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Expiratory Rib Cage Compression, Secretion Clearance and Respiratory Mechanics in Mechanically Ventilated Patients: A Randomized Crossover Trial

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ABSTRACT

Background

Expiratory rib cage compression (ERCC) has been empirically used by the physiotherapists with the rationale of improving expiratory flows and, therefore, the airway clearance in mechanically ventilated patients. This study aimed at evaluating the acute mechanical effects and sputum clearance of an ERCC protocol in ventilated patients with pulmonary infection.

Methods

In a randomized crossover study, sputum production and respiratory mechanics were evaluated in 20 mechanically ventilated patients submitted to 2 interventions. Expiratory rib cage compression intervention consisted of a series of manual bilateral expiratory rib-cage compressions followed by a hyperinflation maneuver. Control intervention (CTRL) followed the same sequence, but instead of the compressive maneuver, the patients were kept on normal ventilation. Static (Cst) and effective (Ceff) compliance, and total (Rtot) and initial (Rinit) resistance of the respiratory system were measured pre (baseline), post-ERCC or CTRL (POST1), and post-hyperinflation (POST2). Peak expiratory flow (PEF) and the flow at 30% of the expiratory tidal volume (Flow 30%Vt) were measured during the maneuver.

Results

Expiratory rib cage compression cleared 34.4% more secretions than CTRL (2.24±1.59 vs 1.47±1.45mL; *P*=.04). Respiratory mechanics showed no differences between control and experimental intervention in POST1 for Cst, Ceff, Rtot and Rinit. In POST2 ERCC promoted an increase in Cst (38.7±10.3 vs 42.2±12mL/cmH2O; *P*=.025) and in Ceff (32.6±9.1 vs 34.8±9.4mL/cmH2O; *P*=.044). During ERCC, PEF increased 16.2L/min P<.001) and Flow 30%Vt increased 25.3L/min (P<.001) when compared with CTRL. Six patients (30%) presented expiratory flow limitation during ERCC. The effect size was small for secretion volume (0.2), Cst (0.15) and Ceff (0.12), and negligible for Rtot (0.04) and Rinit (0.04).

Conclusions

Although ERCC increases expiratory flow, it has no clinically relevant effects in improving the sputum production and respiratory mechanics in hypersecretive mechanically ventilated patients. The maneuver can cause expiratory flow limitation in some patients.

Keywords (MeSH)

Physical therapy modalities; intensive care; respiratory therapy, mucociliary clearance, pneumonia.

Trial registration: NCT01525121

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INTRODUCTION

Despite a number of studies have focused on the effect of mechanical ventilation on the pulmonary parenchyma, more attention should be given to mucus clearance, since its mechanisms in ventilated patients are still poor understood. Furthermore, the advances in understanding of simple interventions such as chest physiotherapy may contribute to reduce mucus retention and respiratory infections[1]. Chest physiotherapy is an essential component of the multidisciplinary approach in critical care settings. In this context, a number of devices and manual techniques have been used to remove pulmonary secretions and re-expand collapsed areas.[2] Thoracic manipulative techniques include expiratory rib cage compression or “squeezing”.[3] This technique aims to increase expiratory flow and stretch intercostal muscles by means of a manual thoracic compression applied during exhalation, followed by a rapid release at the onset of inspiration.[4] The rationale of this technique is based on its compressive effect on the airways, increasing the airflow velocity, which increases mucus transport.[5] As the application of mechanical forces on the thorax reduce the transpulmonary pressure, rib cage compression is likely to promote pulmonary and airway collapse, diminishing respiratory compliance and/or causing expiratory flow limitation (EFL).[6,7] Previous animal studies showed no beneficial effects of ERCC on respiratory mechanics and gas exchange.[8-10] Moreover, in the paper of Unoki et al[9] they postulated that the airway and alveolar collapse may be exacerbated, which is in according to the results of Martí et al[10], who found a reduction in static compliance after applying the maneuver in an animal model. These authors also found an increase in mucus clearance with “hard expiratory rib cage compression” (brief and strong bilateral compressions); however, this was not observed in critical care patients, as reported by Unoki et al[4]. Although there is no clear evidence on the physiological or airway clearance benefits of ERCC in humans, Berti et al[11] found a positive effect on the duration of

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mechanical ventilation, as well as on the ICU discharge rate and Murray score when using ERCC along to manual hyperinflation. It is difficult to determine if these benefic clinical outcomes were due to the ERCC or manual hyperinflation or both, especially because Genc et al[12] observed no improvements in static compliance, gas exchange and secretion clearance when ERCC was added to manual hyperinflation in 22 mechanically ventilated patients. It is likely that these conflicting results are due to the design differences between the studies, along to some potential confounding factors which were not considered. The present study was conducted to examine the acute mechanical effects and sputum clearance of an ERCC protocol in ventilated patients with pulmonary infection. We hypothesized that, although ERCC can promote EFL and lung collapse in some circumstances, this technique improves the airway clearance in hypersecretive mechanically ventilated patients.

METHODS

Study Design

This was a randomized crossover study. Participants were recruited from patients admitted to an 11-bed intensive care unit at a tertiary referral hospital. Allocation was concealed from the enrolling investigators and randomization used two blocks of 10. The study coordinator prepared sealed opaque envelopes containing a preassigned treatment order generated by computer, which were opened sequentially by the physiotherapist at the day of interventions. According to the random order, the participants received manual expiratory rib-cage compression (ERCC) or the control intervention (CTRL) on the same day, with a five-hour washout period between them. Both

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interventions were followed by a hyperinflation maneuver using pressure support ventilation. The study protocol is depicted in Figure 1.

Participants

All subjects were under mechanical ventilation, presenting medical diagnosis of pulmonary infection, and hypersecretion (defined as the need for suctioning < 2-hour intervals). Pulmonary infection was defined as a score of ≥ 6, as determined by the Clinical Pulmonary Infection Score (CPIS).[13] CPIS was calculated after individual scoring for each of the following parameters, as follows: a) Temperature: 36.5–38.4°C = 0 point, 38.5–38.9°C = 1 point, ≤36°C or ≥39°C = 2 points; b) White blood cells (×109/l) 4.0-11.0 = 0 points, 11-17 = 1 point, > 17 = 2 points; c) Secretions: none to minimal = 0 point, moderate = 1 point, large amount = 2 points; d) PaO_2/FiO_2: >240 = 0 point, ≤ 240 = 2 points; e) Chest radiograph infiltrates: clear = 0 point, patchy = 1 point, localized = 2 points. Exclusion criteria included individuals with hemodynamic instability (defined by heart rate > 130 bpm and/or mean arterial pressure < 60 mmHg), use of vasopressor drugs, absence of respiratory drive, acute bronchospasm, acute respiratory distress syndrome, immediate postoperative neuro-surgery, untreated pneumothorax and lung hemorrhage. The research protocol was approved by the institution's Ethics Committee, and written informed consent was obtained from the patients' next of kin before the study began.

Intervention

The patients were kept in supine at 30 degree head-up position. Ventilatory mode was changed to volume-controlled, with a tidal volume of 8mL/kg, inspiratory flow of 60 Lpm (square wave) and positive end expiratory pressure (PEEP) of 5 cmH_2O (inspired fraction of oxygen remained unchanged). A first tracheal suctioning was done, and the mucus was discarded. Then, a series of five minutes of bilateral expiratory rib-cage compressions ensued. Aiming to minimize inter-therapist variability, the technique was applied by the same registered and trained physiotherapist. His hands were positioned on the lower ribs, and the force was applied every two breaths only during the expiration, synchronizing the maneuver rate with the patients' respiratory rate. Then, the patients underwent a new suctioning procedure, and a hyperinflation maneuver consisting of a 10-minute period under pressure support ventilation of 35 cmH_2O was done. Control intervention followed the same sequence, but instead of the compressive maneuver, they were kept on normal ventilation with the parameters described above.

Outcome Measurements

Sputum Production

Secretion clearance was the primary outcome, and was measured as sputum volume (mL).[14] Secretions were collected immediately after ERCC and CTRL, using a sputum trap attached to the suction system. Sterile saline (10mL) was flushed through the suction tubing into the trap to clear any secretions in the catheter. The volume of sputum was recorded subtracting the saline volume from

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the total volume in the trap. The suctioning procedure was performed following the American Association for Respiratory Care recommendations: closed suction system, suction catheter with maximal internal-to-external diameter ratio of 0.5, delivery of 100% oxygen 30s immediately before and 1 minute after the procedure, duration of 15s, and vacuum pressure of -150 mmHg.[15]

Respiratory Mechanics

Respiratory mechanics recordings were performed immediately pre (PRE), post-expiratory rib cage compression or CTRL (POST1), and post-hyperinflation (POST2). There were no intervals between these protocol steps and respiratory mechanics recording.

To the measurements the patients were positioned in supine 30 degree head-up position, with hyperinflated cuff and submitted to tracheal suctioning. After that, they underwent 3 sighs with a two-fold increase in tidal volume (“volume history”).[16] According to the end inspiratory occlusion method[17], peak pressure (P1), plateau pressure (P2), ΔP1 (P1 – inflexion point pressure), inspiratory flow (Flow) and tidal volume (Vt) were used to calculate static and effective compliance of the respiratory system [$C_{st,rs} = Vt/(P2 - PEEP)$; $C_{eff,rs} = Vt/(P1 - PEEP)$], and total and initial resistance of the respiratory system [$R_{rs} = (P1 - P2)/Flow$; $R_{init} = \Delta P1/Flow$). During the manual compressive maneuver, a superposition between pre and per-compression flow-volume loops was characterized as EFL.[18,19] In addition, peak expiratory flow and expiratory flow at 30% of the expiratory tidal volume (aiming at recording the flow in intermediate and/or peripheral airways) were also measured during the ERCC. In all steps (PRE, POST1 and POST2) the respiratory signals were collected from the ventilator display (Vela, Infrasonics, San Diego, CA, USA) and the representative value for each respiratory mechanics variable was computed as an average of three consistent measures.

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Statistical Analysis

According to the data from Lemes et al,[20] power calculation indicated that 10 participants would provide sufficient power (80%) to detect a difference of 68% in sputum volume, assuming a standard deviation of 67% and significance of 0.05.

Data were tested for normality and homogeneity of variances (Shapiro-Wilk test and Levene median test), and expressed as mean ± standard deviation (if normally distributed) or as medians in combination with quartiles and percentiles (if not normally distributed). According to data distribution, the Two-way repeated measures analysis of variance and the Tukey test were used to examine the between-interventions differences in respiratory mechanics, and Wilcoxon test was used to compare the sputum production between CTRL and ERCC. The significance level was set at 0.05 and the software Sigmastat 3.1 (Systat Software, San Jose, CA, USA) was used for all analysis. The clinical effect of ERCC was assessed by the effect size statistic, calculated as the mean change found in a variable divided by the standard deviation of that variable.[21] We used the criteria of Cohen[22] to interpret the effect size, where a value of 0.2 is considered a small effect, a value of 0.5 is considered a moderate effect, and a value of 0.8 is considered a large effect. These calculations used the POST2 data, considering CTRL and ERCC results.

RESULTS

Data from the participants of the study are in Table 1. The only center involved in this trial (Military Police Rio de Janeiro State Hospital) has a throughput of 234 critical care patients per year, with 65% managed with mechanical ventilation. All participants completed the measurements and their baseline respiratory mechanics values were similar before CTRL and ERCC (Table 2). One neurosurgical patient presented with Glasgow Coma Score of 9. Three patients were not sedated, and the other 16 subjects were sedated with midazolam (0.02 to 0.2 mg kg-1 h-1) and/or fentanyl (1.0 to 7.0 µg kg-1 h-1). The infusions were titrated with the aim of keeping the level 2-4 in the Ramsay Sedation Scale.[23] The same experienced physiotherapist delivered control and experimental interventions for all patients, and the procedures were well tolerated without unfavorable signs and symptoms (alteration in blood pressure and/or heart rate > or < 20% of resting values, and desaturation of oxihaemoglobin > 10% of baseline levels).[24]

The respiratory mechanics and expiratory flow profiles in CTRL and ERCC are shown in Table 2 and 3, respectively. ERCC cleared 34.4% more secretions than CTRL (*P* = .04) (Figure 2 and Table 2). Respiratory mechanics showed no differences between control and experimental intervention in POST1 for Cst (*P* = .097), Ceff (*P* = .34), Rtot (*P* = .917) and Rinit (*P* = .971). In POST2 ERCC promoted an increase in Cst (*P* = .025) and in Ceff (*P* = .044), but not in Rtot (*P* = .917) and Rinit (*P* = .971). The effect size was small for secretion volume (0.2), Cst (0.15) and Ceff (0.12), and negligible for Rtot (0.04) and Rinit (0.04).

During ERCC, PEF increased 16.2 L/min *P* < .001) and Flow 30% Vt increased 25.3 L/min (*P* < .001) when compared with CTRL (Table 3). Six patients (30%) presented EFL during ERCC. In a post-hoc analysis comparing EFL and NEFL patients there was an increase in PEF during ERCC in NEFL (*P* = .007), but not in EFL (*P* = .193). The Flow 30% Vt increased in EFL (*P* = .043) and in NEFL (*P* = .001), but NEFL presented higher values (*P* = .006). There were correlations between Rtot and Flow 30% Vt during ERCC (r = - 0.635; *P* = .0026) and between Rinit and Flow 30% Vt during ERCC (r = -0.596; *P* = .0056). There were no other associations between expiratory flows during ERCC and respiratory mechanics parameters.

DISCUSSION

Although the lack of evidence, ERCC have been empirically used by the physiotherapists with the rationale of improving expiratory flows and, therefore, the airway clearance.[3] Our study showed that ERCC improves the expiratory flow but has little effect on secretion removal and respiratory mechanics in mechanically ventilated patients with pulmonary infection. These results are in agreement with previous studies that found no benefits with ERCC in animal models,[8-10] and in mechanically ventilated patients.[4,12]

Mucus Clearance

In this study the mucus clearance was computed as sputum volume. Although the measurement of the transport rate of mucus in the airway using a radioactive tracer technique is the most direct outcome parameter,[5] this method is difficult to implement in critical care settings. Thus, the

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quantification of expectorated mucus has been used as an important airway clearance marker in most of physiotherapy studies including mechanically ventilated patients.[12,20,25-28] To a better mechanistic approach, our mucus clearance results are discussed along to the following respiratory mechanics sub-items.

Expiratory Flow Limitation

Airflow transport depends mainly on the airflow velocity, which is determined by the airway diameter and the intrapulmonary pressure created by the expiratory muscles (or by any thoracic compressive maneuver).[5] During a forced expiration or a compressive maneuver, the transmural pressure might reduce the airways diameter causing a disproportion between the driving pressure and the expiratory flow (EFL). If the airway collapse occurs, the downstream flow equals to zero, and the secretion removal is interrupted.[6,29]

As ERCC is mechanically similar to a forced expiratory maneuver, it has the potential to cause EFL and peripheral airways' closure. In our study, maybe because all patients were under mechanical ventilation and positive end expiratory pressure (PEEP) of 5 cmH_2O, only 6 patients (30%) presented EFL during ERCC. It is likely that the protective mechanical effect of PEEP during the maneuver prevented the EFL in many patients and contributed to the overall increase in PEF and Flow at 30% Vt. As expected, this augment was more pronounced in NEFL patients, suggesting that higher PEEP levels can be necessary to stabilize the airways during ERCC in some patients. In this way, aiming at increase the expiratory flow and prevent the airway collapse, during the maneuver the PEEP level should be increased until the point where there is no superposition between the baseline (current ventilation) and ERCC flow-volume loops observed in the ventilator display.

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As the protective mechanical effect of PEEP is not present in spontaneous ventilated patients, is likely that ERCC cause EFL and airways' closure more frequently, mainly in patients with pulmonary obstructive diseases. Yet, is impossible to predict in which patients ERCC will cause a dynamic compressive effect, which in thesis can help the secretion removal, or the airways' collapse, which can interrupt the airways' clearance. Based on this premise, to remove peripheral secretions, the rationale of ERCC should include strategies aiming to avoid the airways' collapse, mainly in patients with reduced airways' stability and/or functional residual capacity (as for instance bed-ridden patients).

Respiratory System and Airways' Resistance

As in previous studies, we didn't find changes in Rrs and Rinit,rs.[10,20,30,31] According to these authors, transitory bronchial constriction, variable secretion distribution pattern in the airways among patients, mucociliar activity, and individualized response to the applied intervention could explain the unchanged respiratory resistance observed after the use of airway clearance techniques. Moreover, because the highest contribution of central and intermediate airways to the respiratory system resistance, if the secretions displace from the periphery to more proximal airways (but are not completely removed by suctioning) there will be an augment in these resistance parameters.[32]

We also found that in patients with higher respiratory and airways' resistance (Rtot and Rinit, respectively) the compressive maneuver was less effective in augmenting the expiratory flows. It suggests that for more severely obstructed patients a higher tidal volume may add some benefit to ERCC in increasing the expiratory flow.

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Static and Effective Respiratory Compliance

Although Cst has been used by several authors to assess the effect of airway clearance techniques in mechanically ventilated patients,[20,25-28], the only study evaluating expiratory rib cage compression as an isolated technique in humans used Ceff instead.[4] The use of this mechanical variable made the interpretation of respiratory mechanics difficult, because this parameter is influenced not only by the elastic properties of the respiratory system (which depends on the recruitment or derecruitment of lung periphery), but also by its resistive and inhomogeneous components (which depends on other factors, as the patterns of secretion distribution in the airways).[17] Nevertheless, these authors also postulate that ERCC can cause any degree of pulmonary collapse, which is in according to the study of Martí et al[10] who found a reduction in Cst after ERCC in an animal model. In our study, Cst and Ceff didn't change immediately after ERCC, but presented a small increase after a hyperinflation maneuver when compared to CTRL. It is likely that ERCC promoted some peripheral airways' desobstruction but associated to any degree of pulmonary collapse (because the compressive effect), so that these events counterbalanced each other resulting in an unchanged Cst in POST1. The subsequent hyperinflation maneuver re-inflated the collapsed areas[33] and, then, the Cst values became higher in ERRC (POST2). These positive effects in respiratory mechanics only after a hyperinflation maneuver point to a new possible recommendation when using ERRC, aiming at re-inflate any pulmonary collapsed areas. However, this recommendation is applicable only if future studies show clinically relevant benefits of ERCC in some context.

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Our results cannot be extrapolated to other clinical settings, because the patients were evaluated in a specific ventilatory mode and parameters. Indeed, the physiological and therapeutic consequences of the chest physiotherapy techniques are greatly influenced by the ventilatory settings, as for example the role of PEEP in preventing airway and lung collapse. Additionally, since there are no favorable evidences on the ERCC short-term effects in the current literature, we can’t expect a positive impact of this technique in clinically relevant outcomes, such as length of stay, weaning outcome, mortality and incidence of ventilator-associated pneumonia. The only study on these outcomes applied ERRC along to manual hyperinflation, making difficult to determine which of these two techniques was responsible for the improvement in the length of stay and time to weaning found by the authors.[11]

Regarding the limitations of the study, arterial blood gases were not recorded and a blinded assessor could have done all measurements. Although these limitations, we believe that our results add an important contribution to the field, since we assessed the isolated clinical and physiological effect of ERCC (not associated to any other technique), confirming the negative results from previous animal and human studies. Moreover, in our study we took special care to ensure that sputum production and respiratory mechanics could reflect the effects of ERCC on lung periphery. In this way, before the interventions all patients were suctioned to remove the secretions from central airways, and respiratory mechanics measurements included static compliance of respiratory system, a recognized marker of peripheral desobstruction.[34] We also raised possible recommendations to the technique, as the use of a hyperinflation maneuver after its application, and the flow-volume curve monitoring during the thoracic compression to avoid airways’ collapse by setting an appropriate PEEP level.

Further studies are necessary to evaluate the effects of ERCC in spontaneous ventilation, in specific diseases and respiratory mechanics conditions, and under different ventilatory modes and parameters. It is likely that other factors, as the compressive force magnitude, the airways' stability

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and the pulmonary volume at the onset of expiration influence the effectiveness of ERCC. Additionally, to a better understanding of the ERCC underlining mechanisms, it is important to consider not only the peak expiratory flow, but also the fluid mechanics principles, which are determining of mucus transport by air-liquid interaction in central and peripheral airways.

In conclusion, although ERCC increases expiratory flow, it has no clinically relevant effects in improving the sputum production and respiratory mechanics in hypersecretive mechanically ventilated patients. The maneuver can cause expiratory flow limitation in some patients.

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LEGEND TO THE FIGURES

Figure 1 - Design and flow of participants through the trial.

Figure 2 – Sputum volume produced after control (CTRL) and expiratory rib cage compression (ERCC) interventions. The solid lines in each box denote the median values, the lower and upper box boundaries represent the 25th and 75th centiles, respectively, the whiskers signal the 10th and 90th centiles, and the closed circles encompass the range of data points.

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Table 1. Basic Characteristics of 20 Mechanically Ventilated Patients with Pulmonary Infection

Age, years	65 (13)
Median	72
Interquartile range	61~77.5
Gender	9 (45)
Male	9 (45%)
Female	11 (55%)
PaO_2/FiO_2	
Mean (SD)	322 (74)
APACHE II at ICU admission	
Mean (SD)	19 (7)
Underlying diseases	
Ventilator associated pneumonia	13 (65%)
Community- acquired pneumonia	5 (25%)
Aspirative pneumonia	2 (10%)
Upper abdominal surgery	2 (13.4%)
Congestive heart failure	2 (5%)
Stroke	1 (5%)
Septic shock	2 (10%)
Neurosurgery	1 (5%)
Kidney failure	1 (5%)
Pulmonary emboly	1 (5%)
Ventilatory mode	
VC-CMV	4 (20%)
PC-CMV	4 (20%)
PSV	12 (60%)
PEEP, cmH_2O	
Median	5
Interquartile range	5~8
FiO_2	0.35 (0.13)
Median	0.3
Interquartile range	0.3~0.38
Duration of ventilation, days	
Mean (SD)	11.2 (4.5)

Abbreviations: PaO2 = arterial partial pressure of oxygen; FiO2 = inspiratory fraction of oxygen; APACHE II = Acute Physiology and Chronic Health disease Classification System II; VC-CMV = Volume-controlled continuous mandatory ventilation; PC-CMV = Pressure-controlled continuous mandatory ventilation; PSV = Pressure support ventilation; PEEP = positive end expiratory pressure.

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Table 2 – Secretion Clearance and Respiratory Mechanics

	PRE		POST1		POST2	
	CTRL	**ERCC**	**CTRL**	**ERCC**	**CTRL**	**ERCC**
Secretion Clearance (n=20)						
Sputum volume (mL)						
Median			1.0	2.0*		
Interquartile range			0.5~1.95	1.0~3.25		
Respiratory Mechanics (n=20)						
Cst,rs (mL/cmH_2O)						
Mean (SD)	38.8 (9.2)	40.2 (12.2)	36.5 (8.4)	39.1 (9.8)	38.7 (10.3)	42.2 (12)*
Ceff,rs (mL/cmH_2O)						
Mean (SD)	32.7 (8)	33.3 (9.9)	31.3 (7.7)	32.4 (8.1)	32.6 (9.1)	34.8 (9.4)*
Rtot,rs (cmH_2O/L/s)						
Mean (SD)	16.4 (5.4)	17.1 (4.6)	17.6 (5.9)	17 (4.6)	16.8 (5.5)	16.4 (5.7)
Rinit,rs (cmH_2O/L/s)						
Mean (SD)	13.8 (4.8)	14.4 (4.1)	15.1 (4.7)	14.2 (3.8)	13.6 (4.5)	14 (5.4)

Abbreviations: CTRL = control intervention; ERCC = expiratory rib cage compression; Cst = static compliance of the respiratory system, Ceff = effective compliance of the respiratory system, Rtot = total resistance of the respiratory system, Rinit = initial resistance of the respiratory system. *statistically different from CTRL ($P < .05$). PRE = baseline; POST1 = immediately after CTRL or ERCC; POST2 = immediately after a hyperinflation maneuver.

Table 3 - Expiratory Flow Profiles During Expiratory Rib Cage Compression in 20 Mechanically Ventilated Patients with Pulmonary Infection

	PRE		**PER**	
	CTRL	**ERCC**	**CTRL**	**ERCC**
PEF (L/min)				
Mean (SD)	46.4 (15.2)	43.6 (17.5)	46.2 (15.3)	59.6 (18.3)**
Flow 30% Vt (L/min)				
Mean (SD)	15 (7.3)	12.6 (6.1)	15.1 (7.5)	38 (15.7)**
Flow Limitation, n (%)				6 (30)

Abbreviations: PEF = peak expiratory flow; Flow 30% Vt = flow at 30% of the expiratory tidal volume. CTRL = control intervention; ERCC = expiratory rib cage compression; PRE = baseline; PER = during CTRL or ERCC. **statistically different from CTRL (*P* < .01).

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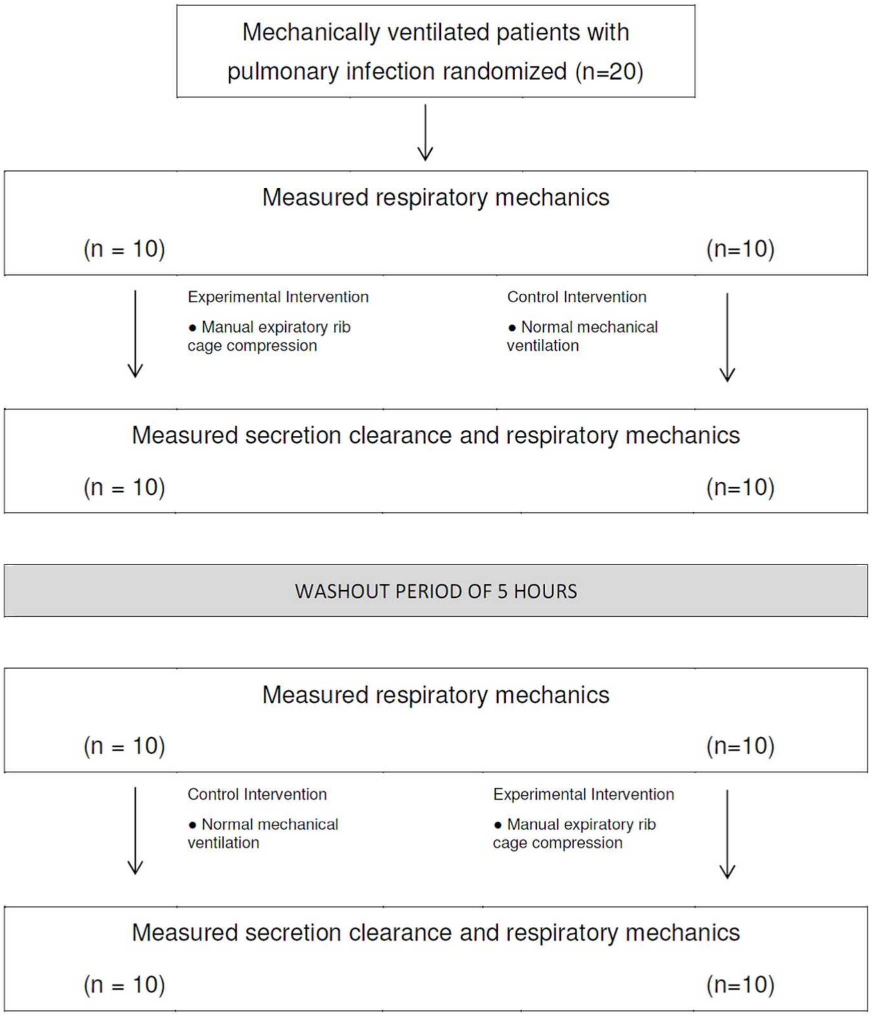

Figure 1 - Design and flow of participants through the trial.
115x121mm (300 x 300 DPI)

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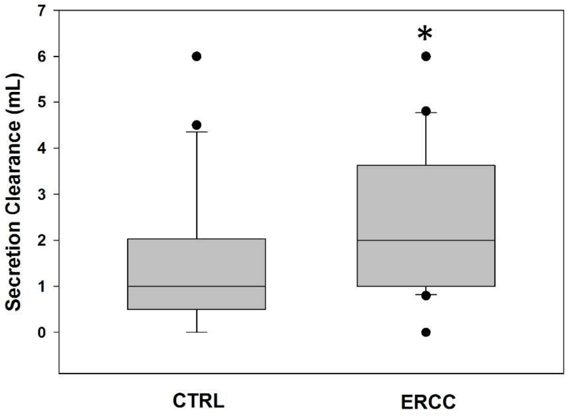

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