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"ULTRAPEEP: Lung ultrasound for the assessment of lung recruitment during esophageal pressure-guided PEEP in ARDS"

Coordinatore: Prof. Nicola Ferri

Supervisore: Prof. Michele Carron

Dottorando: Facchin Francesca

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ABSTRACT

Background: Whereas the importance of low tidal volume to avoid ventilator-induced lung injury (VILI) in patients with ARDS is well known, several uncertainties still exist regarding how to set positive end-expiratory pressure (PEEP). Many approaches have been considered, but no one showed a clear effectiveness in terms of outcome. Recently a ventilator strategy using esophageal pressure to estimate the transpulmonary pressure has been proposed by Talmor and colleagues. Although they found an improvement in arterial oxygenation, it was not explored whether the increase in oxygenation was due to lung recruitment.

Objectives: The aims of this study were: i) to evaluate the differences between the PEEP level and the end-expiratory transpulmonary pressure (PL_{EEO}) by setting PEEP according to ARDS Network and to esophageal pressure (Pes); ii) to assess whether the Pes-guided PEEP is associated with an improvement in oxygenation; iii) to show that Pes-guided PEEP increases lung recruitment estimated by lung ultrasound score (LUS); iv) to determine whether the new setting of PEEP is associated to a change in plasmatic cytokines as markers of VILI.

Methods: 15 patients with moderate and severe ARDS were enrolled. For the first 2 hours, PEEP was set according to the Acute Respiratory Distress Syndrome Network standard-of-care recommendations (phase A). It was then adjusted according to measurements of Pes for the following 2 hours (phase B) to maintain a positive PL_{EEO}. PEEP levels were finally returned equal to phase A for the last 2 hours (phase C). The primary end point was the improvement in lung recruitment assessed with lung ultrasound at the end of phase B.

Measurements and Main Results: Lower PEEP levels and PL_{EEO} were found during phase A compared to phase B [median PEEP was 10 [10.0-12.0] vs 16 cmH_2O [15.5-19.5]; median PL_{EEO} was -3 [-4.0 to -0.5] vs 2 cmH_2O [2-4], $p < 0.02$]. Arterial oxygenation improved in phase B compared to phase A [median PaO_2:FiO_2 during phase A was 149 [120 to 166] vs 166 [153 to 177] during phase

B, $p < 0.05$]. The median LUS was 18 during phase A [11 to 27] and decreased to 15 [11 to 18] during phase B. A decrease in LUS ≥4 (indicating lung recruitment) was found in 10 of 15 patients. Although differences in oxygenation and LUS from phase B to phase C were not significant, the clinical parameters at the end of phase C tended to be similar to phase A. The analysis of plasmatic cytokines revealed no difference between phases.

Conclusions: In patients with moderate and severe ARDS, PEEP-induced LUS reduction and increase in oxygenation seems to indicate that setting PEEP according to the Pes-guided method results in a greater alveolar recruitment than setting PEEP according to ARDS Network strategy. Further investigations are needed to confirm these results and to exclude the presence of PEEP-induced alveolar overdistension.

1. BACKGROUND

1.1. Acute Respiratory Distress Syndrome: Epidemiology and Pathophysiology

Acute Respiratory Distress Syndrome (ARDS) is known since 1967 and is characterized by i) severe hypoxemia; ii) acute onset; iii) radiographic opacities; iv) respiratory failure not fully explained by cardiac failure or fluid overload. (1) (2). ARDS can be caused by different pathophysiological conditions, either pulmonary or non pulmonary. Even though recent advances in clinical management, mortality is still high (40%) and the long term morbidity has a substantial impact in public health (3) (4) (5). No effective pharmacological therapies exist for ARDS and mechanical ventilation is the only treatment with proven efficacy (6).

1.2. ARDS and Ventilator-Induced Lung Injury

Despite the fact that ventilatory support remains the basic therapeutic approach for ARDS, mechanical ventilation can itself exacerbate or cause lung damage (7). The underlying mechanisms of ventilator-induced lung injury (VILI) include: i) alveolar overdistension, due to high transpulmonary pressure (barotrauma) and excessive tidal volume (volutrauma), ii) repetitive opening and closing of alveoli (atelectrauma); iii) release of inflammatory mediators, resulting in a local and systemic inflammatory response (biotrauma) (8).

Recent studies suggest that barotrauma and volutrauma are caused by excessive “stress” and “strain”. Stress, or tension, can be defined as the force which develops in a structure as a reaction to an applied external force of the same entity but opposite direction. The deformation of the structure due to the applied force is called strain (9).

In the lungs during mechanical ventilation stress and strain are periodically changing variables characterized by maximum and minimum values (end-inspiratory and end-expiratory transpulmonary pressure for stress and end-inspiratory and end-expiratory lung volume for strain). While VILI

originates in the lung, it may also affect distal organs by release of mediators from the lung into the systemic circulation; this de-compartmentalization of VILI is presumably one of the causes of multi-organ failure occurring in patients with ARDS resulting in higher mortality rates (8).

1.3. ARDS and bedside Selection of Positive End-Expiratory Pressure

The recognition of these mechanisms has greatly contributed to design lung-protective ventilation strategies (e.g. reduction of plateau pressure and tidal volume; use of positive end-expiratory pressure [PEEP]) (10). These approaches have significantly limited the "stress" and "strain" of lung parenchyma and end-expiration alveolar collapse (11).

Whereas low tidal volume is clearly beneficial in ARDS patients, several uncertainties still exist regarding the use of PEEP (12) (13). Many studies indicate the importance of positive end-expiratory pressure (PEEP) in improving blood oxygenation, preventing VILI, reducing atelectrauma and maintaining lung recruitment. However, strategies to precisely identify the optimal level of PEEP have not been found yet. Three recent large multicenter randomized trials (ALVEOLI, ExPress, LOVS) failed to demonstrate a reduction of mortality using higher versus lower levels of PEEP in patients with ARDS (14) (15) (16) (17). High levels of PEEP could indeed be harmful, causing hemodynamic impairment, lung overdistension and increase in alveolar dead space, in particular with patients with low recruitability. Instead, in patients with high recruitability, the oxygenation response to high PEEP may correlate with the degree of lung recruitment. The PEEP-related increased oxygenation may be due to improvement in ventilation-perfusion (V/Q) matching, not necessarily to lung recruitment (18).

1.4. ARDS and esophageal pressure-guided PEEP

The use of esophageal pressure as a surrogate of pleural pressure can prove useful in setting the best PEEP for individual patients. Talmor et al. demonstrated that esophageal pressure-guided PEEP significantly improves oxygenation and lung compliance in ARDS patients (19). This observation

may suggest that PEEP should be tailored on pulmonary and chest-wall mechanics to maintain a positive transpulmonary pressure (20) (21).

During passive conditions, the pressure applied to move gas into the lung, is delivered by the ventilator and it is equal to the Paw (22). It is important to consider both the lung and chest wall components. In static conditions, when the airway resistance is nil, Paw= P_L + Ppl (where Paw is the airway pressure, P_L is the transpulmonary pressure, and Ppl is the pleural pressure) (9, 23).

Pleural pressure could be estimated with the use of an esophageal balloon catheter, validated to measure esophageal pressure. The calculated P_L can be negative at end-expiration. PEEP could thus be increased until P_L becomes positive at end-expiration to keep airways open (19).

1.5. Thoracic ultrasound and lung recruitment

For the bedside assessment of lung recruitment, a method based on lung ultrasound has been recently validated (24) (25). Lung ultrasonography is emerging as a rapid and non-invasive bedside tool for the detection of specific ultrasound patterns, associated with several pulmonary and pleural disorders (26). In patients with ARDS, lung ultrasound allows the diagnosis of alveolar-interstitial syndrome, lung consolidation, pulmonary abscess, pneumothorax and pleural effusion (24) (27).

The ultrasound detection of multiple and diffuse vertical B lines (comet tails) correspond to moderate decrease in lung aeration, resulting from several heterogeneous conditions with diffuse involvement of the interstitium, impairment of gas exchange, and subsequent respiratory failure (26).

Bouhemad et al have recently demonstrated the accuracy of bedside lung ultrasound for the assessment of aeration changes in ARDS patients (24). By using a recently proposed score (24), they found a statistically significant correlation between lung ultrasound features and PEEP-induced oxygenation increase.

Lung ultrasound score (LUS) could thus be a useful bedside procedure to assess lung response to Pes-guided ventilation. Moreover, bedside lung ultrasound may identify patients in which higher

levels of PEEP, even if physiologically defined, do not correspond to lung recruitment. This is the group of patients who may experience only injurious effects of PEEP.

2. STUDY OBJECTIVES

The aims of this pilot study are to assess whether:

- There are differences between the PEEP level and and the PL_{EEO} by setting PEEP according to ARDS Network strategy and to Pes.
- The Pes-guided PEEP improves oxygenation.
- The Pes-guided PEEP is associated with an increase in lung recruitment estimated by LUS.
- The change in oxygenation is associated to a change in plasmatic cytokines as markers of VILI.

3. MATERIAL AND METHODS

3.1. The Target Population

This pathophysiological pilot study was performed in the Medical Surgical ICU of Padova Teaching Hospital, from February 10th, 2015 to June 19th, 2017.

The study was approved by the institutional review board. Investigators screened all consecutive patients admitted to the ICU during the study period. Patients satisfying all of the inclusion and exclusion criteria were considered eligible.

3.1.1. Inclusion criteria

- Moderate or severe ARDS, defined according to the Berlin definition (2)
- Endotracheal intubation or tracheostomy

3.1.2. Exclusion criteria

- Severe heart failure/cardiogenic shock
- Pulmonary arterial hypertension requiring systemic vasodilators
- Contraindications to esophageal balloon: esophageal pathology (stricture, perforation, high grade of varices), recent history of esophageal or gastric surgery, upper GI tract bleeding, severe coagulopathy and nasal trauma
- Age < 14 years

3.2. Study Design

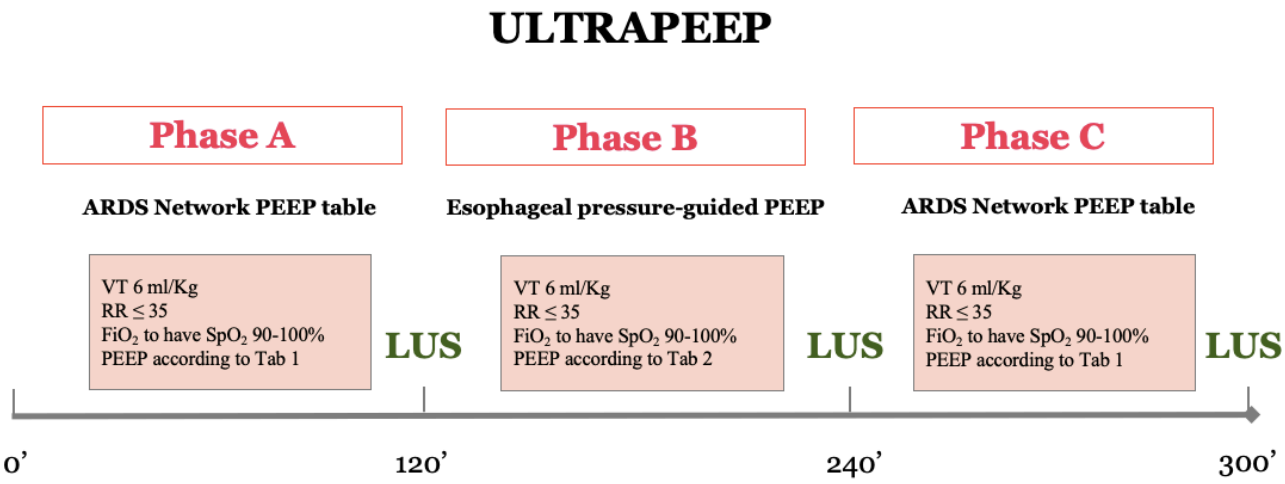

Table 1. Lower PEEP/higher FiO2

FiO_2	0.4	0.5	0.5	0.6	0.6	0.7	0.7	0.8	0.8	0.9	0.9	0.9	0.9	1.0
PEEP	5	5	8	8	10	10	10	12	14	14	14	16	18	18-24

Table 2. Esophageal pressure-guided PEEP

FiO_2	0.4	0.5	0.5	0.6	0.6	0.7	0.7	0.8	0.8	0.9	0.9	1.0
P_{LEEO}	0	0	2	2	4	4	6	6	8	8	10	10

Figure 1. Time course of the protocol. In each patient tidal volume, respiratory rate and FiO_2 were maintained constant. Positive end-expiratory pressure (PEEP) was set according to ARDSNet, following the "Lower PEEP/higher FiO_2" table during phase A and C, and according to transpulmonary pressure, following the "Esophageal pressure-guided PEEP" table during phase B. Each phase lasted 2 hours. At the end of each phase, before changing the ventilation setting, a complete lung ultrasound examination was performed, arterial blood gas, central venous saturation, blood sample for cytokines was withdrawn for analysis and measurements of respiratory mechanics were performed.

3.2.1 Preparation and general management

For this pathophysiological study we enrolled patients with moderate or severe ARDS, within 72 hours of the diagnosis. The study protocol is summarized in Figure 1. All the subjects were supine, with the head of the bed elevated to 30 degrees. Patients were deeply sedated and curarized. The anesthesia was maintained with infusion of propofol (4–5 mg/kg/h) and paralysis with cisatracurium (0.6–1 mg/kg). Heart rate (HR) and cardiac rhythm, mean arterial pressure (MAP), central venous pressure (CVP), transcutaneous O_2 saturation (SpO_2), airway pressure (Paw), end tidal CO_2 ($EtCO_2$) and tidal volume (VT) were constantly monitored. The Oxygenation Index was

calculated as the reciprocal of partial pressure of arterial oxygen and the fraction of inspired oxygen (PaO_2:FiO_2) times mean airway pressure Data acquisition and analysis were done with the PowerLab Data Acquisition System (AD Instruments, Sydney, Australia).

Immediately before the beginning of the study, a nasogastric catheter ("NutriVent™, Sidam, Italia") with esophageal and gastric balloon was placed. Pes was measured during an end-inspiratory (Pes_{EIO}) and an end-expiratory occlusion of the airway. The variation of Pes during tidal inflation (ΔPes) was calculated as the difference between Pes_{EIO} and Pes_{EEO}. Transpulmonary pressure (PL) at end expiration was calculated as the difference between Paw and Pes at end expiration (PL_{EEO} = Paw_{EEO} - Pes_{EEO}). The intragastric pressure was measured only during an end-expiratory occlusion of the airway.

As per our usual clinical protocol, the correct positioning of the distal portion of the nasogastric catheter was confirmed by aspiration of gastric juices, auscultation of air insufflations into the stomach, and by a rise in intrabdominal pressure after external compression of the stomach. The assessment of the correct positioning of the esophageal balloon was performed observing concordant variations of airway esophageal and gastric pressures during an inspiratory occlusion. All study data were recorded directly on the patient's clinical file and transcribed into the Case Report Form.

3.2.2. Study protocol

Phase A: PEEP set according to ARDSNetwork. Each patient, while having heavy sedation and paralysis, underwent mechanical ventilation according to ARDSNet strategy (10) following the "Lower PEEP/Higher FiO_2" table (Table 1). Tidal volume was set at 6 ml per kilogram of predicted body weight (PBW) [PBW for males was calculated as 50 + 0.91 x (cm of height -152.4) and PBW for female as 45.5 + 0.91 x (cm of height -152.4)]. Respiratory rate was kept minor or equal to 35 to maintain $PaCO_2$ between 35 and 50 mmHg, FiO_2 to have a SpO2 90-100%. PEEP levels were set at

5-24 cmH_2O according to ARDSNet recommendation based on the patient's PaO_2 and FiO_2 (Table 1).

120 minutes after the enrolment the following measurements were performed:

1) Lung ultrasound to obtain lung ultrasound score (LUS, see below for details regarding experimental measurements).

2) Paw_{EEO}, Paw_{EIO}, respiratory system compliance (Crs), lung compliance (C_L), Pes_{EEO}, Pes_{EIO}, IGP, alveolar dead space.

3) Arterial blood gases.

4) Central venous oxygen saturation.

Table 1. Lower PEEP/Higher FiO_2

FiO_2	0.3	0.4	0.4	0.5	0.5	0.6	0.7	0.7	0.7	0.8	0.9	0.9	0.9	1.0
PEEP	5	5	8	8	10	10	10	12	14	14	14	16	18	18-24

Phase B: Pes-guided PEEP. Patients were then ventilated with the same tidal volume, respiratory rate and FiO_2 of phase A, while PEEP was set to achieve a PL_{EEO} of 0 to 10 cmH_2O, according to a sliding scale based on the PaO_2 and the FiO_2 (Table 2) (28).

After 120 minutes with this ventilatory setting the same measurements were repeated as in phase A (steps 1 to 4).

Phase C: Returning to baseline. Patients were then ventilated with the same setting of phase A and after 120 minutes the same measurements were repeated as in phase A (steps 1 to 4).

Blood samples for cytokines measurement were collected before starting the study and hourly from the beginning of the study.

Table 2, Esophageal Pressure-guided PEEP

FiO_2	0.4	0.5	0.5	0.6	0.6	0.7	0.7	0.8	0.8	0.9	0.9	1.0
P_{LEEO}	0	0	2	2	4	4	6	6	8	8	10	10

3.3. Data Collection, Outcomes & Follow-up

3.3.1. Duration of the study protocol

Patients enrolled in this study were ventilated according to the study ventilation strategy for 6 hours. At the end of the study the results of pressure measurements were made available to the caregivers, who were free to use or not use them for decisions concerning treatment and ventilator management.

3.3.2. Data collection; Duration of Follow-Up

During the study, we collected physiological data as per study protocol. We also documented the use of sedatives, analgesics, corticosteroids, inotropes and vasopressors. Thereafter, we followed patients to the time of hospital discharge, to record ICU and hospital survival.

3.4. Experimental Measurements

3.4.1. Lung ultrasound to assess lung recruitment

All the patients at the end of each phase were evaluated with lung ultrasound using a 2 to 5 MHz probe. Lung ultrasound was performed scanning along three lines: midclavicular, anterior and mid axillary. For each line, two areas were investigated (upper and lower). Thus emithorax was divided into six areas, for which all the intercostal spaces were examined (26).

Four ultrasound aeration patterns were defined (25) (Figure 2):

i) Normal aeration (N): presence of lung sliding with A lines or fewer than two isolated B lines.

ii) Moderate loss of aeration: multiple well-defined B lines (B_1).

iii) Severe loss of aeration: multiple and coalescent B lines (B_2).

iv) Lung consolidation with tissue pattern (C).

The lung ultrasound score (LUS) was obtained adding up the score of each area, and could range from 0 to 18 (N=0, B1=1, B2=2, C=3) for each hemithorax. LUS was a global picture of lung aeration and can be monitored during the phases. A decrease in score indicates an increase in aeration. A differential LUS of at least 4 points was considered as significant change in aeration between phases.

Ultrasound images and videos were saved and analyzed off-line by independent physicians, unaware of the timing and the ventilatory settings.

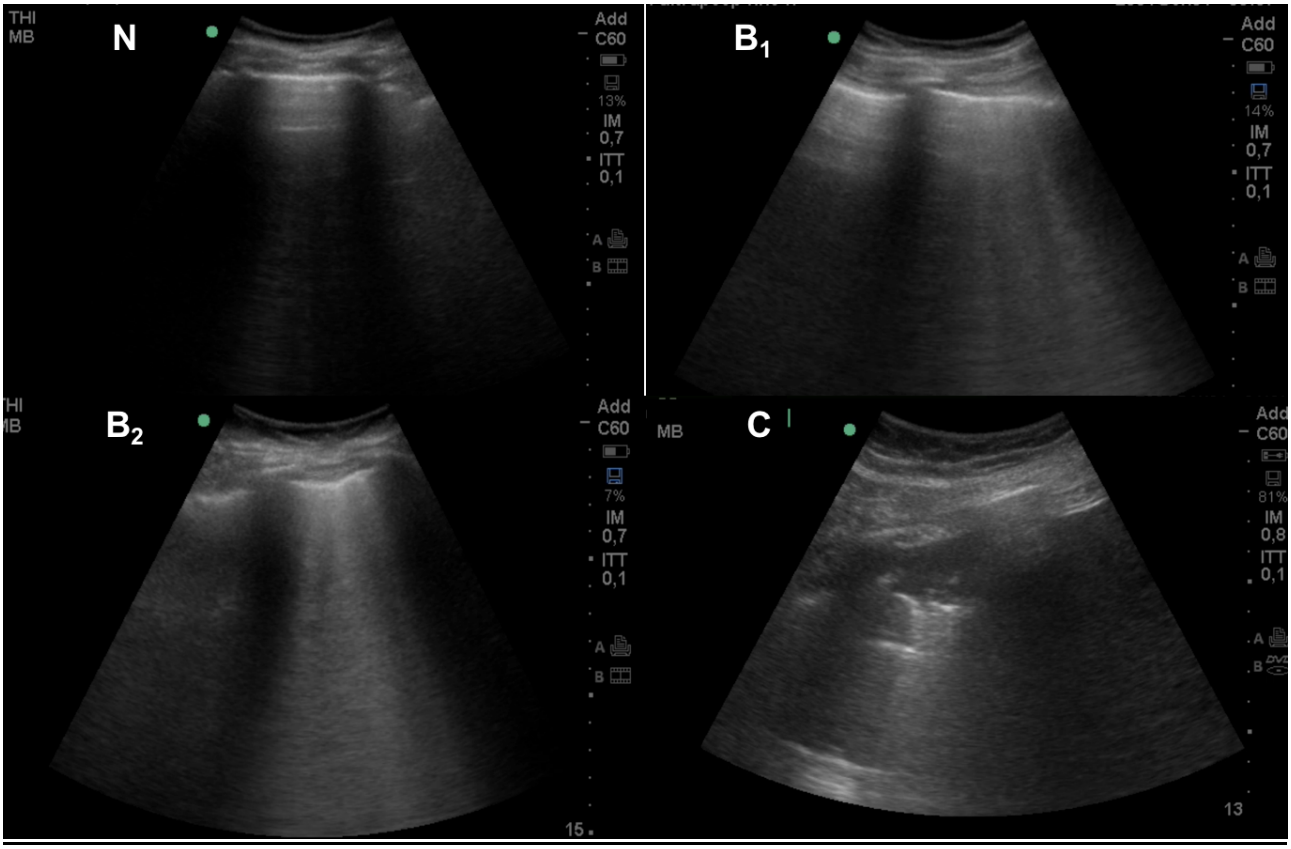

Figure 2. Lung ultrasound patterns. N = normal aeration; B_1 = moderate loss of aeration; B_2 severe loss of aeration; C = consolidation pattern.

3.4.2. Cytokines measurements

Blood samples for cytokines measurement were collected before starting the study and hourly from the beginning of the study (overall 7 samples per patient were collected). EDTA blood samples of 2

mL were drawn from an arterial line, centrifuged at 1500 g for 10 min. The serum aspirated was stored at –70 °C. IL-6, TNFα, IL-10 and IL-1ra were detected in EDTA plasma of 11 (out of 15) patients with commercially available enzyme-linked immunosorbent assays (ELISA) (29). Il-6 and IL-10 were determined by ELISA kits obtained from Biovendor company (Czech Republic) whereas TNFα and IL-1ra were measured using ELISA kits from Thermo Scientific (MA, USA).

3.4.3. Other measurements

- Inspiratory plateau airway pressure was measured at the end of an inspiratory pause of at least 6 seconds.
- Expiratory airway pressure was measured at the end of an expiratory pause of at least 6 seconds.
- Blood samples for gas analysis were drawn from an arterial line.
- Respiratory system compliance (C_{rs}) was calculated according to standard equation: Crs = VT/ (inspiratory Paw - expiratory Paw). Lung compliance was calculated as C_L = VT/ (PLEIO – P_{LEEO}). Physiological dead space (VD) as an index of global V/Q mismatching calculated with Bohr equation: VD= (P_aCO_2 - $P_{\bar{E}}CO_2$)/P_aCO_2

3.5. Statistical Analysis

No power calculation had been performed as this is a pilot study and we tested a physiological concept.

Considering the small size of the sample, all the variables were compared using non parametric tests. Continuous variables were presented as medians and interquartile ranges and compared with the Mann–Whitney test. Correlations between ultrasound lung aeration, PEEP and oxygenation changes were tested using Spearman correlation rank analysis. Statistical analysis was performed using SPSS software version 24 (SPSS, Inc., Chicago, IL, USA).

4. RESULTS

Fifteen consecutive critically ill patients with ARDS were included in the study: 1 had severe ARDS, 14 moderate. In Table 3 the main clinical characteristics of the patient population are summarized. Median age was 55 yrs and median body max index was 29.4. Most patients were severely ill with median SAPS II score at inclusion of 36 [IQR 27 to 44]. Eleven patients were admitted to ICU for pneumonia, median PaO_2:FiO_2 at admission was 141 mmHg [IQR 121 to 155] and median CO_2 was 40.5 mmHg [IQR 36.1 to 42.5].

Table 3. Baseline characteristics of the patients.

Patient No	Age (years)	Gender	BMI (kg/m^2)	SAPS II	Primary diagnosis	P/F at baseline	CO_2 at baseline
1	36	F	27.2	17	Pneumonia	102	48.1
2	69	F	27.3	34	Pneumonia	89	40.6
3	51	M	19.6	44	Pneumonia	156	42.7
4	50	F	26.0	33	Pneumonia	119	69
5	64	F	29.4	91	Cardiac arrest	175	55.4
6	53	M	36.3	46	Pneumonia	167	35.6
7	61	F	26.7	52	Pneumonia	127	26
8	55	F	54.7	24	Pneumonia	146	34
9	36	M	49.1	26	Pneumonia	104	40.5
10	49	M	39.2	29	Esophagitis	133	31
11	76	M	33.8	41	Abdominal surgery	153	41.8
13	68	M	27.8	26	Pneumonia	173	47
12	44	M	32.1	45	Opioid Overdose	154	37.5
14	66	M	27.7	36	Pneumonia	103	41.9
15	77	M	23.7	37	Pneumonia	137	39.7
Median (IQR)	**55 (49-67)**	**60% male**	**29.4 (26.9-37.7)**	**36 (27-44)**	**NA**	**141 (121-155)**	**40.5 (36.1-42.5)**

4.1. Physiological measurements

The ventilator settings and physiological measurements are summarized in Table 4. Patients were ventilated using tidal volume of 6 ml/kg. Ventilator setting other than PEEP were kept constant throughout the experiment.

In Figure 3 we reported the PEEP and the PL_{EEO} obtained with different methods; as shown PEEP and PL_{EEO} were significantly higher during the Pes-guided phase (median PEEP was 16 versus 10 cmH_2O and median PL_{EEO} was 2 versus -3 cmH_2O) ($p<0.02$).

Figure 4 shows the improvement in oxygenation during the Pes-guided PEEP phase: median PaO_2:FiO_2 improved from 149 during the ARDS Network PEEP phase to 166 during the Pes-guided PEEP phase ($p<0.05$). As shown, the difference in oxygenation between phase B and C is not statistically significant.

Although we found an improvement in oxygenation during the Pes-guided ventilation phase, we did not find any difference in CO_2, Crs, C_L and hemodynamic parameters (Table 4).

Table 4. Respiratory Values during the Study Protocol.

Measurement	Phase A ARDSNetwork strategy	Phase B Esophageal pressure-guided strategy	Phase C ARDSNetwork strategy	P value (between phase A and B)
MV mode	VCV	VCV	VCV	NA
VT (ml/kg)	6	6	6	n's.
Pes_{EEO} (cmH_2O)	15 (13-16)	16 (12-18)	15 (14-16)	n.s.
Pes_{EIO} (cmH_2O)	18 (16-18.5)	20 (15-20)	17 (16-18.5)	n.s.
Paw (cmH_2O)	21 (20-27)	28 (26.5-31.5)	22 (20-24.5)	p<0.02
PEEP (cmH_2O)	10 (10-12)	16 (15.5-19.5)	10 (10-13)	p<0.02
P_{LEEO} (cmH_2O)	-3 [-4-(-0.5)]	2 (2-4)	-2 [-4-(-0.5)]	p<0.02
P_{LEIO} (cmH_2O)	4 (2-10)	9 (8-10)	5 (2.5-9)	p<0.02
Ppeak (cmH_2O)	27 (25-30.5)	33 (32-34.5)	28 (27-30)	p<0.02
Pplateau (cmH_2O)	21 (20-26.5)	28n(27.5-31.5)	21 (20-26.5)	p<0.02
Driving Pressure	10 (8.5-13)	9 (8-12.5)	9 (7.5-12)	n.s.
RR (breaths/min)	17 (13.5-20)	18 (14.5-21)	17 (14.5-22)	n.s.
Crs (ml/cmH_2O)	46.9 (33.3-62.8)	42.5 (36.9-56.1)	35.7(34.3-56.2)	n.s.
C_L (ml/cmH_2O)	45 (36.1-70.7)	50 (42.9-79.4)	43 (37.9-80)	n.s.
P/F	149 (120-166)	166 (153-177)	141 (126-178)	p<0.05
$PaCO_2$ (mmHg)	43.5 (37.4-59.6)	47.4 (41.8-54.7)	48 /38.4-54.6)	n.s.
pH	7.43 (7.34-7.45)	7.39 (7.35-7.43)	7.38 (7.34-7.45)	n.s.
$ScVO_2$ (mmHg)	95.9 (95.35-97.3)	96.8 (96.1-98)	96 (94.8-97.45)	n.s.
HR (beats/min)	80 (66-93)	78 (71-89)	74 (62-82)	n.s.
MAP (mmHg)	78 (69-90)	72 (71-89)	77 (72-83)	n.s.
CVP (mmHg)	12 (10-14)	13 (11.5-15)	14 (13.5-14.5)	n.s.
LUS	18 (11-27)	15 (11-18)	15 (10-18)	p<0.05

Values are expressed as medians and interquartile ranges in brackets. MV = mechanical ventilation; VT = tidal volume; Pes = esophageal pressure; EIO = end-inspiratory occlusion; EEO = end-expiratory occlusion; Paw = airway pressure; RR = respiratory rate; Crs = respiratory system compliance; C_L = lung compliance; P/F = PaO_2:FiO_2; ScVO2 = central venous saturation; HR = heart rate; MAP = mean arterial pressure; CVP = central venous pressure; LUS = lung ultrasound score.

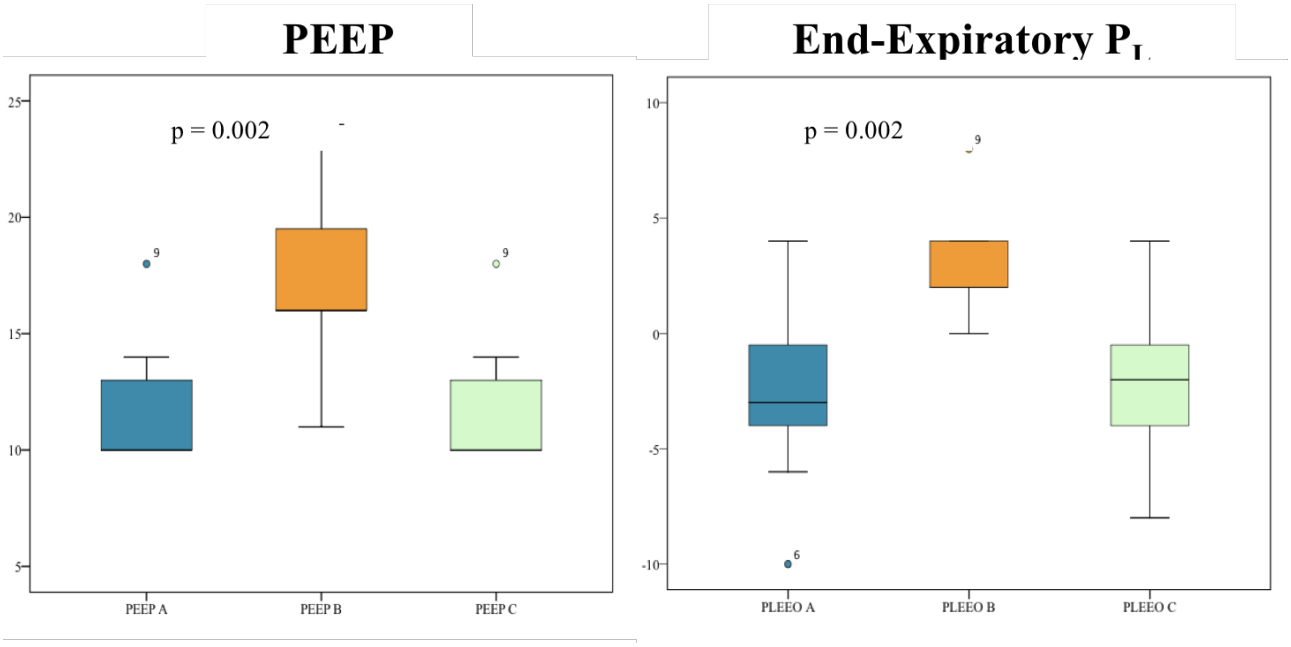

Figure 3. Box Plots of positive end-expiratory pressure (PEEP) and transpulmonary pressure (PL_{EEO}) during the 3 phases. Medians and IQR are represented. In phase A, PEEP was set according to the ARDS Network table "Lower PEEP/Higher FiO_2"; in phase B, PEEP was set according to the esophageal pressure (see Table 1 and 2); in phase C, PEEP was the same as in phase A. The difference in PEEP and PL_{EEO} between phases was statistically significant ($p<0.02$).

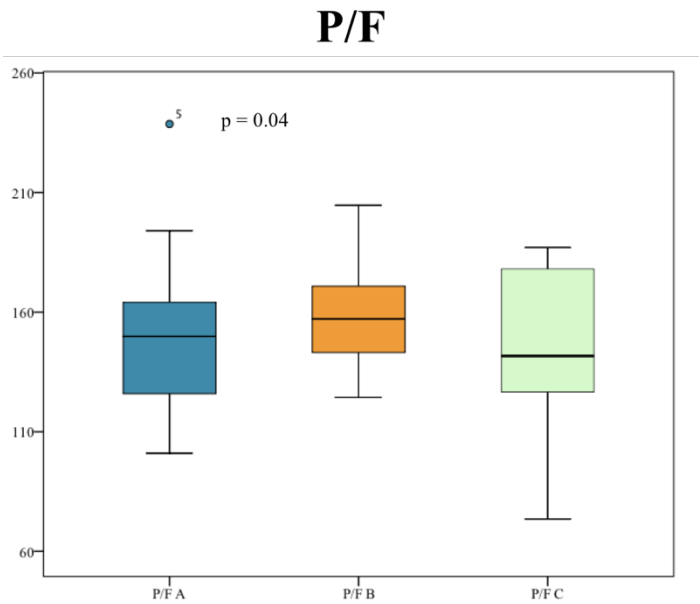

Figure 4. Boxplots of PaO_2:FiO_2 (P/F) during the 3 phases. Medians and IQR are represented. In phase A, PEEP was set according to the ARDS Network table "Lower PEEP/Higher FiO_2"; in phase B, PEEP was set according to the esophageal pressure (see Table 1 and 2); in phase C, PEEP was the same as in phase A. The difference in P/F was significant only between phase A and B ($p<0.05$).

4.2. Esophageal pressure-guided PEEP and lung recruitment

Lung aeration assessed with LUS improved during the Pes-guided ventilation phase. Median LUS was 18 when the PEEP was set according to the "Lower PEEP/higher FiO_2" (Table 1) and 15 during the Pes-guided phase ($p<0.05$). A decrease in LUS ≥4 (indicating lung recruitment) was found in 10 of 15 patients. In 3 patients the score did not change and in 2 it got worse. Four out of 11 patients maintained the obtained improvement in phase C. Four patients were not followed in phase C for technical or clinical issues.

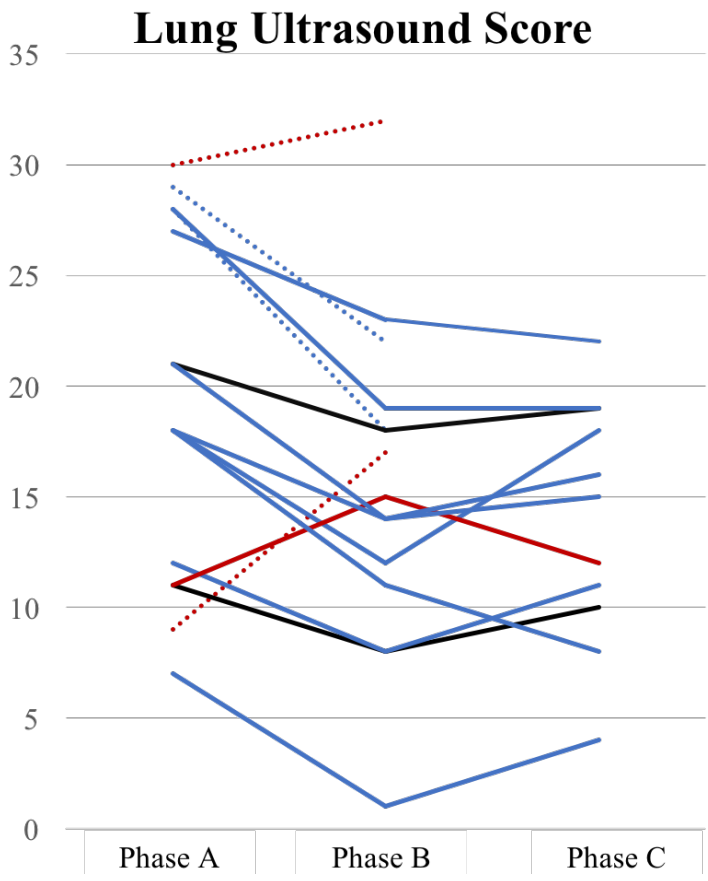

Figure 5. Lung Ultrasound Score (LUS) trend over phases. Patients who were not followed until phase C are represented with dashed lines. Blue lines indicate improvement in lung aeration from the ventilation strategy with PEEP set according to ARDS Network (phase A) to the esophageal pressure-guided phase (phase B), represented by a reduction in LUS. Red lines indicate a decrease in lung aeration and black line indicates a non significant change in LUS (less than 4). Four out of eleven patients maintained the obtained improvement when they returned to ARDS Network ventilation strategy (phase C).

4.3. Plasmatic cytokines analysis

Plasmatic biomarkers were analyzed in 11 patients. Plasma levels of IL-6, TNF, IL-10 and IL1-Ra were not significantly affected by the two strategies (Figure 6).

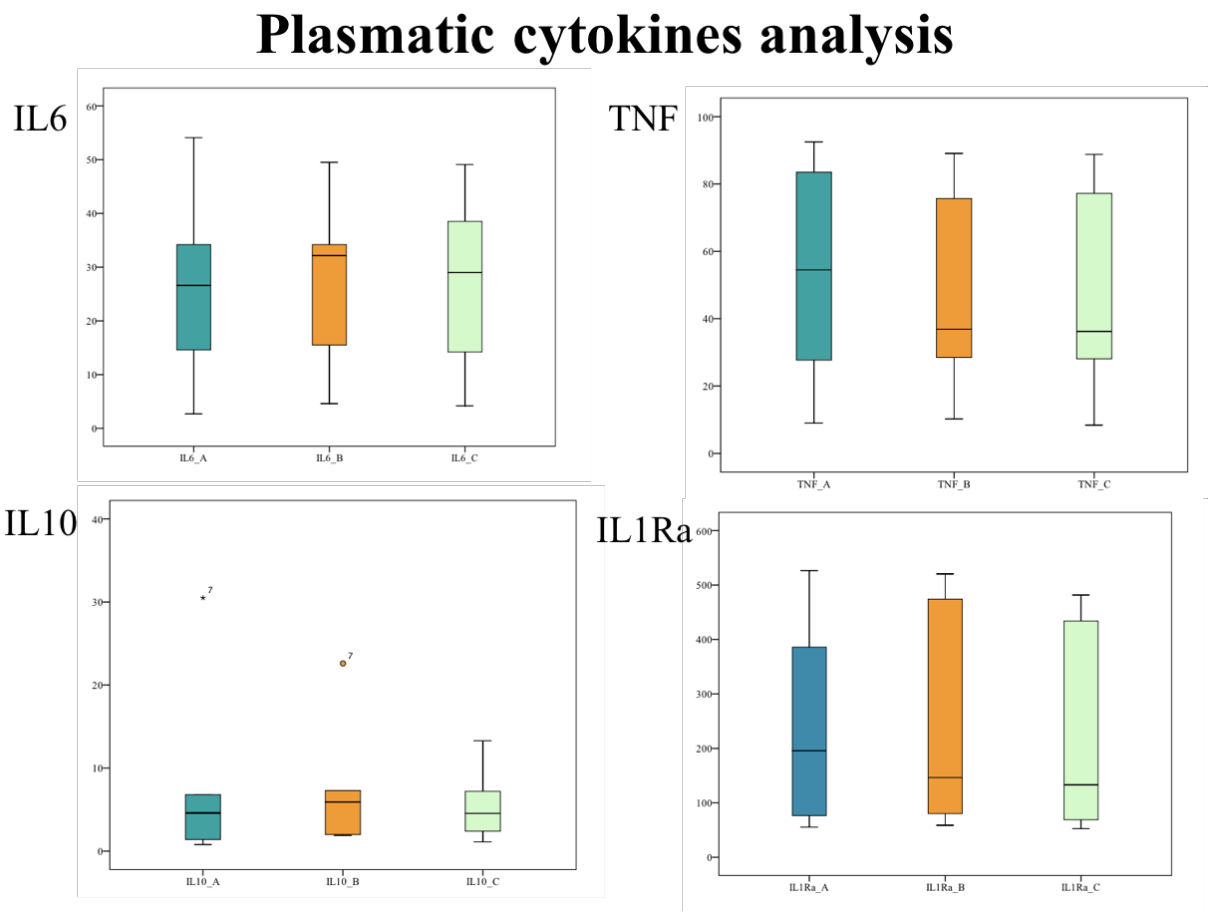

Figure 6. Box plots of the plasmatic cytokines during the during the 3 phases. Medians and IQR are represented. In phase A, PEEP was set according to the ARDS Network table "Lower PEEP/Higher FiO_2"; in phase B, PEEP was set according to the esophageal pressure (see Table 1 and 2); in phase C, PEEP was the same as in phase A. Plasma levels of IL-6, TNF, IL-10 and IL1-Ra were not significantly affected by the three strategies.

5. DISCUSSION

The major findings of our study are that i) PEEP set according to PL is higher compared to PEEP set according to ARDS Network; ii) the Pes-guided PEEP is associated with an increase in oxygenation and in lung recruitment estimated by LUS; iii) no difference in pro-inflammatory cytokines were found between phases.

The EPVent-1 trial demonstrated an improvement in oxygenation and compliance using Pes to estimate P_L. We confirmed these results showing that raising PEEP according to P_L in patients with high chest wall elastance improves oxygenation and lung recruitment. Moreover, we found a median $P_{L_{EEO}}$ during the ARDS Network ventilation phase of -3 cmH_2O, indicating possible alveolar collapse at end expiration.

In patients with higher portion of recruitable lung, a higher PEEP reduces the intra-tidal collapse and reinflation, minimizing the possible atelectrauma. Individualizing PEEP titration by targeting the PL may help to identify patients who most likely need higher airway pressures (either end-inspiratory or end-expiratory) to generate a sufficient level of P_L. These are normally the patients with a higher-than-normal chest wall elastance, who are the most likely to benefit from higher PEEP. Conversely in patients with low chest wall elastance, setting a lower PEEP keeps expiratory P_L low, possibly preventing overdistension.

We found an improvement in oxygenation as measured by the PaO_2:FiO_2 ratio during the ventilation phase with PEEP levels set up according to P_L, confirming the results obtained by Talmor et al. Oxygenation then tended to decrease in phase C even though the difference between phase B and C is not statistically significant.

The PEEP-related improvement in oxygenation was found associated to a significant improvement in lung recruitment measured with lung ultrasound.

The third phase (“Return to baseline”) was set up using the same PEEP levels of phase A, to assess

whether the lung recruitment and the increase in oxygenation was truly due to PEEP. Although in this phase LUS did not significantly differ from LUS in the second one, we found it was trending upward, suggesting the importance of keeping the optimal individualized PEEP to maintain the lungs recruited.

We did not find any significant difference in Crs and C_L, CO_2 and hemodynamics between phases. Oxygenation cannot be the only clinical parameter to be considered to evaluate the clinical improvement and since this is a pilot study dealing with a small sample size, we also observed the individual response to the Pes-guided PEEP. The majority of our patients needed higher PEEP, according to Pes and after its increase they showed improvements in lung recruitment and in their clinical parameters.

Although none of the adopted ventilation strategies was associated with adverse events, we found a group of patients worsened clinical parameters. The higher PEEP in those patients might have raised the dead space without giving lung recruitment but instead increasing the risk of overdistension. Those patients did not show an improvement in lung recruitment, and this suggests that lung ultrasound can be usefully associated with Pes to prevent possible negative effects of PEEP.

Several human and experimental studies showed the role of systemic biomarkers in determining the effects of mechanical ventilation in ARDS patients (30) (31) (7). Stuber et al. found an increase in plasma levels of plasmatic cytokines 1 hour after raising tidal volumes in ventilated ARDS patients (30). We assessed the possible injurious effects of PEEP by analyzing the pro-inflammatory cytokines IL-6 and TNF and the anti-inflammatory IL-10 and IL1-Ra. We did not find significant variation in any of them. We may speculate there is no risk of overdistension using higher levels of PEEP, nonetheless these results have to be confirmed.

Whereas this study is important in defining some of the pathophysiological mechanisms related to the PEEP-induced lung recruitment, several limitations must be addressed. It was a single-center study with a small sample, and findings cannot be generalized until they are confirmed in a larger trial powered to detect changes in appropriate clinical end points.

Even though the measure of absolute values of Pes to assess end-expiration P_L was demonstrated useful in previous studies, it can be influenced by several factors (e.g. weight of mediastinum, lung volume, posture, abdominal pressure). Moreover, some authors suggest that the Pes should be used only as relative value, i.e., only the variations of Pes reflect very precisely the variations of pleural pressure, but the absolute value of Pes is not related to the value of pleural pressure.

Lung ultrasound cannot detect PEEP-induced lung hyperinflation. To avoid ventilator-induced lung injury, we limited tidal volume to keep plateau airway pressure ≤ 32 cmH_2O and end-inspiratory transpulmonary pressure ≤ 20 cmH_2O.

Patients with moderate and severe ARDS are critically ill. Their physiology and hemodynamics may change rapidly, leading to outcomes not necessarily reflecting our intervention. To minimize this risk, we designed the protocol to be as short as possible. Even the gold standard for the measurement of lung recruitability (i.e., the quantitative CT scan analysis) has troubles in detecting the overinflated tissue, and alternative methods (such as volumetric capnography) should be implemented to quantify overinflation.

In conclusion, our data emphasize the importance of PEEP when ventilating patients with ARDS. The use of Pes to tailor PEEP on pulmonary and chest-wall mechanics may have clinical benefit in terms of lung recruitment and oxygenation. This approach needs to be confirmed with further investigation.

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