Effects of different recruitment maneuvers on bacterial translocation and ventilator-induced lung injury

Perihan Ergin Özcan, M.D., Özkan İbrahim Akıncı, M.D., İpek Edipoğlu, M.D., Evren Şentürk, M.D., Sevil Baylan, M.D., Atahan Arif Çağatay, M.D., Kemal H Türköz, M.D., Figen Esen, M.D., Lütfi Telci, M.D., Nahit Çakar, M.D.

1Department of Anesthesiology, Istanbul University Istanbul Faculty of Medicine, Istanbul-Turkey
2Department of Infectious Diseases and Clinical Microbiology, Istanbul University Istanbul Faculty of Medicine, Istanbul-Turkey
3Department of Pathology, Marmara University Faculty of Medicine, Istanbul-Turkey

ABSTRACT

BACKGROUND: Investigated in the present study were the effects of various recruitment maneuvers (RMs) using the same inflation pressure-time product on bacterial translocation from lung to blood, and ventilator-induced lung injury (VILI).

METHODS: Tracheotomy was performed on anesthetized rats, and ventilation was initiated using pressure-controlled mode. Subsequently, Pseudomonas aeruginosa was inoculated through the tracheotomy tube and ventilated for 30 minutes before rats were randomly separated into 4 groups. Group 1 underwent sustained inflation (SI), Group 2 underwent low-pressure SI, Group 3 underwent modified sigh, and Group 4 was a control group. Blood cultures were taken at baseline, 15 minutes after randomization (after each RM for the first hour), and finally at 75 minutes after the last RM. The rats were euthanized and the lungs were extirpated. The left lung was taken for measurement of wet:dry weight ratio, and the right lung was used for pathologic evaluation.

RESULTS: Positive blood cultures were found to be higher in Group 3 at early study periods. Total pathological scores were also higher in Group 3.

CONCLUSION: Higher severity of ventilator-induced lung injury occurred in the modified sigh group, evidenced by bacterial translocation and results of histopathological evaluation.

Keywords: Bacterial translocation; mechanical ventilation; recruitment maneuver; SIGH; ventilator-induced lung injury.

INTRODUCTION

Mechanical ventilation is the most important supportive therapeutic option in cases of acute respiratory distress syndrome (ARDS). However, it is well known, based on experimental and human studies, that mechanical ventilation may cause ventilator-induced lung injury (VILI).[1]

Different recruitment maneuvers (RMs) are important adjuncts of mechanical ventilation in clinical practice, used to enhance oxygenation by opening collapsed sections of the lung. Due to the tendency of unstable alveoli to collapse, it is thought that RMs are likely to reduce VILI that occurs due to repeated opening and closing during ventilation. However, RMs have side effects, and may induce hyperinflation and lung injury.[2] Therefore, to evaluate RMs for efficiency and related risks, optimal pressure level, applicable time period, and applicable rate should be discussed.

RMs can be performed in different ways. Sustained inflation (SI) is applied to raise static airway pressure to a fixed level for a fixed time period. Inflation pressure applied in several RM-related studies has ranged between 30 and 60 cmH₂O, with the time periods ranging from 3 seconds to 3 minutes. These studies have suggested that the magnitude of airway pressure and duration of pressure elevation play major roles in the success of an RM.[3–5] Respiratory effects of different RMs have also been studied.[6]

Bacterial translocation from the lungs to the bloodstream has been used as a marker of VILI in a number of experimental
Repeated collapse and reopening of the alveoli, inflammation, and high ventilation pressures and volumes have been shown to cause VILI.\[11,12\]

Examined in the present study were the effects of different RMs with identical inflation pressure-time products on bacterial translocation from the lungs to the bloodstream, oxygenation, morphologic changes, and wet-dry weight ratio of the lungs. The authors hypothesized that the level of pressure applied was not the only factor triggering VILI, and that the frequency and timing of pressure also had significant impacts on its occurrence.

MATERIALS AND METHODS

The study was conducted following approval of protocol from the Institutional Animal Investigation Committee. Care and handling of the animals were in accordance with European Community guidelines. Thirty-two male Sprague Dawley rats (each weighing 250–300 g) were used. Sample size was calculated in accordance with a previous study.\[10\] Significance level (each weighing 250–300 g) were used. Sample size was calculated in accordance with a previous study,\[10\] Significance level of 5% (α=0.05) and probability of 80% (β=0.20), used to detect a difference of at least 80% increase in bacterial translocation, indicated an appropriate sample size of n=7 subjects.

Animal Preparation

Rats were anesthetized with inhaled mixture of 1–3% isoflurane, 60% O₂, and 40% air, with subsequent intraperitoneal injection of 50 mg/kg ketamine. Following surgical tracheotomy, the rats were kept under pressure-controlled ventilation with Servo 300 ventilator (Siemens AG, Solna, Sweden).

Ventilation parameters:

(i) peak inspiratory pressure (PIP) of 10 cmH₂O
(ii) positive end-expiratory pressure (PEEP) of 0 cmH₂O
(iii) respiratory rate of 60 breaths/minute
(iv) inspired fraction of oxygen (FiO₂) of 1.0
(v) inspiratory-to-expiratory time ratio (I/E) of 1/2

Intraperitoneal ketamine (50 mg/kg) and vecuronium bromide (0.5 mg/kg) were also used for maintenance of anesthesia and muscle relaxation.

The carotid artery was cannulated using 24-gauge Insyte-W catheter (Becton Dickinson Infusion Therapy Systems Inc., Sandy, UT, USA) in aseptic conditions. Blood pressure was monitored using Mercury disposable transducer (Mennen Medical, Inc., Southampton, PA, USA), and blood samples were obtained for blood gas analyses and blood cultures. Rectal body temperatures were continuously monitored, and normothermia was maintained using heating lamp and pad.

Bacterial Preparation

Regarding preparation of the bacterial solution, Pseudomonas aeruginosa (ATCC 27853) were thawed and cultured over-night, incubated in brain-heart infusion broth (Becton Dickinson, Inc., Sparks, MD, USA) in 37°C to obtain stationary-phase microorganisms. A tube containing an inoculum of 1x10⁵ colony-forming units/mL of Pseudomonas aeruginosa was individually prepared for each rat on every study day and kept on ice until used.

Experiment Protocol

After completion of monitorization, rats were ventilated with baseline ventilator settings for 15 minutes in order to achieve stabilization. Baseline ventilator parameters (tidal volume [Vₜ], PIP, mean airway pressure [MawP], PEEP) and hemodynamic parameters (mean arterial pressure [MAP], heart rate [HR]) were recorded. Blood samples were obtained for baseline blood gas analyses and blood culture. Colloid solution (hydroxyethyl starch 450/0.7, 6%; Eczacıbaşı Baxter, İstanbul, Turkey) was infused to replace blood loss after each blood draw. Blood pressure was kept within normal range by saline infusion.

After first blood samples were obtained, 500 μL of saline containing 10^5 cfu/mL Pseudomonas aeruginosa was instilled through the tracheostomy tube, and 5 mL of air was injected to distribute the bacteria through the lungs.

PEEP level was increased to 3 cmH₂O, and all rats were ventilated for 30 minutes prior to being randomly separated into 4 groups, as follows:

- **Group 1 (Pressure group):** SI of 40 cmH₂O PEEP - 20 sec, 4 times/h
- **Group 2 (Time group):** Low-pressure SI of 20 cmH₂O PEEP - 40 sec, 4 times/h
- **Group 3 (Modified sigh group):** Modified sigh of 40 cmH₂O PIP - 3 cmH₂O PEEP for 1 minute, f: 60/minute, I/E:1/2, 4 times/h
- **Group 4 (Control group):** 10 cmH₂O PIP - 3 cmH₂O PEEP

In all groups, ventilator settings between RMs were maintained at baseline (PIP: 10 cmH₂O; PEEP: 3 cmH₂O; f:60/min; FiO₂: %100; I/E:1/2).

Inflating pressure-time product (the product of the pressure value over the time it is applied) was equal in the 3 RMs (Fig.1). The method applied in Group 3 was not completely in line with sigh. For this reason, in order to establish an equal product in all groups, high PIP (40 cmH₂O) was imposed at a frequency level of 60/minute for 1 minute. Because of this, the RM of Group 3 is described as “modified sigh.”

Microbiologic Evaluation

Blood cultures were incubated at 37°C overnight and kept in the incubator for at least 10 days. Blood cultures were analyzed to determine whether culture positivity had been obtained each day, regardless of turbidity or opacity. Bacteremia was defined as ≥1 colony of Pseudomonas aeruginosa appearing in 100 μL of blood sample cultured on agar plate.
Isolated strains were identified using standard microbiological methods. *Pseudomonas aeruginosa* strains were identified by the presence of large colonies, grape-like odor, oxidase-positive colonies, the ability to grow at 42°C, and characteristic pigmentation. Once isolated strains were identified, E-test strips (AB Biodisk Na Inc., Solna, Sweden) were used to determine whether susceptibility pattern was similar to that of *Pseudomonas aeruginosa*.

### Morphological Evaluation

The rats were euthanized using intra-arterial sodium thiopental (120 mg/kg), the thorax was opened under aseptic conditions, and the lungs were extirpated with the heart. The left lung was used for the measurement of wet:dry weight. Microbalances were used to determine wet weight; the lung was then kept in an incubator at 100°C for 24 hours and weighed again to determine dry weight.

The right lung was sent to the pathology laboratory in 10% formalin. A pathologist blinded to the study groups performed histological examination. The lung was serially sectioned in caudal-to-coronal fashion from the apex to the base and embedded in paraffin blocks. Following routine dehydration and clearing processes, 3–4 µm sections from each paraffin block were taken and stained with hematoxylin and eosin. All fields of slides were read. Sections were evaluated with grading scale of 0 to 3 for 6 parameters (perivascular edema, peribronchial lymphocytic infiltration, intra-alveolar hemorrhage, intra-alveolar macrophage infiltration, interstitial mononuclear cell infiltration, interstitial polymorphonuclear leukocyte infiltration; 0 = none, 1 = focal and rare, 2 = widespread, 3 = whole-lung involvement).

Six blood cultures were taken: at baseline, 15 minutes after RM, and 75 minutes after final maneuver. In addition, ventilator variables and hemodynamic parameters were recorded simultaneously. Blood gas analyses were performed at baseline and at experiment conclusion, when the final blood culture was taken. Schematic diagram of the experiment is shown in Fig. 2. Primary outcome was evaluation of bacterial translocation, and secondary outcomes concerned oxygenation, morphologic changes, and wet:dry weight.

### Statistical Analysis

Morphologic evaluation scores, and pH, PaO₂, PaCO₂, MawP, and MAP values are reported as mean±SD. Intergroup comparisons were performed using Kruskal-Wallis one-way analysis of variance. Dunn’s multiple comparisons test was used for post-hoc analysis when p<0.05. Wilcoxon Mann-Whitney U test was used for intragroup analysis. Chi-square test was used to compare positive blood cultures among groups. Kaplan-Meier curves and log-rank test were used to analyze bacterial translocation. In all analyses, p<0.05 was considered statistically significant.
RESULTS

Blood Cultures

All rats survived until the end of the experiment. Positive blood cultures were observed 15 minutes after the first maneuver in 1 rat Group 1, 2 rats in Group 2, and 5 rats in Group 3 (Table 1). In Group 3, positive blood cultures were observed in all rats after the third maneuver. Group 4 and Group 3 culture results differed at all times except baseline. Blood culture results obtained following third RM revealed that the number of positive cultures in Group 3 was significantly different than in Group 2 (OR: 0.02; 95% CI: 0.0009 to 0.55, p=0.007) and Group 4 (OR: 0.003; 95% CI: 0.0000 to 0.19, p=0.0002). By the end of the experiment, all blood cultures were positive in Groups 1, 2, and 3, but no positive blood cultures were present in Group 4. In Fig. 3, the Kaplan-Meier curve shows percentage of positive blood cultures in terms of time in each group (p=0.0012, log-rank test).

Blood Gas Analysis, and Hemodynamic and Mechanical Ventilation Variables

Baseline pH, PaO₂, PaCO₂, MawP, and MAP were similar in all groups (Table 2). When baseline and end-of-experiment values were compared, PaO₂ was lower in Groups 1, 2, and 3, though the difference was significant only in Group 3 (p=0.004). By the end of the experiment, the PaO₂ value in Group 4 was significantly higher than that in Groups 1, 2, and 3. In addition, PaCO₂ was decreased in all experimental groups. However, no significant differences between baseline and final PaCO₂ values were observed.

<table>
<thead>
<tr>
<th>Table 1. Time course of positive blood culture results</th>
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<tbody>
<tr>
<td>Group 1 (n=8)</td>
</tr>
<tr>
<td>Baseline</td>
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<tr>
<td>1st RM</td>
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<tr>
<td>2nd RM</td>
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<tr>
<td>3rd RM</td>
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<tr>
<td>4th RM</td>
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<td>75 min after last RM</td>
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</table>

RM: Recruitment maneuver. *p<0.05, significantly different from Group 3; †p<0.05, significantly different from Group 3; ≠p<0.05, significantly different from Group 1; §p<0.05, significantly different from Groups 1, 2, and 3.

<table>
<thead>
<tr>
<th>Table 2. Oxygenation, and hemodynamic and mechanical ventilation variables</th>
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<tbody>
<tr>
<td>Group 1</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Baseline</td>
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<tr>
<td>End of experiment</td>
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<tr>
<td>PaO₂ (mmHg)</td>
</tr>
<tr>
<td>Baseline</td>
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<tr>
<td>End of experiment</td>
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<tr>
<td>PaCO₂ (mmHg)</td>
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<tr>
<td>Baseline</td>
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<tr>
<td>End of experiment</td>
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<tr>
<td>MAP (mmHg)</td>
</tr>
<tr>
<td>Baseline</td>
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<tr>
<td>End of experiment</td>
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<tr>
<td>MawP (cmH₂O)</td>
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<tr>
<td>Baseline</td>
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<tr>
<td>End of experiment</td>
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<tr>
<td>VT (mL)</td>
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<tr>
<td>Baseline</td>
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<td>End of experiment</td>
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MAP: Mean arterial pressure; MawP: Mean airway pressure; VT: Tidal volume. *p<0.05, significantly different from Group 4; †p<0.005, significantly different from baseline.
In addition, MAP was monitored continuously throughout the experiment, though only 6 MAP values for each rat were included in statistical analysis. Compared to baseline values, MAP had decreased in all groups by the end of the experiment. These decreases were statistically significant in Groups 1, 2, and 3, but not in the control group.

A total of 4 mL blood was obtained for blood gas analyses and blood cultures throughout the study, and the same amount of colloid was used to replace blood loss. The amounts of NaCl used during the study period were 4.9±1.6 mL in Group 1, 4.5±1.6 mL in Group 2, 5.5±1.6 mL in Group 3, and 3.4±1.3 mL in Group 4.

Wet:dry weight ratio was higher in Group 3 (5.3±0.7), compared to Group 1 (4.5±1.5), Group 2 (4.6±2.1), and Group 4 (4.6±0.3), though the differences were not statistically significant.

### Histology Results

The right lung was evaluated for 6 pathological changes (Table 3). One of the most striking results was that the highest scores were seen in Group 3. Intra-alveolar hemorrhage, intra-alveolar macrophage, and polymorphonuclear leukocyte infiltration were significantly different among the groups (p<0.05).

### DISCUSSION

Primary findings were as follows:

1. RMs had positive blood cultures.
2. Modified sigh had negative effect on bacterial translocation, histopathology, and oxygenation.

Investigated in the present study were the influences of different RMs with the same inflation pressure-time product on bacterial translocation from the lungs to the bloodstream, and the modified sigh group was found to be at highest risk. Many experimental studies have shown that bacteria translocate from the lungs to the bloodstream via mechanical ventilation.[7–10] Mechanical ventilation strategies associated with high VT and high airway pressure can produce microvascular injury in the lungs, leading to pulmonary edema, which results in lymphatic flow acceleration that allows bacteria to enter systemic circulation.[13,14] Deterioration of mucociliary activity caused by mechanical ventilation can also facilitate passage of bacteria by disrupting bacterial clearance. The bacteria may be forced to spread out of the airway due to the physical impact of positive pressure, which is reported to be more significant in small animals.[1]

The highest rate of bacterial translocation via repeated RMs was observed in the modified sigh group. This rate, observed particularly in the early stages, indicated that repetitive opening and closing can cause more severe lung injury than exposure to high pressure or prolonged exposure to pressure. In an experimental study, Tschumperlin et al. demonstrated that repeated intermittent distention in alveolar cell culture leads to more inflammatory mediator secretion than fixed continuous distension.[15] During histopathologic examination in the present study, intra-alveolar hemorrhage and intra-alveolar macrophage infiltration were found to be more significant in the modified sigh group. Though not statistically significant, other histopathologic parameters indicating lung injury were also found to be higher in the modified sigh group.

### Table 3. Quantitative pathology scores of groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Perivascular edema</td>
<td>0.33±0.50</td>
<td>0.50±0.70</td>
<td>0.80±0.78</td>
<td>0.25±0.50</td>
<td>0.44</td>
</tr>
<tr>
<td>Peribronchial lymphocyte infiltration</td>
<td>1.55±1.01</td>
<td>1.40±0.69</td>
<td>2.00±0.66</td>
<td>0.75±0.50</td>
<td>0.06</td>
</tr>
<tr>
<td>Intra-alveolar hemorrhage</td>
<td>0.88±0.33</td>
<td>1.10±0.73</td>
<td>1.60±0.84</td>
<td>0.50±0.57</td>
<td>0.04</td>
</tr>
<tr>
<td>Intra-alveolar macrophage</td>
<td>0.33±0.70</td>
<td>0.60±0.69</td>
<td>1.50±0.84</td>
<td>0.50±1.00</td>
<td>0.02</td>
</tr>
<tr>
<td>Interstitial mononuclear cell infiltration</td>
<td>2.00±0.86</td>
<td>2.20±0.91</td>
<td>2.30±0.67</td>
<td>1.00±0.00</td>
<td>0.052</td>
</tr>
<tr>
<td>Interstitial PNL infiltration</td>
<td>1.55±1.01</td>
<td>1.80±0.78</td>
<td>2.40±0.69</td>
<td>1.00±0.81</td>
<td>0.048</td>
</tr>
<tr>
<td>Total</td>
<td>1.11±0.98</td>
<td>1.26±0.95</td>
<td>1.76±0.90</td>
<td>0.66±0.63</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

PNL: Polymorphonuclear leukocytes; *p<0.05, significantly different from Group 3; †p<0.0001, significantly different from Group 3; ‡p<0.05, significantly different from Group 3.
In the 3 experimental groups, different numbers of positive blood cultures were observed after the first maneuver. This early bacteremia supports the hypothesis that application of mechanical ventilation can cause susceptibility to bacteremia. Lin et al. similarly reported that translocation of the instilled bacteria can lead to positive blood culture in spite of preventive artificial ventilation strategies (V_{f}: 7 mL/kg; PEEP: 5 cmH\textsubscript{2}O).[16] As mentioned in the methodology of the present study, there was an interval of 1 hour between the initiation of mechanical ventilation and sampling of the first positive blood culture. This interval apparently provided the necessary time for translocation.

In a previous study conducted by the present study group, an RM (45 cmH\textsubscript{2}O/30 sec) had been applied every 15 minutes for 2 hours on rats that had been administered intratracheal Pseudomonas aeruginosa, and positive blood culture was not detected.[19] However, in the present study, the rats were administered mechanical ventilation for 45 minutes prior to the first RM. This interval may have allowed bacteria to spread across the alveoli. Due to several environmental factors, reduced bacterial lag time increasing the number of bacteria may also have played a role in the translocation. Another important difference is that mechanical ventilation frequency was 60/minute, and decrease in PaCO\textsubscript{2} values was observed at the end of the study, compared to the baseline values. Cakar et al. used mechanical ventilation with a frequency rate of 30/minute, and PaCO\textsubscript{2} increased in the RM group. Stretch injury due to mechanical ventilation could have been avoided by hypercapnic acidosis, and no growth may have been noticed in blood cultures, a suspicion that has been supported in the literature.[17,18] A third difference between previous studies and the present is that, despite fluid resuscitation, MAP decreased, compared to baseline, in all groups except for the control group by the end of the study. It is thought that hypotension due to impaired circulation and repetitive opening and closing may have made the modified sigh group more susceptible to lung injury.

That the highest rate of oxygenation deterioration, the earliest and most pronounced growth on blood culture, and the highest, though still not significant, wet: dry ratio were observed in the modified sigh group has led the authors to suspect that the mechanism somehow causes increased damage to lungs, in spite of inflation pressure-time product and baseline ventilation parameters being identical to those of the other RMs. Though the 3 experimental groups were exposed to high pressure for the same amount of time, the method was different. PIP of 40 cmH\textsubscript{2}O and PEEP of 3 cmH\textsubscript{2}O caused repetitive opening and closing in the modified sigh group for 1 minute. It is thought that, because high levels of pressure elicit opening, the repetitive opening and closing result in damage to the alveoli. While duration of exposure to high pressure was the same, a constant level of pressure was applied with no variation in the pressure and time groups. Compared to the time group, growth was observed earlier and in greater amounts in the pressure group, though there was no statistically significant difference. However, the literature supports that of these 2 important RM components, pressure has more impact than time.[19–21]

Many interesting points have been made in the “pressure vs time” debate. Pressure and time are primary factors in recruitment and derecruitment of the alveoli. Based on the mathematical model developed by Bates and Irwin, alveoli not only have critical opening and closing pressure, but duration of opening and closing changes in acutely injured lungs.[22]

Several limitations affected the present study. First, such differentiations between the time and the pressure groups were not made, because volumetric measurements were not taken. Second, results of 40 cmH\textsubscript{2}O/40 sec and 20 cmH\textsubscript{2}O/20 sec groups were not compared with 40 cmH\textsubscript{2}O/20 sec and 20 cmH\textsubscript{2}O/40 sec groups. Third, the same maneuvers used in an ARDS model may have produced different results.

In conclusion, all RMs eventually resulted in bacteremia. Higher severity of VILI occurred in the modified sigh group, evidenced by bacterial translocation and histopathological evaluation, despite it having the same inflation pressure-time product as the other RM groups.

Acknowledgments
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Conflict of interest: None declared.

REFERENCES

Farklı yeniden kazandırma manevralarının bakteri translokasyonu ve ventilatör ilişkili akciğer hasarına etkisi

**ÖzET**

**DENEYSEL ÇALIŞMA - ÖZET**

**AÇIKLAMA**

**AMAC:** Bu çalışmada, aynı basınç-zaman ürünü farklı yeniden kazandırma manevralarının kana bakteri geçişine ve ventilatör ilişkili akciğer hasarına etkisi araştırıldı.

**GÖREV VE YONTEM:** Sıçanlara anestezi uygulandıktan sonra trakeotomi açıldı ve basınç kontrollü ventilasyon modunda ventilasyona başlandı. Ardından Pseudomonas aeruginosa içeren solüsyon trakeotomi kanulünden verilip, ventilasyona bu şekilde 30 dakika devam edildikten sonra sıçanlar rasgele dört gruba ayrıldı. Grup 1: “Sustained inflation” (SI); Grup 2: Düşük basınç SI (LPSI); Grup 3: Modifiye Sigh; Grup 4: Kontrol grubu. Kan kültürleri bakteri verilmeden önce, ilk bir saat her yeniden kazandırma manevrasından sonra ve son manevradan 75 dakika sonra toplam altı kez alındı. Daha sonra sıçanlar arter içine verilen sodium tiyopental ile sakrifiye edilerek toraks açılıp akciğerler çıkarıldı. Sol akciğer yaş kuru ağırlık (WW/DW) oranı için, sağ akciğer patolojik inceleme için kullanıldı.

**BULGULAR:** Kan kültürlerine bakıldığında Grup 3 de daha erken dönemde kan kültürlerinde üreme tespit edildi. Grup 3 de patolojik skorlar daha 2015;151:1568–75. CrossRef

**OLGU SUNUMU**

**GEREÇ VE YÖNTEM:** Sıçanlara anestezi uygulandıktan sonra trakeotomi açıldı ve basınç kontrollü ventilasyon modunda ventilasyona başlandı. Ardından Pseudomonas aeruginosa içeren solüsyon trakeotomi kanulünden verilip, ventilasyona bu şekilde 30 dakika devam edildikten sonra sıçanlar rasgele dört gruba ayrıldı. Grup 1: “Sustained inflation” (SI); Grup 2: Düşük basınç SI (LPSI); Grup 3: Modifiye Sigh; Grup 4: Kontrol grubu. Kan kültürleri bakteri verilmeden önce, ilk bir saat her yeniden kazandırma manevrasından sonra ve son manevradan 75 dakika sonra toplam altı kez alındı. Daha sonra sıçanlar arter içine verilen sodium tiyopental ile sakrifiye edilerek toraks açılıp akciğerler çıkarıldı. Sol akciğer yaş kuru ağırlık (WW/DW) oranı için, sağ akciğer patolojik inceleme için kullanıldı.

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