**On Line Data Supplement**

**ENROLLMENT:**

The electronic medical record of patients admitted to the Medical and Surgical ICUs at MGH were screened daily for inclusion and exclusion criteria.

**Inclusion criteria**

* 18 years or older
* BMI ≥ 35 kg/m2
* Matching the Berlin definition for the diagnosis of ARDS(1)
* Waist circumference > 88 cm (for women)
* Waist circumference > 102 cm (for men)
* Presence of an arterial line
* Presence of a central venous catheter

**Exclusion criteria**

* Known presence of esophageal varices
* Recent esophageal trauma or surgery
* Severe thrombocytopenia (Platelets count ≤ 5,000/mm3)
* Severe coagulopathy (INR ≥ 4)
* Presence or history of pneumothorax
* Pregnancy
* Patients with poor oxygenation index (PaO2/FiO2< 100 mmHg with at least 10 cmH2O of PEEP)
* Pacemaker and/or internal cardiac defibrillator
* Hemodynamic parameters: systolic blood pressure <100 mmHg and >180 mmHg, or if systolic blood pressure is between 100-180 mmHg on high dose of IV continuous infusion of norepinephrine (>20 μg per minute), or dobutamine (>10 μg per minute), or dopamine (>10 μg per Kg per minute), or epinephrine (>10 μg per minute).

Once an eligible subject was identified, an attending physician of the unit where the patient was admitted approached the patient’s surrogate (court-appointed guardian, healthcare proxy/attorney, spouse, adult child or other close family member) and explained to him/her all the details of the research protocol. Then, if he/she was willing, an investigator went through further details and answered questions regarding the research study. Written informed consent was obtained from the patient’s surrogate.

**STUDY PROTOCOL:**

After assessing proper sedation level (Richmond Agitation and Sedation Score -4/-5)(2) patients were paralyzed by administration of 0.2 mg/Kg of cisatracurium besylate (Nimbex, AbbVie, Cambridge, MA, USA). If throughout the study protocol any sign of spontaneous breathing effort was noticed a further dose of paralytic was administered (0.05mg/kg predicted body weight, (PBW)). Patient’s bed was positioned at 0° above ground level.

Patients were ventilated in volume-controlled ventilation, at tidal volume of 6 mL/kg PBW, respiratory rate was maintained as set per clinical management, I:E = 1:2 and FiO2 was 100%, these ventilator settings were maintained throughout the study protocol.

Recruitment maneuvers were performed in pressure-controlled ventilation respiratory rate = 10 breath/min, I:E = 1:1, pressure control 10 cmH2O, lung recruitment was performed by progressive increase in PEEP by 5 cmH2O every 30 seconds targeting a maximum Plateau Pressure of 50 cmH2O in order to obtain an end inspiratory transpulmonary pressure ( PLI) > 20 cmH2O while avoiding a decrease in compliance below 20 mL/cmH2O.

If hemodynamic instability or decrease in oxygen saturation occurred during the recruitment maneuver the maneuver was interrupted and previous ventilator settings restored until stability was obtained.

**MEASUREMENTS:**

**Measurement system:**

Data from airway and esophageal pressure, gas flow and the capnogram were recorded on a dedicated system (Windaq, DATAQ Instruments, Akron, OH, USA). Sampling rate was 300 Hz. Data analysis was performed offline utilizing *Labview 7.0* derived analysis software. To measure the changes in intra-thoracic pressures and lung volumes within a breathing cycle a resampling and interpolation process was used. A single “average” respiratory cycle was obtained for each patient at different ventilatory settings. Airway and esophageal pressures at end-inspiration and at end-expiration were obtained at zero flow. Tidal volume was calculated as the integral of the expiratory flow-time waveform. Partitioned respiratory system elastances were calculated.

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**Esophageal Manometry**

A nasogastric tube equipped with an esophageal balloon was used for this study (Avea GS smarthCath; Carefusion, Yorba Linda, CA).

Correct positioning of the esophageal catheter was ensured with an occlusion test(3). After inflating the catheter according to manufacturer recommendations, during a prolonged expiratory hold airway and esophageal pressures were recorded during slow bilateral chest compression. The two waveforms were plotted in a 2 by 2 diagram; agreement was graphically and numerically checked. If the ratio between the variation in esophageal and airway pressures was between 0.8 and 1.1 the positioning was considered correct and the catheter was firmly secured in place.

To take into account the effect of esophageal elastance over the measurement of esophageal pressure we reproduced in our patients the methods applied in a recent paper by Mojoli et al.(4). This procedure was performed to identify the minimum volume to inflate the esophageal balloon avoiding any extra pressure exerted by the esophageal wall (VMIN) and to identify the volume that allows the best estimation of the intra-tidal changes in esophageal pressure (VBEST) used to calculate chest wall elastance.

Firstly, we measured the pressure-volume curve (P-V curve) of the esophageal balloon *ex-vivo* where the surrounding pressure was maintained at the atmospheric level. The balloon was deflated to generate a negative transmural pressure (-20 cmH2O) and then progressively inflated by steps of 0.5 mL (Figure E2)(4). The *in-vivo* test was performed at the beginning of the study protocol right after securing the catheter in place. The PEEPARDSnet level was applied and a P-V curve was performed firstly deflating the volume to generate a negative pressure (-20 cmH2O) and subsequently inflating the balloon by steps of 0.5 mL up to a total of 6.5 mL: this volume is the sub-maximal catheter inflation volume that was chosen to avoid the risk of overstretching the esophagus and the balloon catheter.

VMIN was identified as the first inflation step fulfilling simultaneously three conditions: a) The volume was above the *ex-vivo* minimum volume producing non-stressed conditions in the balloon-wall (linear portion with zero slope in the *ex-vivo* P-V curve), b) The *in-vivo* P-V curve was already at its linear, central portion (producing minimum slope), and c) the esophageal pressure at end-expiration equaled or was greater than PEEP.

VBEST was identified as the inflation volume fulfilling simultaneously three criteria: a) The volume was above the *ex-vivo* minimum volume producing non-stressed conditions in the balloon-wall (linear portion with zero slope in the *ex-vivo* P-V curve), b) The *in-vivo* P-V curve was already at its linear, central portion (producing minimum slope), and c) The change in the esophageal pressure between end-inspiration and end-expiration was the maximum among all the volume tested.

The catheter was inflated at VBEST throughout all the study protocol. To calculate partitioned respiratory system elastances the measurements of esophageal pressure while the esophageal catheter was inflated at VBEST were used.

To calculate transpulmonary pressures the measurement of esophageal pressure (recorded with the catheter inflated at VBEST) was corrected according to the following formula:

PesABSOLUTE = PesMEASURED – Esophageal Elastance \* (VBEST – VMIN)

**Volumetric Capnography**

Flow and partial pressure of expired CO2 was measured using infrared absorption technology by a mainstream analyzer positioned distally to the Y-piece and connected to a capnograph (Respironics NM3, Philips, Andover, MA, USA). The signal was averaged over 30 breaths at the three main time-points of the study protocol. Physiologic dead space was calculated by applying Enghoff modification of the Bohr equation(5).

$$\frac{Vd}{Vt}=\frac{PaCO\_{2}-PECO\_{2}}{PaCO\_{2}}$$

Where PaCO2 is the partial pressure of CO2 in the arterial blood sample and PECO2 is calculated as the area under the curve of the volumetric capnogram divided by the expiratory volume. The Fowler dead space (VDF), reflecting the anatomic dead-space volume of the conducting airways, was determined by calculating the expired gas volume until the inflection point of phase II was reached in the volumetric capnogram(6).

**Airways Resistance**

Airways resistance was calculated by applying the least square fitting method to the airway pressure and flow data recorded at each PEEP step during the PEEPDECREMENTAL trial. The least square fitting method relies on the application of the equation of motion of the respiratory system:

$$Paw\left(t\right)=V^{'}aw\left(t\right)\*R\_{RS}+\left(\frac{Vol\left(t\right)}{C\_{RS}}\right)+PEEPtot$$

At any time (t) throughout a breathing cycle the airway pressure (Paw) is equal to the sum of the work performed to overcome: the resistance of the respiratory system (V’aw \* RRS), the elastic recoil of the respiratory system (Vol/CRS) and the total PEEP (PEEPtot).

By knowing three variables (Paw, V’aw and Vol) collected simultaneously throughout a breathing cycle it is possible to derive the other three unknown variables (RRS, CRS, PEEPtot) by performing the following equation:

$$y=x\_{1}\*a+x\_{2}\*b+k$$

**Electrical Impedance Tomography**

The Enlight 1800 (Timpel SA, São Paulo, Brazil) Electrical Impedance Tomography device was used.

Changes in absolute air content were determined by changes in impedance (ΔZ) inside regions of interest(7). Accordingly changes in end expiratory lung volume (EELV) were correlated to changes in end-expiratory lung impedance (EELZ). The correlation factor was determined by the relationship between tidal volume and tidal variation in impedance (tidal ΔZ). Consequently changes in EELV (mL) between two time-points can be derived using the following formulas:

$$ΔEELV \left(mL\right) : ΔEELZ \left(Z\right) = Tidal Volume \left(mL\right) : Tidal ΔZ(Z)$$

$$ΔEELV \left(mL\right)= \frac{ Tidal Volume \left(mL\right)\* ΔEELZ \left(Z\right)}{Tidal ΔZ(Z)} $$

Lung collapse and overdistension were calculated for each electrical impedance tomography pixel by comparing pixel-compliance at different PEEP levels. Each pixel-compliance was determined dividing tidal ΔZ by the variation in pressure during the respiratory cycle (Compliance PIXEL = ΔZ / ΔP)(8). Briefly, to estimate collapse and overdistension, values of pixel compliance were compared among different PEEP levels. Therefore, for a given pixel, if aeration increased and compliance worsened, it meant the onset of new overdistension. On the other hand, if aeration increased and compliance improved, it meant collapse reversal. The maximum compliance and aeration are depicted among all the PEEP steps and used as reference for the comparison. To compare lung morphology between the same PEEP levels obtained during the Incremental and Decremental PEEP titration all measurement were referenced to the best pixel-compliance obtained during the PEEPDECREMENTAL trial after the recruitment maneuver since the recruitment maneuver allows the measurement of ventilation distribution in all the “recruitable” pixels.

To compare lung morphology among PEEPARDSnet, PEEPINCREMENTAL and PEEPDECREMENTAL, the aerated lung area (at Optimal PEEPDECREMENTAL) was divided in 4 regions of interest (ROI) each one covering 25% of the total lung area(9)(figure E3). Then, we measured the distribution of ventilation as percentage of tidal ventilation distributed to each ROI.

**STATISTICAL ANALYSIS:**

**Sample Size Calculation:**

We anticipated enrolling 14 patients in this two-treatment crossover study based on the change in respiratory system elastance at titrated PEEP levels measured in a previous study in a cohort of obese non-ARDS patients(10). The probability of 90 percent that the study would detect a treatment difference, at a two-sided 0.05 significance level, was based on the assumption that the difference in lung elastance between treatments (PEEPDECREMENTAL vs PEEPINCREMENTAL) would have been 1.7 cmH2O/L with a standard deviation of 1.8 cmH2O/L.

**On Line Data Supplement Figures Caption**

**Figure E1. Esophageal Balloon Calibration Pressure-Volume Curve.**

In-Vivo End-Inspiratory Esophageal Pressure (PesI - Empty *Squares*), End-Expiratory Esophageal Pressure (PesE - Empty *Circles*) and a In-Vitro Esophageal Balloon (*Triangles*) pressure-volume curves are presented in the graph. To normalize the starting volume (0 mL) the balloon was deflated until reaching -20 cmH2O intra-balloon pressure. Balloon inflation was performed by stepwise increase in volume of 0.5 mL. Before each step inflation, the balloon was deflated to -20 cmH2O. In the In-Vivo curve, the slope of the shaded part of the curves represents esophageal elastance. The equation describing the PesE its flat part (shaded area) is: y=1.434x+14.371. Data are expressed as Mean±SD.

**Figure E2. Electrical Impedance Tomography Regions of Interest (ROI)**

To compare distribution of ventilation within the lung among the three study steps PEEPARDSnet, PEEPINCREMENTAL and PEEPDECREMENTAL, the aerated lung area (at Optimal PEEPDECREMENTAL) was divided in 4 regions of interest (ROI) along the ventro-dorsal axis each one covering 25% of the total lung area

**Figure E3. Representative Lung CT Scan Images Performed Ahead of the Study Procedures**

*Panel A.* 61 years old male 42 kg/m2 BMI, admitted to the ICU for post operative (knee surgery) respiratory failure. *Panel B.* 40 years old female 72 kg/m2 BMI, admitted to the ICU for hypoxemic respiratory failure.

*Panel C.* 29 years old 59 kg/m2 BMI, admitted to the ICU for hypoxemic respiratory failure due to pneumonia.

CT scan has been always performed to rule out pulmonary embolism. CT scan images were selected at approximate mid lung to be representative the largest lung parenchymal area.

**Figure E4. Correlation between PLE values at different PEEP levels during PEEPINCREMENTAL and PEEPDECREMENTAL trial.**

*Panel A.* PLE values at different level of PEEP measured during the PEEPINCREMENTAL (empty triangles-dashed line) and PEEPDECREMENTAL (filled squares-continuous line) trial. PEEP levels are expressed as relative values compared to the optimal PEEP level found both in the incremental and decremental PEEP trial. *Panel B.* Bland-Altman correlation between PLE values measured at the same level of PEEP during the PEEPINCREMENTAL and PEEPDECREMENTAL trial. Data are expressed as Mean±SD. PLE = End-expiratory transpulmonary pressure

**Figure E5. Chest Radiographs Before and After the Study Procedures**

Portable chest radiographs obtained within 24 hours before (*Panel A and C*) and 24 hours after (*Panels B and D*) the study procedures in a 68 year-old male BMI= 59 kg/m2 (Panel A and B) and a 66 year-old female BMI=62 kg/m2 (Panel C and D). Note increased lung volumes in panel B compared to A, and in panel D compared to C. Atelectasis demonstrated in Panel C is resolved in panel D. No signs of macroscopic lung over distension or barotrauma are present.

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