used 7 patients in each group, and we will use these data analysis as an example of our data presentation plan and to show the most important measurements of our study. At all time points (3, 10 and 20 minutes) after intervention there is a significant difference in Com_{dyn} between the two groups (p<0.001). The Com_{dyn} is much higher in the RM group vs control group (table 2). The significant difference in lung Com_{dyn} depicts in figure 1a too.

Post intervention the peak inspiratory pressure at all time points (3, 10 and 20 minutes) is significantly lower in the RM group compared to control group (p=0.021, table 2, figure 1b).

PO_2/F_iO_2 and PO_2 are also significantly higher in the RM group vs the control group (p<0.001) at all time points post intervention (3, 10 and 20 minutes) while PO_2 is also significantly higher in the RM vs control group postoperatively (p<0.001). SpO_2 is significantly difference between the two groups at 10 and 20 minutes post intervention and postoperatively (p=0.04) – table 2

There are no significant differences in expiratory tidal volume, minute volume, end tidal CO_2, PCO_2 and in hemodynamic data between the two groups (tables 2, 3).

Table 2. Respiratory Mechanics and Gas Exchange in the two groups of pregnant women (recruitment vs control, respectively-**Example**). Data are presented as mean ± SD. The P-value corresponds to the tests of between subject main effects using the mixed Anova test (recruitment vs control group). The two groups were also compared separately at each time point using the independent two-sample t-test (*) and (**). (*) denotes significant differences from the control when $P<0.01$ while (**) is used whenever $P<0.05$. PaO_2, arterial blood oxygen; $PaCO_2$, arterial blood CO_2; SpO_2, arterial blood oxygen saturation; FiO_2, oxygen concentration.

| | Baseline | After intervention (minutes) | | | Postoperative | P-value |
		3 min	10 min	20 min		
Peak Inspiratory Pressure (cmH_2O)					-	0.021
Recruitment	23.9 (2.9)	19.9 (2.5)*	20.2 (2.9)*	20.3 (3.0)*		
Control	23.0 (2.8)	22.1 (2.8)	22.5 (2.7)	22.2 (2.7)		
Com$_{dyn}$ (ml/cmH_2O)					-	<0.001
Recruitment	33.6 (6.8)	51.4 (12.8)*	49.9 (11.2)*	49.7 (10.9)*		
Control	31.1 (7.9)	29.6 (7.2)	30.6 (8.1)	30.9 (8.1)		
Pa$_{O2}$ (mmHg)						<0.001
Recruitment	211.7 (10.6)	242.7 (26.9)*	242.1 (22.0)*	243.5 (27.6)*	108.4 (6.7)*	
Control	214 (18.4)	219.8 (22.6)	214 (22.3)	212 (38.4)	98.4 (5.3)	
PaO2/FiO2					-	<0.001
Recruitment	422.2 (22.1)	481.1 (48.6)*	475.3 (42.7)*	474 (50.3)*		
Control	426.4 (43.3)	429 (39.2)	426 (41.5)	422.5 (37.5)		
ET$_{CO2}$ (mmHg)					-	0.5
Recruitment	30.9 (3.4)	29.2 (3.4)	28.4 (3.8)	28.1 (3.6)		
Control	30.4 (2.9)	30.0 (3.0)	29.4 (2.8)	28.6 (2.7)		
Expiratory Tidal Volume (ml)						0.5
Recruitment	584.5 (63,1)	588 (65.3)	578 (66.2)	581 (65.1)	576 (62.8)	
Control	575.9 (54.2)	582 (66.3)	585 (58.4)	579 (66.3)	585 (64.8)	
Minute Volume (lit)						0.38
Recruitment	7.0 (7.74)	7.1 (0.63)	6.9 (0.65)	7.1 (0.68)	6.9 (0.65)	
Control	6.8 (0.63)	6.9(0.67)	6.8 (0.72)	7.1 (0.65)	6.8 (0.59)	
PCO$_2$ (mmHg)						0.08
Recruitment	33.9 (2.2)	32.7 (3.0)	32.7(3.8)	32.7 (3.7)	35.6 (1.9)**	
Control	34.4 (1.9)	33.7 (2.9)	33.8 (2.9)	33.6 (3.2)	36.5 (1.4)**	
SpO$_2$						0.04
Recruitment	98.8 (0.7)	99 (0.7)	99.1 (0.8)**	98.9 (0.8)**	98.3 (0.8)*	
Control	99 (0.7)	98.9 (0.7)	98.7 (0.6)	98.6 (0.5)	97.7 (0.7)	

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Table 3. Systolic Arterial Pressure, Diastolic Arterial Pressure, Mean Arterial Pressure and Heart rate between the two groups of pregnant women (recruitment vs control, n=40 and n=41 respectively-**Example**). Data are presented as mean ± SD. The P-value corresponds to the tests of between subject main effects using the mixed Anova test (recruitment vs control group). The two groups were also compared separately at each time point using the independent two-sample t-test.

| | Baseline | After Intervention (minutes) | | | P- Value |
		3 min	10 min	20 min	
Systolic Arterial Pressure (mmHg) Recruitment Control	124.5 (6.2) 125.7 (9.1)	121.8 (8.4) 122.7 (9.5)	122.8 (9.8) 120.1 (10.7)	121.9 (10) 119.2 (10.5)	0.63
Diastolic Arterial Pressure (mmHg) Recruitment Control	73.6 (10.5) 72.7 (8.0)	70.5 (10.0) 70.6 (7.6)	70.3 (9.6) 71.1 (7.6)	69.6 (9.0) 71.1 (6.3)	0.8
Mean Arterial Pressure (mmHg) Recruitment Control	88.8 (10.20 88.4 (9.50	83.4 (10.1) 85.8 (7.4)	84.4 (10.3) 83.8 (7.2)	83.8 (10.3) 82.4 (7.2)	0.98
Heart Rate (beats per minute) Recruitment Control	95.1 (12.3) 96.2 (6.7)	92.5 (12.4) 91.6 (6.2)	91.6 (11.6) 91.5 (6.6)	91.4 (12.0) 91.0 (7.0)	0.96

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Figure 1a and 1b: RM=recruitment maneuver group, Baseline=before intervention, 3, 10 and 20 minutes=post intervention time.

Difference in Dynamic Compliance between the two groups
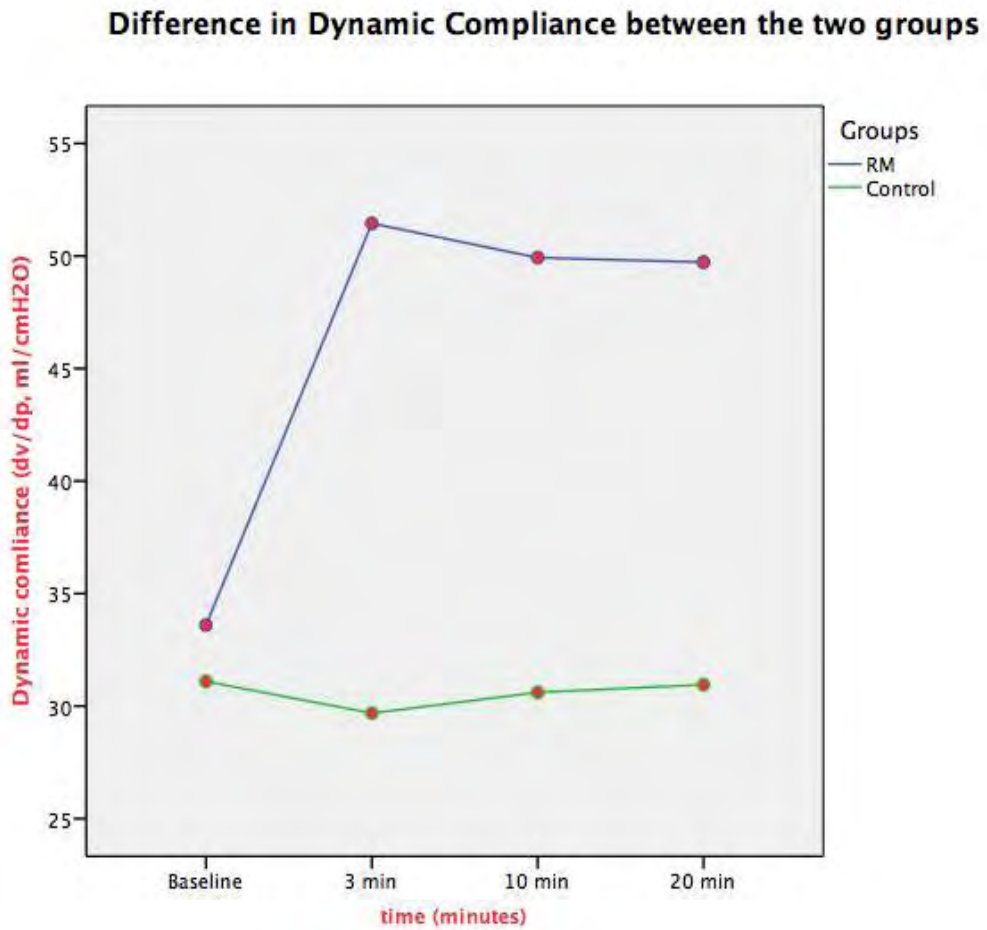
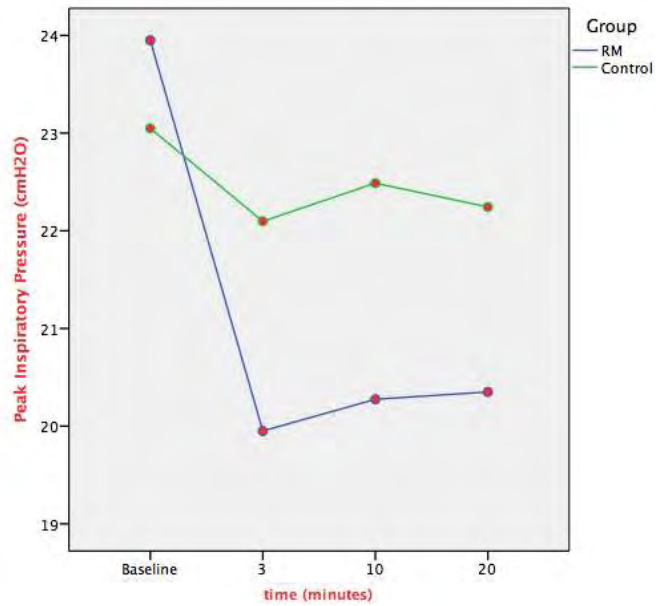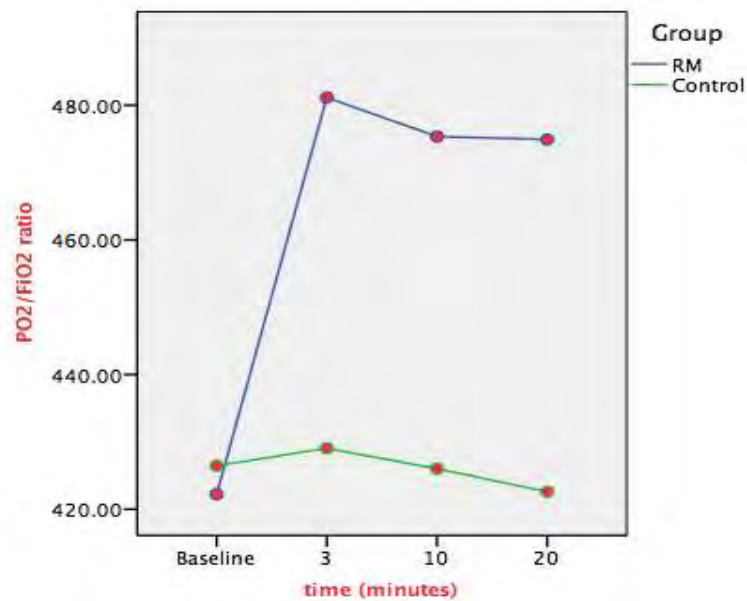**Difference in Peak Inspiratory Pressure between the two groups**

Figure 2:

Difference in PO2/FiO2 ratio between the two groups

Discussion:

Lung-protective ventilation, which refers to the use of low tidal volumes PEEP, and which may also include the use of RM, has been shown to reduce mortality among patients with the acute respiratory distress syndrome and is now considered best practice caring of many critically ill patients[2,9,15,19,23,24]. Over the last years many physicians have questioned the benefits of using lung-protective ventilation in the surgical setting, and in many surgical procedures the results are very positive[2,9,15-18,22,25-31].

Previous studies

In a recent multicenter, double-blind, parallel-group trial with 400 patients the investigator concluded that as compared with a practice of nonprotective mechanical ventilation, the use of a lung-protective ventilation strategy in intermediate-risk and high-risk patients undergoing major abdominal surgery was associated with improved clinical outcomes and reduced health care utilization[2]. In this trial the primary outcome was a composite of major pulmonary and extra-pulmonary complications occurring within the first 7 days after surgery. In a systematic review published in 2014 the investigators developed the benefits of lung protective ventilation during surgery and general anesthesia and offers some recommendations (Lung-protective ventilation, which refers to the use of lower tidal volume and limited Pplateau to minimize overdistension, and PEEP to prevent alveolar collapse at end-expiration) for mechanical ventilation in the surgical context[9]. In another systematic review and meta-analysis which published in 2012 the investigators concluded that although the ideal intraoperative ventilation strategy in obese patients remains obscure there is some evidence that RM added to PEEP compared with PEEP alone improves intraoperative oxygenation and compliance without adverse effects. There was no evidence of any difference between PCV and VCV[16]. In three other prospective clinical trials the RM proved very effective in restoration of lung compliance and lung mechanics in laparoscopic surgery[15,17,18] while one more study in normal weight and obese patients undergoing laparoscopic surgery had the same beneficial results[22]. One more study published in 2012 showed ventilation improvement during thoracic surgery[30] while RM strategy improved arterial oxygenation after cardiopulmonary bypass too[25]. In 2010 Tusman et al. insisted that the application of RMs during anaesthesia normalizes lung function along the intraoperative period and that there is physiological evidence that patients of all ages and any kind of surgery benefit from such an active intervention[29].

Atelectasis is the main cause of postoperative pulmonary complication while the danger is mainly noted in high-risk patients[1,3]. Pregnant women are considered "healthy women" and low risk for GA, but on the other hand mimic they obese patients. This will be the first study, where it will be perform RM in this special population.

Limitations of the study

In our study we will check for differences in Com_{dyn} but not in the static compliance, which is also a very important record of the pulmonary performance. The reason is the lack of the special instruments. Many other records could be performed for respiratory mechanics too.

As we will use both RM and PEEP we will not be able to know if the compliance increasement (if exist) will be due to RM or to PEEP.

While this study will probably demonstrate a restoration in pulmonary compliance and in oxygenation, it will not be powered to describe pulmonary outcomes, such as postoperative atelectasis or pneumonia.

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Appendix 1

Clinical Trial Methodology, Elias Zintzaras

Permuted blocks:

We consider a block of 10 patients and two treatments RM and Control. Then we define an array of 10 numbers, from 1 to 10:

1 2 3 4 5 6 7 8 9 10

A permutation of the array may produce the following blocks:

Block 1: 3 5 1 4 9 8 10 2 6 7

Block 2: 1 6 8 9 10 2 4 5 7 3

Block 9: 4 7 9 2 8 10 6 1 3 5

We need 9 blocks (90 patients)

We assign digits 1-5 to A

Digits 6-10 to B

Block	Sequence	Treatment	Patients
	3	A	1
	5	A	2
	1	A	3
	4	A	4
Block 1	9	B	5
	8	B	6
	10	B	7
	2	A	8
	6	B	9
	7	B	10
	1	A	11
	6	B	12
	8	B	13
	9	B	14
Block 2	10	B	15
	2	A	16
	4	A	17
	5	A	18
	7	B	19
	3	A	20
	3	A	21
	1	A	22
	7	B	23
	9	B	24
Block 3	2	A	25
	4	A	26
	10	B	27
	8	B	28
	5	A	29
	6	B	30

	9	B	31
	3	A	32
	2	A	33
	10	B	34
Block 4	7	B	35
	1	A	36
	4	A	37
	8	B	38
	5	A	39
	6	B	40
	5	A	41
	1	A	42
	9	B	43
	7	B	44
Block 5	2	A	45
	10	B	46
	3	A	47
	4	A	48
	6	B	49
	8	B	50
	9	B	51
	1	A	52
	3	A	53
	7	B	54
Block 6	2	A	55
	4	A	56
	5	A	57
	8	B	58
	10	B	59
	6	B	60
	10	B	61
	5	A	62
	1	A	63
	8	B	64
Block 7	3	A	65
	2	A	66
	9	B	67
	4	A	68
	6	B	69
	7	B	70
	3	A	71
	8	B	72
	10	B	73
	1	A	74
Block 8	9	B	75
	2	A	76
	7	B	77
	6	B	78
	4	A	79
	5	A	80
	7	B	81
	1	A	82
	4	A	83
	8	B	84
Block 9	3	A	85
	10	B	86
	2	A	87
	5	A	88

	9	B	89
	6	B	90

Appendix 2

We will use power analysis – Two independent groups (comparison of means)-Clinical Trial Methodology, Elias Zintzaras

- *Sample size in two independent groups.*
- *The formula for calculating the sample size is:* $n \geq 2\left(\frac{s}{\Delta}\right)^2 (1.96 + 1.28)^2$
- *S = SD (from previous trials it was found that the between subject variance of compliance is 25)*
- *Δ =20 (difference in compliance that is considered clinically significant)*

Consequently,

$$*n \geq 2\left(\frac{25}{20}\right)^2 (1.96 + 1.28)^2 \rightarrow n \geq 33 \text{ patients on each group}$$

* If we include +10% to compensate for potential loss then the final sample size should be at least 37 patients on each group

Appendix 3 - Demographic intra- and post-operative patient's data

Patient ID:	X	
Age (years)	X	
BMI (kg/m^2)	X	
Smoking status (n)	X	X
Duration of anesthesia (min)	X	
Time to delivery (min)	X	
Crystalloids (ml)	X	
Colloids (ml)	X	
Blood (units)	X	
Ephedrine dose (mg)	X	
Length of hospital stay (days)	X	
Pulmonary complications (n)	X	

Appendix 4 - Mechanical ventilation patient's data

Patient ID:	Baseline	3 minutes	10 minutes	20 minutes	Postoperative
Minute volume (ml)	X	X	X	X	X
Tidal volume (ml)	X	X	X	X	X
Compliance (dyn)	X	X	X	X	X
P (peak)	X	X	X	X	X
End tidal CO$_2$	X	X	X	X	X

Appendix 5 - Haemodynamic patient's data

Patient ID:	Baseline	3 minutes	10 minutes	20 minutes	Postoperative
SBP (mmHg)	X	X	X	X	X
DBP (mmHg)	X	X	X	X	X
MAP (mmHg)	X	X	X	X	X
BPM	X	X	X	X	X

Appendix 6 – Patient's Blood gas data

Patient ID:	Baseline	3 minutes	10 minutes	20 minutes	Postoperative
PO_2 (mmHg)	X	X	X	X	X
PCO_2 (mmHg)	X	X	X	X	X
PH	X	X	X	X	X
SpO_2 (%)	X	X	X	X	X
PO_2/FiO_2	X	X	X	X	X

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