筑　波　大　学

博 士 （医 学） 学 位 論 文

The effects of open vs. closed endotracheal suctioning on lung injury in lavage-induced surfactant-depleted model

（閉鎖式・開放式吸引が肺傷害に与える影響）

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Contents

List of abbreviations:

ARDS	acute respiratory distress syndrome
VILI	ventilator–induced lung injury
PEEP	positive end expiratory pressure
OS	open endotracheal suctioning
CS	closed endotracheal suctioning
HC	healthy control
P/F ratio	PaO_2/FIO_2 ratio
PIP	peak inspiratory pressure
ELISA	Enzyme-Linked Immunosorbent Assay
IL-6	interleukin-6
TNF-α	tumor necrosis factor –alpha
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
SD	Standard deviation

Introduction

Acute respiratory distress syndrome (ARDS) is one of the most challenging problems in critical care medicine, with substantial mortality and significant long-term morbidity [1]. ARDS is a clinical syndrome characterized by severe hypoxemia, stiff lungs, and decreased respiratory system compliance. In its early phase, ARDS is characterized by acute and diffuse endothelial and epithelial injury termed diffuse alveolar damage [2], which leads to increased vascular permeability with protein-rich exudative edema. Although originally thought to be relatively homogeneous, a number of recent studies have highlighted the marked heterogeneity of the pathological process with consolidation in the dependent regions of the lung and relatively normal aeration of the nondependent regions [3, 4].

Mechanical ventilation is a life-saving tool for patients with ARDS. However, as with any therapy, it also has the potential to cause or aggravate progressive tissue damage or lung injury, a phenomenon often referred to as ventilator–induced lung injury (VILI) [5-7] . This phenomenon is particularly true in patients with ARDS because of the widespread, heterogeneous distribution of consolidated/atelectatic regions, which produce a small lung volume available for ventilation [3, 7]. Using computed tomography, Gattinoni et al. [3] showed that the lungs of patients with ARDS are highly

asymmetrical along the vertical axis with a small non-dependent lung region continuously open to ventilation, and a dependent consolidated, atelectatic region. In between, there is a region that can be recruited or derecruited depending on the particular ventilator strategy used [3]. In such patients, mechanical ventilation could lead to injury due to overdistention as more of the tidal volume is distributed to the small, relatively normal alveolar regions [3, 8, 9] and/or repeated recruitment or derecruitment of alveolar units that may be exacerbated with ventilation (atelectrauma) [3, 10-13]. Notably, repeated recruitment and de-recruitment can up-regulate a cytokine response in ARDS patients such as tumor necrosis factor-α and interleukin-6 [14]. VILI is characterized by vascular leakage and inflammatory responses that ultimately lead to pulmonary dysfunction [15]. Such inflicted injuries may subsequently stimulate a cascade of biological responses, leading to further lung injury (biotrauma) [16, 17]. Importantly, biotrauma will not only aggravate ongoing lung injury, but can also lead to multiple organ failure. The key to a successful clinical management of patients with ARDS is preventing further advancement of VILI. For this reason, the main goal of the latest strategies for lung protective ventilation has been prevention of alveolar over-distension and derecruitment.

In order to achieve optimal alveolar recruitment, patients with ARDS are often exposed to high levels of positive end-expiratory pressure (PEEP). Exposure of ARDS patients to unintended sudden withdrawal of PEEP (due to transportation of patients, alternating PEEP, endotracheal suctioning, etc.) may aggravate lung injury/collapse and decrease oxygenation. Although endotracheal suctioning is known to be one of the causes of repeated derecruitments during mechanical ventilation, it is still routinely performed in patients with ARDS. There are two methods of endotracheal suctioning, based on selection of catheter: open endotracheal suctioning (OS) and closed endotracheal suctioning (CS) (Fig. 1). OS is the traditional procedure for endotracheal suctioning, which requires disconnecting the patient from the ventilator, followed by insertion of a suction catheter into the trachea. On the other hand, CS allows passage of a suction catheter through the artificial airway, without disconnecting the ventilator (Fig. 1). Maggiore et al. reported that OS induced alveolar derecruitment in patients with ARDS [18]. In the presence of ARDS, the massive loss of lung volume induced by the disconnection of the patient from the ventilator is the predominant mechanism of hypoxemia [19]. Furthermore, the high negative suctioning pressure required for removing bronchial secretions contributes to the loss of lung volume. In contrast, CS is effective to prevent alveolar derecruitment by avoiding ventilator disconnection, thereby

maintainig appropriate oxygenation [18]. On the other hand, a previous study reported that CS also causes desaturation and derecruitment during mechanical ventilation in pediatric patients [20]. The short term effects of endotracheal suctioning are clear (*i.e.*, desaturation and loss of lung volume), but long term and repetitive effects, especially lung injury or molecular alternations, are not clear. Thus, it is unclear whether repeated endotracheal suctioning can exacerbate lung injuries during mechanical ventilation. Additionally, no study to date has investigated the effects of repeated OS vs. repeated CS on: a) lung morphology and molecular profile of crucial cytokines at the circulatory and pulmonary tissue levels; and b) the profile of hemodynamic and respiratory parameters in lavage-induced surfactant-depleted lung injury models during mechanical ventilation.

The facts stated above led us to hypothesize that repeated endotracheal suctioning, especially open suctioning of longer time span, could cause continuous alveolar derecruitment, resulting in gradual reductions in arterial oxygenation and, subsequently, exacerbate lung injury with atelectrauma. The aim of the present study was to assess whether repeated derecruitments induced by OS exacerbates lung injury compared to CS during mechanical ventilation with high PEEP in lavage-induced surfactant-depleted lung injury models. It is anticipated that data generated from the present study will

clarify the effects of repeated OS vs. CS on VILI.

Materials and methods (Fig. 2)

Animal preparation

Thirty six male Japanese White rabbits weighing between 2.8 and 3.5 kg were anesthetized using sodium pentobarbital (75 - 150 mg, bolus infusion) and restrained in a supine position. Under local anesthesia using 1.0% lidocaine solution (0.25 mg/kg), the ventral side of the neck was carefully dissected and a tracheostomy was performed, and an endotracheal tube (3.5 mm internal diameter) placed in the trachea and tied in order to stabilize it. The animals were then ventilated with a LTV-1000 ventilator (CareFusion, San Diego, CA) in pressure-controlled mode with PEEP of 2 cm H_2O, inspiratory time of 0.5 sec and inspired oxygen fraction of 1.0. Airway pressure was adjusted constantly to achieve constant expiratory tidal volume of 6 mL/kg. Initial respiratory rate was set to achieve normo-carbia. Mechanical ventilation was continued in the same manner throughout the experiment, except for the adjustments of PEEP level described later. Anesthesia and muscle paralysis were maintained by continuous infusion of sodium pentobarbital (5 mg/kg/h) and pancuronium (0.1 mg/kg/h) via infusion pump through the ear vein. Normal saline (3 mL/kg/h) was then continuously

infused as maintenance fluid.

The experimental protocol of the present study was approved by the Ethics Committee of the Animal Resource Center of the University of Tsukuba. The animals were cared for in accordance with the guidelines for ethical animal research.

The animals were divided into four groups, *i.e.*, a) OS with lung injury (OS); b) CS with lung injury (CS); c) a control group with lung injury, but without endotracheal suctioning (Control); d) and a healthy control group with 6 hours of ventilation, but without lung injury and endotracheal suctioning (HC) (Fig. 2). In our primary study protocol, groups were CS and OS, Only. A Control and HC groups were also added. Animals in the control and HC groups were, however, not randomly assigned to their respective groups. In order to evaluate and validate the results of the present study all the experiments were repeated using newly added control and HC groups.

Lavage-induced surfactant-depleted lung injury model

The lavage-induced surfactant-depleted lung injury model is a frequently used experimental model of ARDS [12, 13, 21, 22]. Bayat et al. [22] reported that after the lavage-induced surfactant-depletion, animals developed significantly increased area of atelectasis, associated with poor aeration in dependent lung, which could promote the

local concentration of mechanical stresses. An increase in positive end-expiratory pressure significantly reduced poor aeration and recruited atelectasis, but ventilation redistribution persisted and lung remained derecruited. Depletion of surfactant causes lung injury by two mechanisms: first, by facilitating alveolar collapse and increasing mechanical injury to the alveolar walls during repeated cycles of opening/closure during mechanical ventilation, and second, by impairing alveolar host defenses [21]. The saline lavage by itself has little consequence in terms of permeability changes or inflammation [21]. In addition, lavage-induced surfactant-depleted lung injury model is hemodynamically stable [21, 22]. Therefore, this model is optimal to examine the present hypothesis.

After 30 min of stabilization, baseline data were recorded and induction of lung injury was started. Lung injury was induced whole lung lavage using a modified technique described previously by a number of investigators [12, 13, 22, 23]. With the animals in the supine position, the endotracheal tube was disconnected from the ventilator, and saline solution at 38°C (15 mL/kg) was gravity-instilled via the endotracheal tube. The animals were gently rotated from side to side in order to help spread saline solution uniformly. After instillation was completed, the animals were mechanically ventilated with a pressure not exceeding 28 cm H2O for a minute or until

severe bradycardia (<40 beats/min). Subsequently saline solution was drained out of the lung by gravity and then actively suctioned with a suction catheter. After the first lavage, and between subsequent lavages, the animals were ventilated for 5 min with a peak inspiratory pressure (PIP) of 12 cm H2O, a PEEP of 2 cm H2O. Arterial blood gases were monitored after every lavage, and lavage was repeated until the arterial blood gas, drawn 5 min later, showed PaO2/ FIO2 ratio (P/F) < 100. Clinically, ARDS is defined as PaO2/ FIO2 < 200 (regardless of PEEP), with bilateral infiltrates observed on frontal chest radiograph, with no clinical evidence of left heart failure [24]. After confirmation of a stable severe lung injury by another arterial blood gas 30 min later (P/F <100), the experimental protocol was begun, as described below.

Ventilation protocols (Fig. 2)

After lung injury was achieved, intermittent mandatory pressure control ventilation was set as follows: a) the fraction of inspired oxygen was set at 1.0; b) tidal volume was set at 6 mL/kg, c) inspiratory time was at 0.5 sec, d) PEEP was set at 10 cm H_2O (PEEP level was adopted from lower inflection point of previous studies with some minor modifications [12, 13]), e) the mandatory respiratory rate was set at 30/min and f) the

inspiratory pressure limit was set at 28 cm H_2O (the PIP was limited to 28 cm H_2O in order to prevent early deaths from pneumothorax, which occurred in most animals during a pilot study when higher PIP values were used). The mandatory respiratory rate was subsequently adjusted to maintain the $PaCO_2$ in the range of 60 - 100 mm Hg, where possible, with a rate of 30 – 40 /min [25].

Endotracheal Suctioning Protocols (Fig. 2)

CS was performed twice every 30 minutes during ventilation, using a 6 French closed suctioning catheter system (Trachcare, Ballard Medical products, Draper, UT), which was connected to the endotracheal tube under the following conditions: a) Suctioning time and pressure of 10 sec and 140 mm Hg (20 Kpa), respectively; and b) Suction depth of 2 cm (length of adapter) plus length of tracheal tube [26]. OS was performed with the same catheter (Trachcare) under the same conditions, except with a disconnected ventilator circuit from the animal. After OS, ventilator circuit was reconnected at the previous settings.

Data collection (Fig. 2)

The right carotid artery was catheterized for blood gas sampling and monitoring of

arterial pressure. Heart rate and mean arterial pressure were monitored using Philips IntelliVue MP50 Patient Monitor (Philips Medizin Systeme GmbH, Böblingen, Germany). Body temperature was monitored continuously using a rectal probe and was maintained between 38 and 39°C using a heating pad. Arterial blood gas variables including pH, PaO_2, $PaCO_2$, HCO_3, and lactate level were measured with blood drawn from the carotid artery using an ABL 720 blood gas analyzer (Radiometer Copenhagen, Copenhagen, Denmark). Expiratory tidal volume and peak inspiratory pressures (PIP) were recorded from the ventilator display. Effective tidal volume was calculated by subtracting the compression volume of the ventilator circuit from the tidal volume. All data (blood gas variables, ventilator and circulatory parameter) were collected at baseline, at injury, and hourly just before suctioning for a total of 6 h (Fig. 2). Serum samples were collected at baseline, at injury, 2, 4 and 6 h. After completion of the 6 h ventilation, animals were killed with bolus injections of sodium pentobarbital (50 mg/kg). The left lung was rapidly removed and snap-frozen in dry ice.

Enzyme-Linked Immunosorbent Assay (ELISA)

The concentrations of selected inflammatory cytokines, *i.e.*, interleukin (IL) -6 and tumor necrosis factor (TNF) -α, in the homogenized left lung tissue and serum at 6 h

ventilation were determined using rabbit specific commercial ELISA kits that was based on the cytokine ELISA protocol of USCN Life Science & Technology (Missouri City, TX) [27]. All antibodies were washed out 4× with phosphate-buffered saline (1% PBS). Cytokines were assessed using polyclonal TNF-α and IL-6 goat anti-rabbit antibodies (USCN Life). Samples were run in duplicate, and concentrations were calculated from a standard curve. All values of lung tissue were normalized to protein content.

Reverse transcription real-time PCR

The mRNA expression of IL-6 and TNF-α were assessed by Real Time PCR. Total RNA was isolated using an RNA purification kit (Qiagen, Hilden, Germany) and was used for PCR assay to detect mRNA expression. Reverse transcription (RT) of total RNA (2 μg) was performed in a final volume of 100 μ L containing 1 ×TaqMan RT buffer, 5.5 mM $MgCl_2$, 500 mM/L each deoxy-unspecified nucleoside 5'-triphsophate, 2.5 mM random hexamers, 0.4 U/μ L RNase inhibitor, and 1.25 U/μ L multiscribe RT. The action mixture was covered and amplification was initiated by 1 min denaturation at 95°C for 1 cycle, followed by multiple (45 – 50) cycles at 95°C for 15 sec and 60°C for 60 sec using a Lightcycler 480 PCR system (Roche Applied Science). Real Time PCR were carried out as described elsewhere [28], using rabbit specific TaqMan kits

Applied Biosystems, assay-ID Oc04097053_m L for IL-6 mRNA, Oc03397715_m L for TNF-α mRNA and Oc03823402_g1 for Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. For internal control, GAPDH was used.

Histological Analysis

The right lungs were inflated with 4% formaldehyde at a pressure of 20 cm H_2O via trachea and were fixed in 4% formaldehyde for >24 h. Subsequently the lungs were divided into 4 regions with a #11 blade scalpel. Each region was then sectioned, stained with Hematoxylin-Eosin and scored by two investigators (K. H. and K. M.) blinded to experimental conditions. Samples were assigned an injury score in each of the 5 categories (edema, hemorrhage, neutrophil infiltration, bronchiolar epithelial desquamation, and hyaline membrane formation) based on severity (0 = not present, 1 = modest and limited, 2 = intermediate, 3= widespread or prominent, 4 = severe and present throughout), using a method modified from previous studies [29, 30]. Regional composite lung injury scores were calculated by summing the category scores within each lung region. Total lung injury scores were calculated by summing the regional composite lung scores within each animal [31, 32].

Alveolar wall thickness

Multiple digital images (at least 2 images per dorsal portion of left lower lobe) were systematically taken at a ×100 magnification of the entire cross section of paraformaldehyde-paraffin-embedded lungs. Images were overlaid with a 10 × 10 grid (100 μm^2), and the alveolar wall thickness was evaluated from every second image (*i.e.*, in a checkerboard fashion) (Fig. 3). The images were printed at an enlargement of photographic paper. An overlay consisting of lines, each 2 cm long, was printed on each image. Alveolar wall thickness was directly measured length by the part in which each alveolar wall-grid line intersection serves as a sample point (Fig. 3).

Statistical Analysis

Baseline, the mRNA expression and alveolar wall thickness variables were expressed as mean ± standard deviation (SD). Intra-intergroup differences were compared by one way analysis of variance adjusted by Bonferroni's correction. Hemodynamic and gas exchange variables were expressed as mean ± SD. Repeated-measures analysis of variance was used to determine intragroup differences. Specific intergroup differences and time points of this difference were determined by using Bonferroni's correction for multiple comparisons. Lung injury score and cytokine concentrations were expressed as

medians and interquartile range (IQR) (25th and 75th percentiles) and the data were analyzed using Kruskal-Wallis one way analysis of variance. The data from each group were compared with the previous time point starting from baseline injury by a test of within-subjects differences of repeated-measures analysis of variance by IBM-SPSS version 19.0 software (IBM-SPSS Inc., Chicago, IL).

Results

Baseline characteristics. Baseline characteristics of the animals in the study groups are shown in Table 1. There were no differences in body weight, hemodynamic variables and gas exchange parameters before the induction of lung injury.

Gas Exchange. After lung injury was induced, P/F ratio was reduced to a mean of 63 ± 13, 73 ± 20 and 64 ± 9 for the CS, OS and Control groups, respectively (p = 0.511). After PEEP levels were increased to 10 cm H_2O, mean P/F increased to >400 in all groups (Fig. 4A and Table. 2). In the CS, control and HC groups, mean P/F remained over 400 throughout the study period. However, in the OS group, P/F decreased continuously and dropped to a mean of 297 ± 124 at 4 h, to 294 ± 95 at 5 h and to 264 ± 71 at 6 h (all p = 0.000 vs. P/F at 1 h after injury). This P/F level was significantly lower than that in the CS groups (p = 0.013, p = 0.005 and p = 0.000 at 4, 5 and 6 h,

respectively) (Fig. 4A and Table. 2).

At injury, $PaCO_2$ for all groups increased significantly (Fig. 4B and Table. 2) compared to the baseline level. Overall, $PaCO_2$, pH and arterial lactate levels did not differ significantly among all groups at baseline and throughout the 6 h study period (Fig. 4B and Table. 2). PIP significantly increased after the increase in PEEP to 10 cm H_2O, compared with baseline levels. PIP levels were significantly higher than those of the HC group in the OS, CS and control groups after injury. Thereafter, PIP showed a similar trend for the 3 h period after injury. However, in the OS group PIP levels were significantly higher than in the other groups at the 4, 5 and 6 h post-injury interval (Table 2).

Hemodynamic variables. Overall, there was no significant difference in mean arterial pressure and heart rate among all groups (Table 2).

Histological Analysis. The total lung injury scores were higher in all other groups compared to the HC group ($p < 0.007$) (Fig. 5 and 6). Regional composite lung injury scores were also shown (Fig. 5). The neutrophil infiltration score was higher in the OS group compared to the HC group ($p < 0.007$). The hemorrhage score was higher in the OS and CS group compared to the HC group ($p < 0.007$). Scores of each lung injury item as well as total scores were not significantly different between CS and OS groups

(Fig. 5).

Alveolar wall thickness. Total numbers of measurement of alveolar wall thickness were 978 sites. OS group had thicker alveolar wall compared to all other groups. CS and control groups had thicker alveolar wall compared to HC group. There were no significant differences between CS and control groups (Fig. 7).

Expression pattern of IL-6 and TNF-α protein: There were no significant differences observed in pulmonary and serum protein concentrations of IL-6 and TNF-α between OS and CS groups, as demonstrated by ELISA (Fig. 8). Pulmonary and serum concentrations of IL-6 and pulmonary concentrations of TNF-α were higher in all other groups group compared to HC groups ($p < 0.005$). The median values for IL-6 pulmonary concentrations (pg/mg) in the CS, OS, control and HC groups were 207 (170 - 449), 233 (141 - 294), 147 (96 - 212) and 75 (74 - 86), respectively (Fig. 8A). IL-6 serum concentrations (pg/mL) in the CS, OS, control and HC groups were 220 (201 - 281), 219 (205 - 235), 220 (212 - 260) and 179 (171 - 216), respectively (Fig. 8B). TNF-α pulmonary concentrations (pg/mg) in the CS, OS, control and HC groups were 485 (348 - 815), 564 (262 - 898), 372 (352 - 489) and 183 (160 - 287), respectively (Fig. 8C). These results were confirmed and complemented by data generated from mRNA expression (Fig. 8). Consistent to IL-6 and TNF-α protein levels, the pulmonary mRNA

expression levels of these cytokines were not significantly different between CS and OS groups (Fig. 8D and E).

Discussion

The key findings of the present study are that: a) repeated open endotracheal suctioning causes gradual and time-dependent reductions in arterial oxygenation over the course of endotracheal suctioning; b) repeated derecruitments induced by multiple OS do not exacerbate lung injury, based on evidence from histological analysis using lung injury scoring system; c) expression levels of the crucial serum and pulmonary inflammatory cytokines remained unchanged throughout the process of repeated OS compared to CS during mechanical ventilation in an lavage-induced surfactant-depleted lung injury model. This is the first study that uses a longer time course, *i.e.*, intermittent endotracheal suctioning over 6 hours, to investigate the effects of repeated suctioning under a well-controlled experimental setting.

Endotracheal suctioning is the most common secretion management procedure performed in mechanically-ventilated patients, even though lung volume loss, hypoxemia and hemodynamic compromise are known risk factors of such procedures

[18, 33-36]. Also, progressive atelectasis in ARDS can exacerbate hypoxemia. In addition, it may produce lung and systemic injuries through the release of cytokines and right-ventricular failure [37]. The present findings on reductions in arterial oxygenation are similar to those of other groups that have evaluated the effects of CS [18, 34, 38-40]. Consistent to the present results, previous studies have also found that reductions in oxygenation related to endotracheal suctioning are greater with OS than with CS [18, 34, 38-40]. Taken together, these findings imply that reductions in arterial oxygenation is unaffected by either single or repeated OS in ARDS. However, it is important to note that while the present study used up to 6 h period to measure arterial oxygenation, the previous studies only used 10-30 min maximum after endotracheal suctioning [18, 34-44]. Therefore, previous studies were unable to elucidate whether transient fluctuations in arterial oxygenation occurred immediately following endotracheal suctioning and how long the trend in arterial desaturation persisted. The present study provides the first evidence that repeated OS causes gradual reductions in arterial oxygenation over a prolonged time span of 6 hours. Specifically, the present study showed statistically significant reduction in arterial oxygenation at 4, 5 and 6 hours of endotracheal suctioning, suggesting a clear time-dependent reduction in arterial oxygen level through repeated OS.

However, repeated derecruitments induced by OS in the present model did not exacerbate lung injury, based on the molecular expression of crucial inflammatory cytokines compared to CS. One notable and unique feature of the present study is detection of crucial inflammatory cytokines related to ARDS, both at serum and pulmonary levels (lavage-induced lung injury with surfactant-depletion), *i.e.*, both protein and mRNA expression. To date, no study using similar experimental setting has performed such molecular analysis using repeated endotracheal suctioning. The potential inflammatory cytokines *i.e.,* TNF-α and IL-6 were unchanged after 6 h of repeated endotracheal suctioning between the CS and OS groups at both the circulatory and pulmonary levels. The current finding is consistent with that of a recent study where oleic acid-induced ARDS model lacked significant changes in IL-6 and TNF-α at circulatory level in CS compared to OS [45]. However, unlike the present study, this previous study did not evaluate levels of pulmonary cytokines, and, further, it only performed endotracheal suctioning once [45]. Thus, it seems that although the induction method of ARDS was different in the current study from that of Zhao F et al. [45] in which a different number of endotracheal suctioning protocols were used, the expression of serum IL-6 and TNF-α were essentially similar. The facts stated above led us to conclude that the mechanism underlying this gradual and time-dependent decrease

in oxygenation of the OS group may not subsequently stimulate a cascade of biological responses, leading to further lung injury (biotrauma). However, the observation period in the present study still may be too short [46-48]. Future studies should focus in depth on the changes of molecular pattern of potential inflammatory cytokines in these lung injury models with repeated endotracheal suctioning over a longer period of time.

Furthermore, repeated derecruitments induced by OS in the present model did not exacerbate lung injury, based on the histological analysis. OS group had a higher score of hemorrhage and neutrophil infiltration. These findings may explain the progressive reduction in oxygenation of the OS group. However, there was no significant difference between OS and CS groups. During mechanical ventilation, repeated derecruitments (induced by altering PEEP or disconnected from ventilator) of initially recruited lung accentuate lung injury [12, 13]. Previous studies demonstrated that the bronchioles are the major site of this injury [12, 13]. However, the effects of repeated endotracheal suctioning during mechanical ventilation in ARDS subjects on the aggravation of further lung injury is yet to be investigated. The present study showed that no significant differences in lung injury score (bronchiolar epithelial desquamation) existed in the lungs that have already been derecruited, irrespective of repeated endotracheal suctioning, *i.e.*, either open or closed. It is important to note that the same region of the

lung was carefully and blindly evaluated morphologically and that no significant difference in injury score was found between the endotracheal suctioning groups. These findings contradict data showing detrimental effects of OS in ARDS subjects that have undergone mechanical ventilation.

However, the present findings on OS-induced reduction in arterial oxygenation are consistent with results of previous studies [18, 34, 38-40]. One of the major reasons may be alveolar wall thickness by interstitial edema. Interstitial edema causes impairment of diffusion capacity. Especially, this impairment of dorsal portion of the lung is also to serve decreased oxygenation because of ventilation/perfusion mismatch. However, the significance of alveolar wall thickness in early phase ARDS subjects/model are yet to be investigated and not well known. At least, alveolar wall thickness in the HC group was normal compared with previous studies [49, 50]. Therefore, future studies should investigate the effects of alveolar wall thickness in early phase ARDS subjects/model.

In addition, the present study demonstrated that PIP levels were higher in the OS group compared to all other groups. This finding suggests that the OS group had decreased lung compliance. However, this was not directly measured in this study due to methodological reasons. Therefore, it is likely that continuous alveolar derecruitment

is responsible for this progressive reduction in oxygenation. Indeed, a much greater end-expiratory lung-volume change with OS than with CS has been documented [18, 33]. Furthermore, advocates of CS have argued that lung volume recovers more quickly following suctioning [43, 44]. However, repeated derecruitments induced by OS in the present model did not exacerbate total lung injury score. When large tidal volumes are delivered, this can lead to repeated over-distension of alveoli and further aggravate injury [7, 51, 52]. Furthermore, when lung protective ventilation is used, the aggravation of lung injury may depend on the degree of the reduction in aerated lung volume and the tidal volume used [53]. In the present study, the degree of the reduction in aerated lung volume following lung lavage was not severe as indicated by the mean P/F ratio above 400 on the high PEEP in the experimental groups. Therefore, it seems that notwithstanding continuous alveolar derecruitment, the low tidal volume setting in the present experimental protocol during mechanical ventilation might prevent the acceleration of lung injury. Future studies should focus on the changes of lung volume (with inductive plethysmography or magnetometers) with repeated endotracheal suctioning over a longer period of time.

Conflicting reports exist concerning the effectiveness of CS in removing secretions

compared to OS. Although CS is a safe method of endotracheal suctioning, previous studies reported that CS was less effective than OS in removing secretions [19, 54]. Therefore, sometimes there is still a need to perform OS, as well as recruitment maneuver after OS in order to restore lung volumes and to prevent desaturation in clinical settings [18, 36]. In addition, recruitment maneuver may prevent VILI to open atelectasis [13, 55]. In this study, recruitment maneuver was not performed in order to evaluate the effects of open vs. closed suctioning independently. If recruitment maneuver was performed in this study, OS might not have caused progressive reductions in arterial oxygenation. However, recruitment maneuver may induce lung stress and strain, which include several factors, such as the level of pressure, time to reach inspiratory pressure and frequency, leading to VILI [55, 56].

Repeated derecruitments induced by OS in the present model did not exacerbate lung injury, based on the morphological as well as the molecular expression of crucial inflammatory cytokines compared to CS. However, this study demonstrated that CS prevents gradual reductions in arterial oxygenation, whereas the use of repeated OS caused progressive reductions in arterial oxygenation. Recently, patients undergoing mechanical ventilation are managed according to lung-protective strategies in order to

avoid high alveolar pressure using small tidal volumes and to keep alveoli open at end-expiratory level with sufficient PEEP [7, 51, 52]. With the increased use of high PEEP, when ventilator circuit is disconnected, patients can be exposed to the risk of sudden derecruitment and continuous desaturation that could be harmful to ARDS patients. The present findings suggest that routine use of CS is preferable, especially for the patients requiring high PEEP, to avoid gradual reductions in arterial oxygenation with the use of repeated OS.

Limitations of this study

One of the notable limitations of the present study is that animals were received a muscle relaxant, which may have inhibited the animal's efforts to maintain lung volume and altered regional differences in lung volume and ventilation. In addition, the fraction of inspired oxygen was set at 1.0. Indeed, the rate of absorption of gas from an unventilated lung area increases with an increasing FIO_2 [57], thereby exacerbating desaturation. However, in clinical practice, we often need to use high FIO_2 in patients with severe hypoxemia as well. In addition, it is interesting to note that despite this limitation, the present data are consistent with those of previous studies [19, 20, 38-40], thus giving relevance and importance to the present data. In addition, due to technical

limitations, lung volume (with inductive plethysmography or magnetometers) nor lung compliance was not measured directly, and thus one could argue that the loss of lung volume induced by endotracheal suctioning is somewhat speculative. Despite this limitation, it seems that, based on the literature discussed above [19], the reductions in arterial oxygenation observed here might originate from alveolar derecruitment.

Secondly, the lavage-induced surfactant-depleted lung injury model was used. More studies involving different animal species with different endotracheal suctioning protocols using various models of lung injuries should be conducted. In addition, the observation period in the present study still may be too short [46-48]. Future studies should focus on examining the effects of repeated endotracheal suctioning over a longer period of time on the aggravation of lung injury in ARDS, which will more likely simulate the prevailing conditions in clinical settings.

Conclusion

Repeated OS during mechanical ventilation does not exacerbate lung injury in the repeatedly derecruited lung over a long time (6 hours) by repeated endotracheal suctioning compared to CS based on both histological and molecular analyses. Reductions in arterial oxygenation induced by repeated OS causes a gradual and time-dependent decline in lavage-induced surfactant-depleted lung injury model during mechanical ventilation compared to CS and this finding makes the routine use of CS preferable, especially for the patients requiring high PEEP, to avoid gradual reductions in arterial oxygenation with the use of repeated OS.

References

1. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, Hudson LD: **Incidence and outcomes of acute lung injury**. *N Engl J Med* 2005, **353**(16):1685-1693.
2. Meduri GU: **Host defense response and outcome in ARDS**. *Chest* 1997, **112**(5):1154-1158.
3. Gattinoni L, D'Andrea L, Pelosi P, Vitale G, Pesenti A, Fumagalli R: **Regional effects and mechanism of positive end-expiratory pressure in early adult respiratory distress syndrome**. *JAMA* 1993, **269**(16):2122-2127.
4. Roupie E, Dambrosio M, Servillo G, Mentec H, el Atrous S, Beydon L, Brun-Buisson C, Lemaire F, Brochard L: **Titration of tidal volume and induced hypercapnia in acute respiratory distress syndrome**. *Am J Respir Crit Care Med* 1995, **152**(1):121-128.
5. Dreyfuss D, Saumon G: **Ventilator-induced lung injury: lessons from experimental studies**. *Am J Respir Crit Care Med* 1998, **157**(1):294-323.
6. Slutsky AS: **Lung injury caused by mechanical ventilation**. *Chest* 1999, **116**(1 Suppl):9S-15S.
7. Lionetti V, Recchia FA, Ranieri VM: **Overview of ventilator-induced lung injury mechanisms**. *Curr Opin Crit Care* 2005, **11**(1):82-86.
8. Dreyfuss D, Saumon G: **Role of tidal volume, FRC, and end-inspiratory volume in the development of pulmonary edema following mechanical ventilation**. *Am Rev Respir Dis* 1993, **148**(5):1194-1203.
9. West JB, Mathieu-Costello O: **Stress failure of pulmonary capillaries: role in lung and heart disease**. *Lancet* 1992, **340**(8822):762-767.
10. Muscedere JG, Mullen JB, Gan K, Slutsky AS: **Tidal ventilation at low airway pressures can augment lung injury**. *Am J Respir Crit Care Med* 1994, **149**(5):1327-1334.
11. Hudson LD: **Protective ventilation for patients with acute respiratory distress syndrome**. *N Engl J Med* 1998, **338**(6):385-387.
12. Suh GY, Koh Y, Chung MP, An CH, Kim H, Jang WY, Han J, Kwon OJ: **Repeated derecruitments accentuate lung injury during mechanical ventilation**. *Crit Care Med* 2002, **30**(8):1848-1853.
13. Koh WJ, Suh GY, Han J, Lee SH, Kang EH, Chung MP, Kim H, Kwon OJ: **Recruitment maneuvers attenuate repeated derecruitment-associated lung injury**. *Crit Care Med* 2005, **33**(5):1070-1076.
14. Ranieri VM, Suter PM, Tortorella C, De Tullio R, Dayer JM, Brienza A, Bruno F,

Slutsky AS: **Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial**. *JAMA* 1999, **282**(1):54-61.

15. Hegeman MA, Hennus MP, Cobelens PM, Kavelaars A, Jansen NJ, Schultz MJ, van Vught AJ, Heijnen CJ: **Dexamethasone attenuates VEGF expression and inflammation but not barrier dysfunction in a murine model of ventilator-induced lung injury**. *PLoS One* 2013, **8**(2):e57374.
16. Tremblay L, Valenza F, Ribeiro SP, Li J, Slutsky AS: **Injurious ventilatory strategies increase cytokines and c-fos m-RNA expression in an isolated rat lung model**. *J Clin Invest* 1997, **99**(5):944-952.
17. Tremblay LN, Slutsky AS: **Ventilator-induced injury: from barotrauma to biotrauma**. *Proc Assoc Am Physicians* 1998, **110**(6):482-488.
18. Maggiore SM, Lellouche F, Pigeot J, Taille S, Deye N, Durrmeyer X, Richard JC, Mancebo J, Lemaire F, Brochard L: **Prevention of endotracheal suctioning-induced alveolar derecruitment in acute lung injury**. *Am J Respir Crit Care Med* 2003, **167**(9):1215-1224.
19. Lasocki S, Lu Q, Sartorius A, Fouillat D, Remerand F, Rouby JJ: **Open and closed-circuit endotracheal suctioning in acute lung injury: efficiency and effects on gas exchange**. *Anesthesiology* 2006, **104**(1):39-47.
20. Wolf GK, Grychtol B, Frerichs I, van Genderingen HR, Zurakowski D, Thompson JE, Arnold JH: **Regional lung volume changes in children with acute respiratory distress syndrome during a derecruitment maneuver**. *Crit Care Med* 2007, **35**(8):1972-1978.
21. Matute-Bello G, Frevert CW, Martin TR: **Animal models of acute lung injury**. *American journal of physiology Lung cellular and molecular physiology* 2008, **295**(3):L379-399.
22. Bayat S, Porra L, Albu G, Suhonen H, Strengell S, Suortti P, Sovijarvi A, Petak F, Habre W: **Effect of positive end-expiratory pressure on regional ventilation distribution during mechanical ventilation after surfactant depletion**. *Anesthesiology* 2013, **119**(1):89-100.
23. Lachmann B, Robertson B, Vogel J: **In vivo lung lavage as an experimental model of the respiratory distress syndrome**. *Acta Anaesthesiol Scand* 1980, **24**(3):231-236.
24. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R: **The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and**

clinical trial coordination. *Am J Respir Crit Care Med* 1994, **149**(3 Pt 1):818-824.

25. Hickling KG, Town IG, Epton M, Neill A, Tie A, Whitehead M, Graham P, Everest E, A'Court G, Darlow B *et al*: **Pressure-limited ventilation with permissive hypercapnia and minimum PEEP in saline-lavaged rabbits allows progressive improvement in oxygenation, but does not avoid ventilator-induced lung injury**. *Intensive Care Med* 1996, **22**(12):1445-1452.
26. **AARC Clinical Practice Guidelines. Endotracheal suctioning of mechanically ventilated patients with artificial airways 2010**. *Respir Care* 2010, **55**(6):758-764.
27. Hoekstra LT, van Lienden KP, Verheij J, van der Loos CM, Heger M, van Gulik TM: **Enhanced tumor growth after portal vein embolization in a rabbit tumor model**. *J Surg Res* 2013, **180**(1):89-96.
28. Popivanova BK, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fujii C, Mukaida N: **Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis**. *J Clin Invest* 2008, **118**(2):560-570.
29. Sinclair SE, Kregenow DA, Lamm WJ, Starr IR, Chi EY, Hlastala MP: **Hypercapnic acidosis is protective in an in vivo model of ventilator-induced lung injury**. *Am J Respir Crit Care Med* 2002, **166**(3):403-408.
30. Zhou ZH, Sun B, Lin K, Zhu LW: **Prevention of rabbit acute lung injury by surfactant, inhaled nitric oxide, and pressure support ventilation**. *Am J Respir Crit Care Med* 2000, **161**(2 Pt 1):581-588.
31. Kristof AS, Goldberg P, Laubach V, Hussain SN: **Role of inducible nitric oxide synthase in endotoxin-induced acute lung injury**. *Am J Respir Crit Care Med* 1998, **158**(6):1883-1889.
32. Su X, Bai C, Hong Q, Zhu D, He L, Wu J, Ding F, Fang X, Matthay MA: **Effect of continuous hemofiltration on hemodynamics, lung inflammation and pulmonary edema in a canine model of acute lung injury**. *Intensive Care Med* 2003, **29**(11):2034-2042.
33. Brochard L, Mion G, Isabey D, Bertrand C, Messadi AA, Mancebo J, Boussignac G, Vasile N, Lemaire F, Harf A: **Constant-flow insufflation prevents arterial oxygen desaturation during endotracheal suctioning**. *Am Rev Respir Dis* 1991, **144**(2):395-400.
34. Cereda M, Villa F, Colombo E, Greco G, Nacoti M, Pesenti A: **Closed system endotracheal suctioning maintains lung volume during volume-controlled**

mechanical ventilation. *Intensive Care Med* 2001, **27**(4):648-654.

35. Lu Q, Capderou A, Cluzel P, Mourgeon E, Abdennour L, Law-Koune JD, Straus C, Grenier P, Zelter M, Rouby JJ: **A computed tomographic scan assessment of endotracheal suctioning-induced bronchoconstriction in ventilated sheep**. *Am J Respir Crit Care Med* 2000, **162**(5):1898-1904.
36. Dyhr T, Bonde J, Larsson A: **Lung recruitment manoeuvres are effective in regaining lung volume and oxygenation after open endotracheal suctioning in acute respiratory distress syndrome**. *Crit Care* 2003, **7**(1):55-62.
37. Duggan M, McCaul CL, McNamara PJ, Engelberts D, Ackerley C, Kavanagh BP: **Atelectasis causes vascular leak and lethal right ventricular failure in uninjured rat lungs**. *Am J Respir Crit Care Med* 2003, **167**(12):1633-1640.
38. Lee CK, Ng KS, Tan SG, Ang R: **Effect of different endotracheal suctioning systems on cardiorespiratory parameters of ventilated patients**. *Ann Acad Med Singapore* 2001, **30**(3):239-244.
39. Maggiore SM, Iacobone E, Zito G, Conti C, Antonelli M, Proietti R: **Closed versus open suctioning techniques**. *Minerva Anestesiol* 2002, **68**(5):360-364.
40. Johnson KL, Kearney PA, Johnson SB, Niblett JB, MacMillan NL, McClain RE: **Closed versus open endotracheal suctioning: costs and physiologic consequences**. *Crit Care Med* 1994, **22**(4):658-666.
41. Brown SE, Stansbury DW, Merrill EJ, Linden GS, Light RW: **Prevention of suctioning-related arterial oxygen desaturation. Comparison of off-ventilator and on-ventilator suctioning**. *Chest* 1983, **83**(4):621-627.
42. Tingay DG, Copnell B, Grant CA, Dargaville PA, Dunster KR, Schibler A: **The effect of endotracheal suction on regional tidal ventilation and end-expiratory lung volume**. *Intensive Care Med* 2010, **36**(5):888-896.
43. Choong K, Chatrkaw P, Frndova H, Cox PN: **Comparison of loss in lung volume with open versus in-line catheter endotracheal suctioning**. *Pediatr Crit Care Med* 2003, **4**(1):69-73.
44. Kalyn A, Blatz S, Sandra F, Paes B, Bautista C: **Closed suctioning of intubated neonates maintains better physiologic stability: a randomized trial**. *J Perinatol* 2003, **23**(3):218-222.
45. Zhao F, Wang W, Fang Y, Li X, Shen L, Cao T, Zhu H: **Molecular mechanism of sustained inflation in acute respiratory distress syndrome**. *J Trauma Acute Care Surg* 2012, **73**(5):1106-1113.
46. Krebs J, Pelosi P, Tsagogiorgas C, Haas J, Yard B, Rocco PR, Luecke T: **Time course of lung inflammatory and fibrogenic responses during protective**

mechanical ventilation in healthy rats. *Respiratory physiology & neurobiology* 2011, **178**(2):323-328.

47. Reis Miranda D, Gommers D, Struijs A, Dekker R, Mekel J, Feelders R, Lachmann B, Bogers AJ: **Ventilation according to the open lung concept attenuates pulmonary inflammatory response in cardiac surgery**. *Eur J Cardiothorac Surg* 2005, **28**(6):889-895.
48. Derks CM, Jacobovitz-Derks D: **Embolic pneumopathy induced by oleic acid. A systematic morphologic study**. *Am J Pathol* 1977, **87**(1):143-158.
49. Shaw JO, Henson PM: **Pulmonary intravascular sequestration of activated neutrophils: failure to induce light-microscopic evidence of lung injury in rabbits**. *Am J Pathol* 1982, **108**(1):17-23.
50. Klut ME, van Eeden SF, Whalen BA, Verburgt LM, English D, Hogg JC: **Neutrophil activation and lung injury associated with chronic endotoxemia in rabbits**. *Exp Lung Res* 1996, **22**(4):449-465.
51. Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, Wheeler A, Wiedemann HP, Arroliga AC, Fisher CJ, Komara JJ *et al*: **Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome.** *N Engl J Med* 2000, **342**(18):1301-1308.
52. Bowton DL, Kong DL: **High tidal volume ventilation produces increased lung water in oleic acid-injured rabbit lungs**. *Crit Care Med* 1989, **17**(9):908-911.
53. Terragni PP, Rosboch G, Tealdi A, Corno E, Menaldo E, Davini O, Gandini G, Herrmann P, Mascia L, Quintel M *et al*: **Tidal hyperinflation during low tidal volume ventilation in acute respiratory distress syndrome**. *Am J Respir Crit Care Med* 2007, **175**(2):160-166.
54. Lindgren S, Almgren B, Hogman M, Lethvall S, Houltz E, Lundin S, Stenqvist O: **Effectiveness and side effects of closed and open suctioning: an experimental evaluation**. *Intensive Care Med* 2004, **30**(8):1630-1637.
55. Rocco PR, Pelosi P, de Abreu MG: **Pros and cons of recruitment maneuvers in acute lung injury and acute respiratory distress syndrome**. *Expert Rev Respir Med* 2010, **4**(4):479-489.
56. Mekontso Dessap A, Voiriot G, Zhou T, Marcos E, Dudek SM, Jacobson JR, Machado R, Adnot S, Brochard L, Maitre B *et al*: **Conflicting physiological and genomic cardiopulmonary effects of recruitment maneuvers in murine acute lung injury**. *Am J Respir Cell Mol Biol* 2012, **46**(4):541-550.

57. Joyce CJ, Baker AB, Kennedy RR: **Gas uptake from an unventilated area of lung: computer model of absorption atelectasis**. *J Appl Physiol* 1993, **74**(3):1107-1116.

Table 1. Baseline characteristics

	OS group n = 13	CS group n = 13	Control group n = 7	HC group n = 3	*p* value
Body weight, kg	**3.1 ± 0.3**	**3.0 ± 0.3**	**2.8 ± 0.2**	**3.0 ± 0.2**	**0.169**
Lavage, times	**3 ± 1**	**3 ± 1**	**3 ± 1**	**Non**	**0.929**
MAP, mmHg	**131 ± 16**	**118 ± 13**	**116 ± 7**	**119 ± 2**	**0.063**
HR, beats /min	**236 ± 65**	**217 ± 48**	**280 ± 68**	**213 ± 183**	**0.349**
RR, breaths /min	**23.5 ± 5.6**	**22.7 ± 6.2**	**22.3 ± 2.7**	**24.0 ± 1.0**	**0.950**
P/F ratio	**460 ± 51**	**477 ± 54**	**427 ± 36**	**412 ± 38**	**0.085**
$PaCO_2$, mmHg	**44.4 ± 4.6**	**46.1 ± 5.5**	**40.6 ± 10.2**	**44.5 ± 4.3**	**0.363**

OS, open endotracheal suctioning; CS closed endotracheal suctioning; HC healthy control; MAP, mean arterial pressure; HR, heart rate; RR, respiration rate; P/F ratio, PaO_2/FIO_2 ratio. Values are mean ± standard deviations.

Table 2. Sequential changes in variables of lung mechanics and hemodynamics

Variables	group	baseline	injury	1 h	2 h	3 h	4 h	5 h	6 h
PIP, cmH_2O	OS	13.0 ± 3.0	19.3 ± 3.0*	22.6 ± 2.1*	23.0 ± 2.6	23.0 ± 2.1	23.8 ± 2.1§	24.6 ± 2.6†§	24.9 ± 2.9†
	CS	13.2 ± 1.7	19.4 ± 2.6*	21.2 ± 1.6	21.3 ± 2.3	21.3 ± 2.3	21.5 ± 1.9	21.5 ± 2.2	21.8 ± 2.2‡
	Control	13.2 ± 2.0	20.5 ± 2.1*	20.8 ± 1.0	21.3 ± 1.0	21.0 ± 1.3	20.3 ± 1.4	19.8 ± 1.2	19.2 ± 1.5‡
	HC	13.7 ± 1.5	17.7 ± 0.6	17.3 ± 0.6§	19.3 ± 1.2	19.6 ± 2.3	19.3 ± 2.5	19.0 ± 2.0	18.3 ± 1.2‡
Arterial pH	OS	7.43 ± 0.03	7.05 ± 0.14*	7.15 ± 0.13*	7.14 ± 0.13	7.11 ± 0.12	7.09 ± 0.15	7.09 ± 0.17	7.11 ± 0.14
	CS	7.40 ± 0.05	7.11 ± 0.15*	7.16 ± 0.11	7.15 ± 0.10	7.16 ± 0.14	7.12 ± 0.14	7.14 ± 0.08	7.17 ± 0.09
	Control	7.48 ± 0.08	7.20 ± 0.08*	7.22 ± 0.06	7.23 ± 0.06	7.26 ± 0.03	7.26 ± 0.05	7.24 ± 0.05	7.22 ± 0.06
	HC	7.41 ± 0.06	7.22 ± 0.04	7.24 ± 0.02	7.23 ± 0.03	7.22 ± 0.03	7.24 ± 0.05	7.24 ± 0.05	7.20 ± 0.04
PaO_2, cmH_2O	OS	456 ± 51	64 ± 13*	460 ± 44*	453 ± 75	375 ± 86*†	296± 123*†‡	294 ± 95†‡	263 ± 72†§
	CS	475 ± 56	73 ± 20*	446 ± 47*	439 ± 59	429 ± 74	422 ± 70	419 ± 84	438 ± 94
	Control	435 ± 32	64 ± 9*	429 ± 62*	436 ± 45	428 ± 37	437 ± 33	437 ± 25	442 ± 31
	HC	412 ± 38	393 ± 41§	406 ± 30	427 ± 36	420 ± 53	448 ± 5	430 ± 59	437 ± 27
$PaCO_2$, cmH_2O	OS	44.5 ± 4.8	96.0 ± 14.4*	79.8 ± 11.4	79.2 ± 11.4	85.5 ± 16.2	88.3 ± 20.5	89.2 ± 21.7	88.4 ± 13.0
	CS	45.9 ± 5.7	95.1 ± 12.5*	81.0 ± 21.4	85.3 ± 16.9	85.5 ± 17.2	85.2 ± 15.3	82.3 ± 21.7	80.7 ± 15.0
	Control	40.6 ± 10.2	95.3 ± 19.0*	88.0 ± 6.6	80.8 ± 7.2	81.3 ± 2.9	79.5 ± 5.6	82.0 ± 6.3	87.5 ± 9.5
	HC	44.1 ± 4.2	88.8 ± 27.5*	81.2 ± 20.3	90.6 ± 16.9	80.9 ± 3.3	79.8 ± 7.8	76.5 ± 3.0	82.1 ± 2.0
Base excess	OS	4.2 ± 1.6	-2.7 ± 7.9	-1.0 ± 4.2	-0.6 ± 6.9	-3.1 ± 6.1	-2.7 ± 7.1	-3.2 ± 7.5	-2.2 ± 5.4
	CS	3.6 ± 3.4	-2.8 ± 5.7	-1.0 ± 4.3	-0.8 ± 6.6	-1.7 ± 8.0	-2.5 ± 8.6	-2.5 ± 7.0	-1.7 ± 6.9
	Control	5.9 ± 1.8	6.5 ± 4.8	6.5 ± 4.7	5.8 ± 6.1	5.4 ± 4.9	5.9 ± 4.1	5.7 ± 5.4	5.3 ± 6.1

	HC	**3.9 ± 1.1**	**3.4 ± 4.9**	**5.7 ± 5.8**	**5.4 ± 0.9**	**3.1 ± 1.1**	**4.5 ± 22**	**3.7 ± 2.8**	**1.6 ± 2.8**
HCO_3, mmol/L	**OS**	**28.8 ± 2.0**	**28.9 ± 5.9**	**26.7 ± 6.4****	**26.7 ± 5.0**	**28.4 ± 8.1**	**26.9 ± 6.2**	**27.8 ± 7.9**	**26.9 ± 8.2****
	CS	**27.4 ± 3.7**	**29.3 ± 5.1**	**28.6 ± 3.7****	**28.6 ± 3.7**	**29.5 ± 5.6**	**27.9 ± 7.2**	**27.7 ± 6.9**	**27.7 ± 6.8**
	Control	**29.3 ± 3.0**	**36.2 ± 4.4**	**37.2 ± 3.8**	**38.4 ± 3.8**	**35.4 ± 5.9**	**34.8 ± 4.1**	**34.7 ± 4.0**	**35.2 ± 3.5**
	HC	**28.4 ± 0.5**	**31.4 ± 6.9**	**35.5 ± 7.7**	**35.5 ± 7.8**	**35.1 ± 1.5**	**31.9 ± 2.1**	**33.7 ± 2.6**	**33.7 ± 2.6**
Lactate, mmol/L	**OS**	**1.4 ± 0.9**	**4.5 ± 4.0**	**4.1 ± 5.1**	**4.1 ± 5.0**	**4.3 ± 5.8**	**4.4 ± 5.7**	**4.8 ± 6.8**	**5.2 ± 7.5**
	CS	**1.6 ± 0.5**	**4.4 ± 2.6**	**2.4 ± 0.7**	**3.5 ± 3.4**	**4.4 ± 4.4**	**5.3 ± 4.6**	**4.7 ± 3.6**	**6.2 ± 7.6**
	Control	**0.9 ± 0.1**	**3.1 ± 1.2**	**1.6 ± 0.7**	**1.2 ± 0.5**	**1.2 ± 0.6**	**1.3 ± 0.7**	**1.3 ± 0.7**	**1.4 ± 0.8**
	HC	**1.2 ± 0.6**	**1.0 ± 0.3**	**1.3 ± 0.6**	**1.4 ± 0.7**	**2.0 ± 1.6**	**1.9 ± 1.2**	**2.1 ± 1.4**	**1.8 ± 1.0**
MAP, mmHg	**OS**	**131 ± 17**	**118 ± 20**	**98 ± 14***	**102 ± 16**	**101 ± 14b**	**100 ± 20**	**97 ± 11**	**95 ± 16**
	CS	**119 ± 13**	**121 ± 18**	**101 ± 14***	**102 ± 12**	**94 ± 15**	**94 ± 16**	**94 ± 16**	**91 ± 15**
	Control	**116 ± 7**	**119 ± 11**	**93 ± 10***	**95 ± 11**	**96 ± 9**	**102 ± 13**	**106 ± 13**	**105 ± 9**
	HC	**118 ± 2**	**111 ± 6**	**105 ± 13**	**112 ± 26**	**100 ± 15**	**96 ± 6**	**102 ± 16**	**105 ± 15**
HR, beats/min	**OS**	**241 ± 64**	**209 ± 31**	**217 ± 43**	**215 ± 24**	**200 ± 19**	**211 ± 36**	**211 ± 37**	**207 ± 31**
	CS	**214 ± 49**	**195 ± 29**	**221 ± 49**	**218 ± 32**	**227 ± 43**	**224 ±49**	**208 ± 34**	**212 ± 27**
	Control	**272 ± 49**	**242 ± 53**	**244 ± 30**	**253 ± 37**	**252 ± 37**^{c}	**243 ± 39**	**232 ± 39**	**235 ± 20**
	HC	**299 ± 38**	**207 ± 140**	**231 ± 11**	**228 ± 24**	**225 ± 11**	**253 ± 15**	**247 ± 45**	**235 ± 44**

OS, open endotracheal suctioning; CS, closed endotracheal suctioning; HC, Healthy control; PIP, peak inspiratory pressure; MAP, mean arterial pressure; HR, heart rate. $^{*}p < 0.05$ compared with previous value within the same group; $^{\dagger}p < 0.05$ compared with 1 hour after injury within the same group; $^{\ddagger}p < 0.05$ vs. CS and Control groups; $^{\S}p < 0.05$ vs. all other groups; $^{**}p < 0.05$ vs. Control group. Values are mean ± standard deviations

Figure and figure legends:

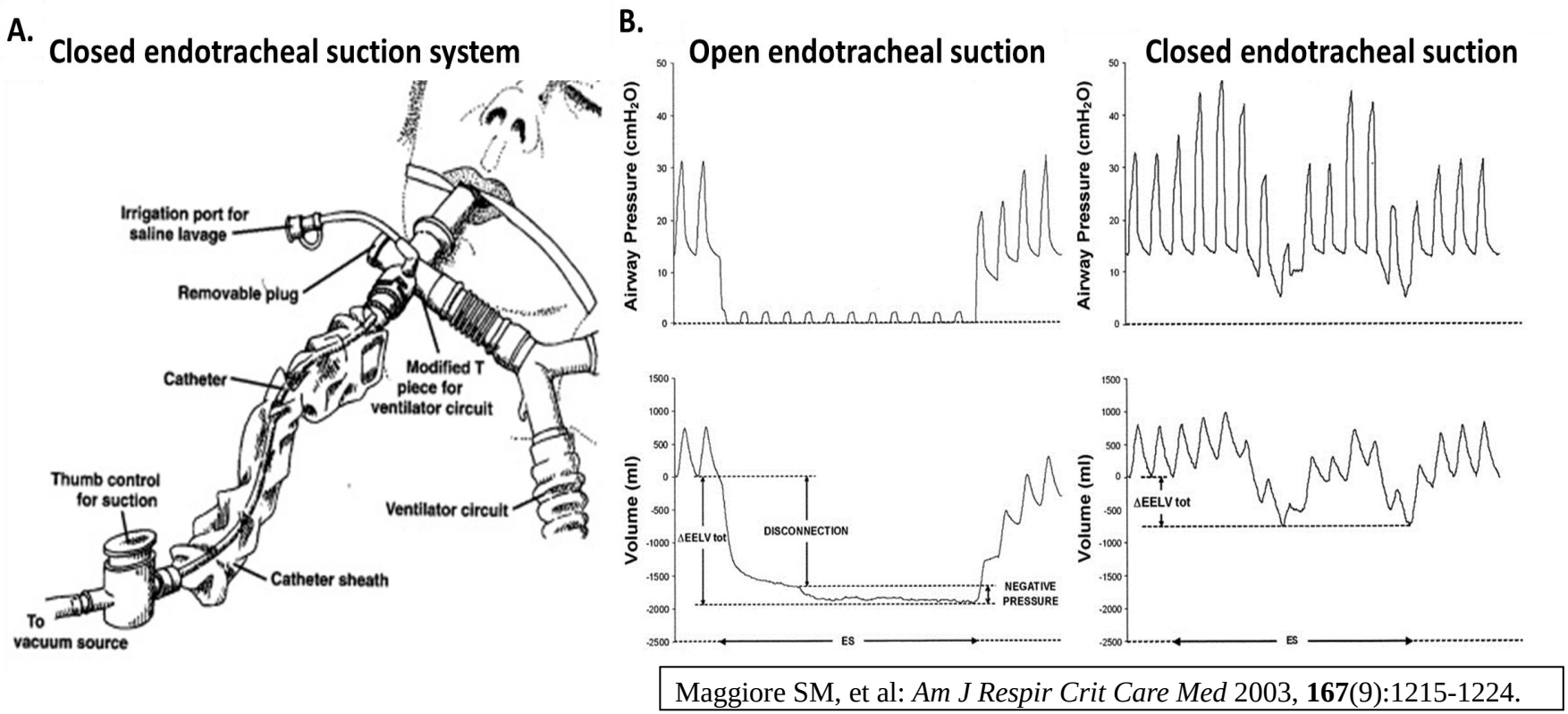

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Figure 1. Closed endotracheal suctioning system (A). Tracings of airway pressure and volume, measured by thoracic respiratory inductive plethysmography, during endotracheal suctioning procedures (B).

(A) Closed endotracheal suctioning system is not disconnected from ventilator during endotracheal suctioning. Therefore, positive

end-expiratory pressure was maintained during closed endotracheal suctioning. In addition, endotracheal suctioning performed with the closed system, while triggering pressure-supported breaths during suctioning could maintain lung volume (B). Previous study results were shown [18]. Changes in total end-expiratory lung volume (EELVtot) were measured as the difference between the value of endexpiratory lung volume of the cycle immediately preceding the suctioning procedure and the minimum value recorded during suctioning. When suctioning was performed after disconnecting the patient from the ventilator, a first drop in lung volume was observed after disconnection (DISCONNECTION) followed by a second drop (NEGATIVE PRESSURE) when negative pressure was applied. In this patient, disconnection from the ventilator contributed more than negative pressure to the total lung volume fall recorded during the entire suctioning procedure. Positive end-expiratory pressure was totally lost during open endotracheal suctioning, whereas it was maintained during closed endotracheal suctioning.

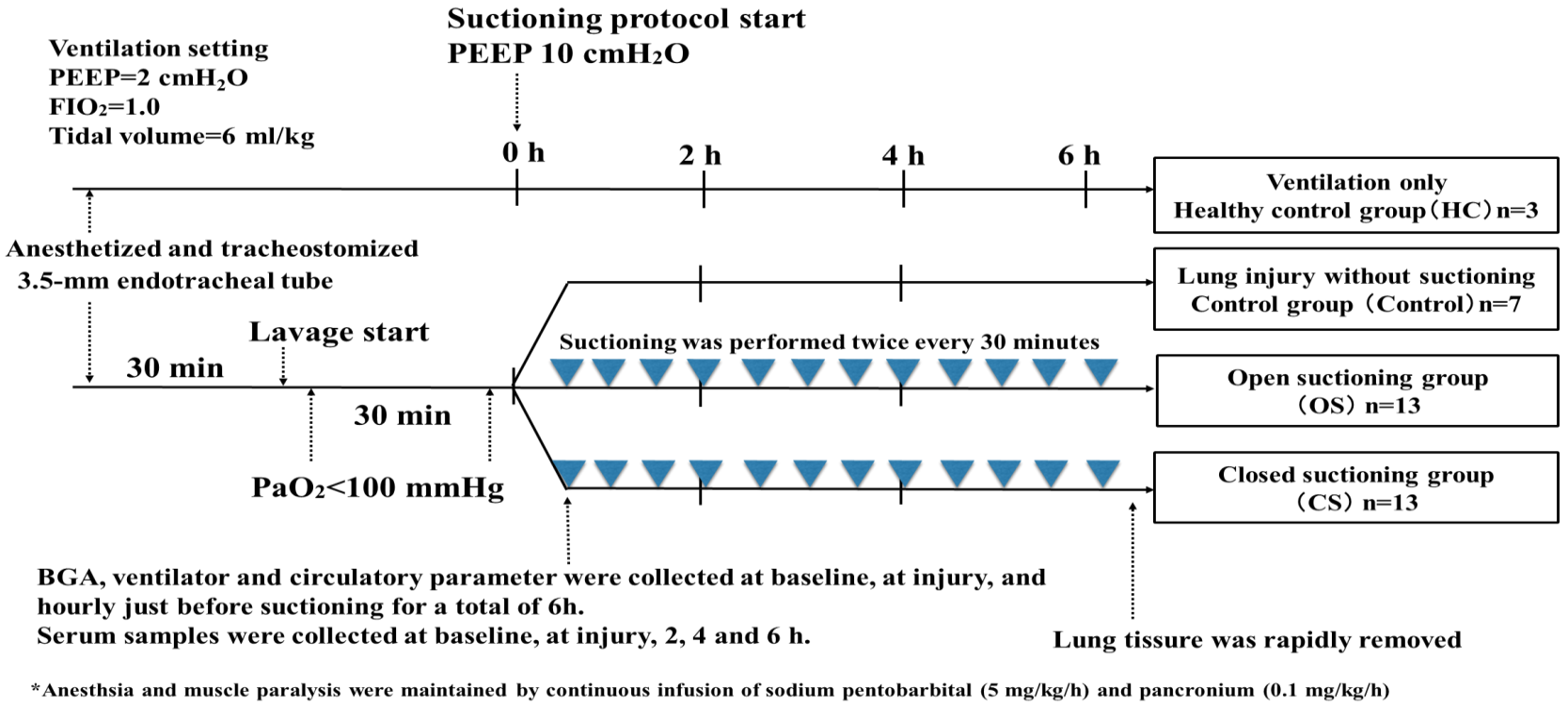

Figure 2. Experimental Flow.

Schematic diagram of the experimental protocol used in this study. In the healthy control group, the animals were ventilated with 10 cmH_2O positive end-expiratory pressure, for 6 hrs of the study. The control group was mechanically ventilated for 6 hours. In the suctioning groups, endotracheal suctioning was performed twice every 30 minutes during 6 hour suctioning protocol (triangle). After completion of the 6 h ventilation, animals were killed and the left lung was rapidly removed and snap-frozen in dry ice.

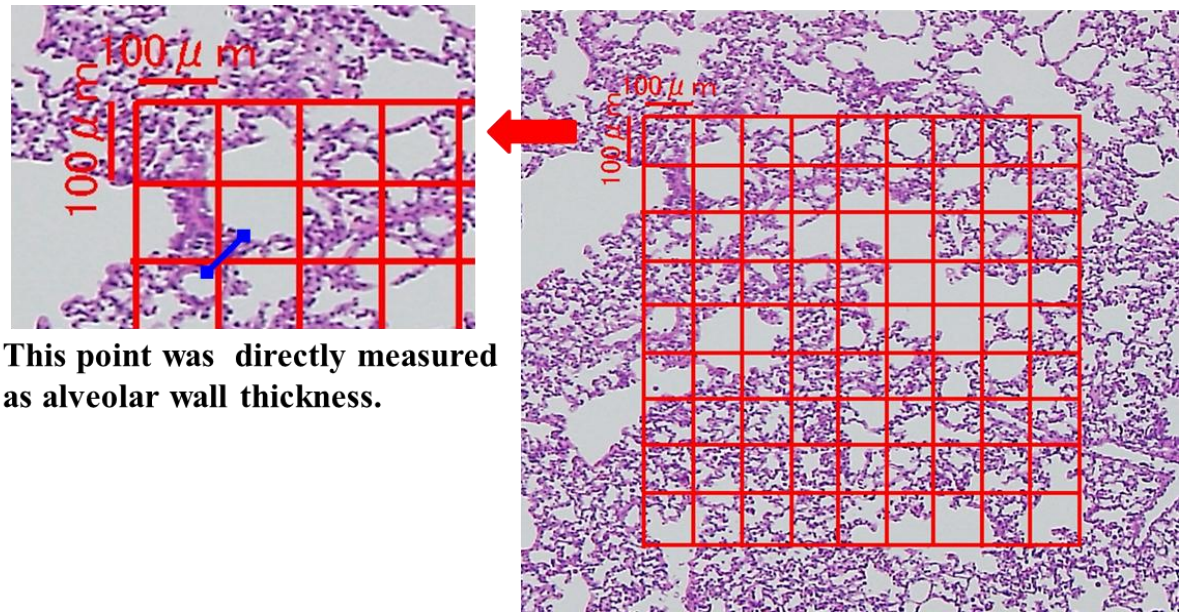

Figure 3. Methods of measurement of alveolar wall thickness.

Multiple digital images (at least 2 images per dorsal portion of left lower lobe) were systematically taken at a ×100 magnification of the entire cross section of paraformaldehyde-paraffin-embedded lungs. Images were overlaid with a 10 × 10 grid (100 μm^2), and the alveolar wall thickness was evaluated from every second image (*i.e.*, in a checkerboard fashion). The images were printed at an enlargement of photographic paper. An overlay consisting of lines of each 2 cm long was printed on each image. Alveolar wall thickness was directly measured length by the part in which each alveolar wall-grid line intersection serves as a sample point.

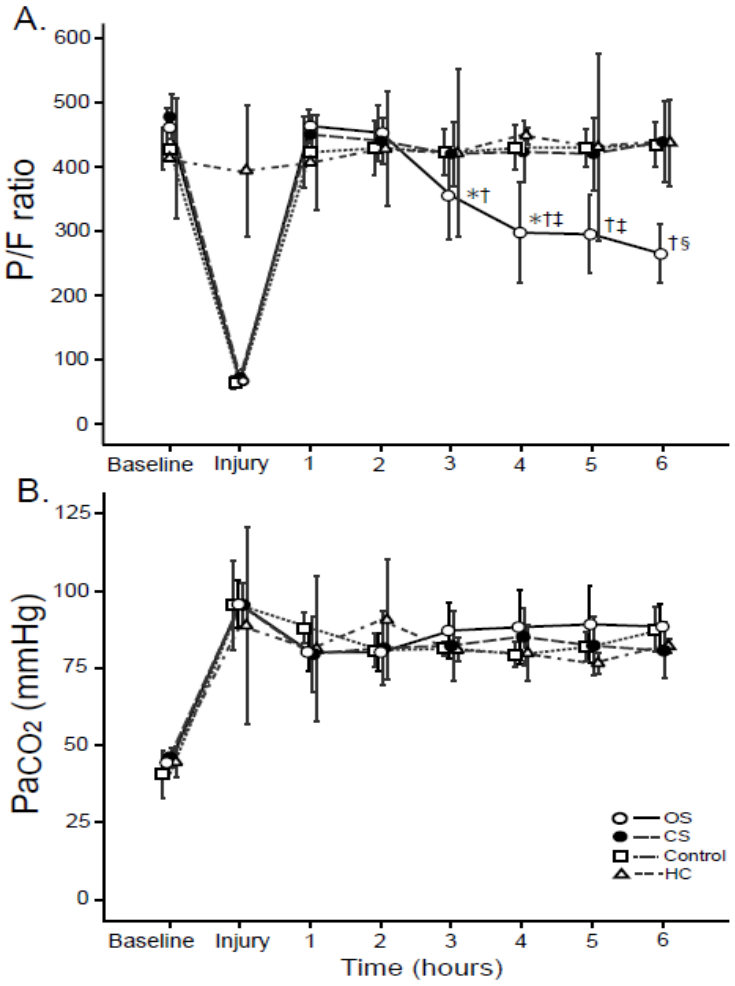

Figure 4. Changes in (A) PaO_2/FIO_2 (P/F) ratio and (B) $PaCO_2$ in the study groups.

OS, open endotracheal suctioning (*open circle*); CS, closed endotracheal suctioning (*closed circle*); Control, control group with lung injury, but without endotracheal suctioning (*square*); HC, healthy control group with 6 hour ventilation, but without lung injury and endotracheal suctioning (*triangle*). Data are shown as means with 95% confidence intervals. (A) OS group shows progressive decline in P/F, whereas all other groups maintained at mean P/F of >400 up to the end of the study. $^{*}p < 0.05$ vs. compared with previous value within the same group; $^{\dagger}p < 0.05$ compared with 1 hour after injury within the same group; $^{\ddagger}p < 0.05$ vs. CS and Control groups. $^{\S}p < 0.05$ vs. all other groups.

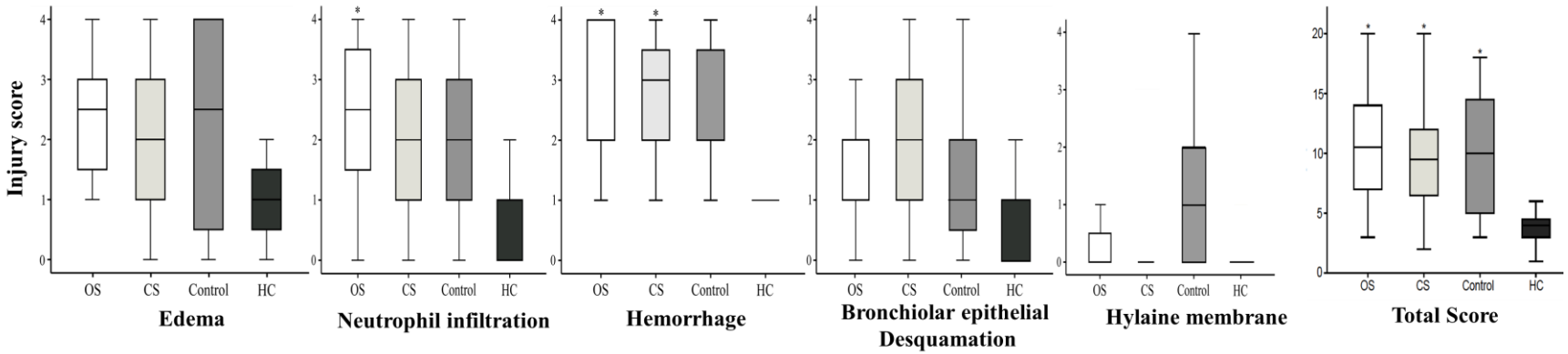

Figure 5. Box-and-whiskers graph of quantitative histological analysis showing the lung injury score.

The ends of the boxes indicate the 25th and 75th percentiles and the lines in the bars indicate the median value. The 10th and 90th percentiles were indicated with whiskers. OS, open endotracheal suctioning; CS, closed endotracheal suctioning; Control, control group with lung injury, but without endotracheal suctioning; HC, healthy control group with 6 hour ventilation, but without lung injury and endotracheal suctioning. *$p < 0.05$, compared with healthy control (HC) group

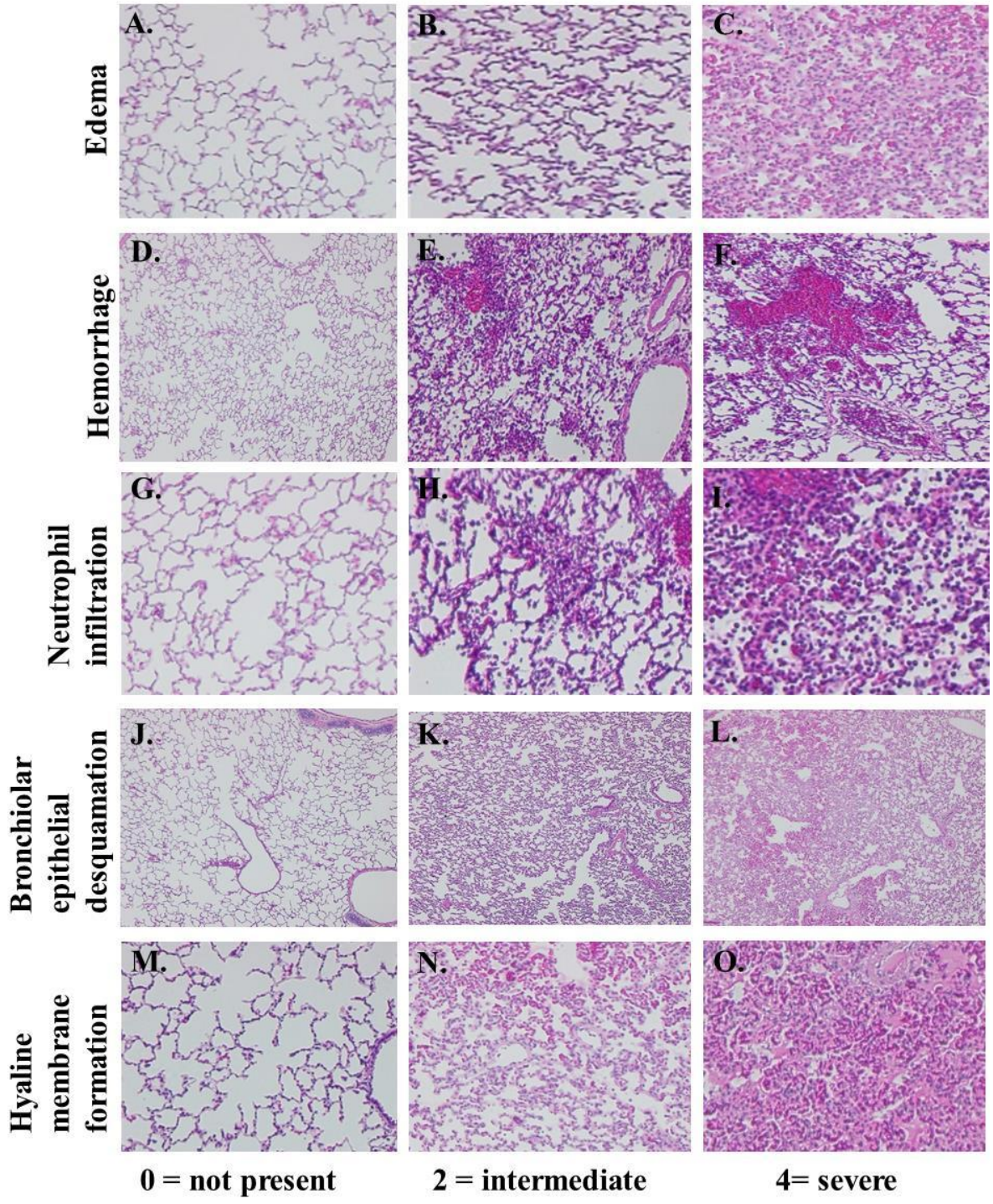

Figure 6. Histology of the lung

Representative lung micrographs stained with hematoxylin and eosin (D-F and J-L magnification: ×200, A-C, G-I and M-O magnification: ×400). A, D, G, J and

M: Histology of the healthy control group, which showed that alveolar walls were very thin, and the majority of the alveoli contained no cellular structure. B, K and O: Histology of the control group, which showed minimal edema, inflammatory cell infiltration. E, H and L: Histology of the closed endotracheal suctioning groups, which showed hemorrhage and more inflammatory cells. C, F, I and N: Histology of the open endotracheal suctioning group. Severe inflammatory cells infiltration and hemorrhage were observed.

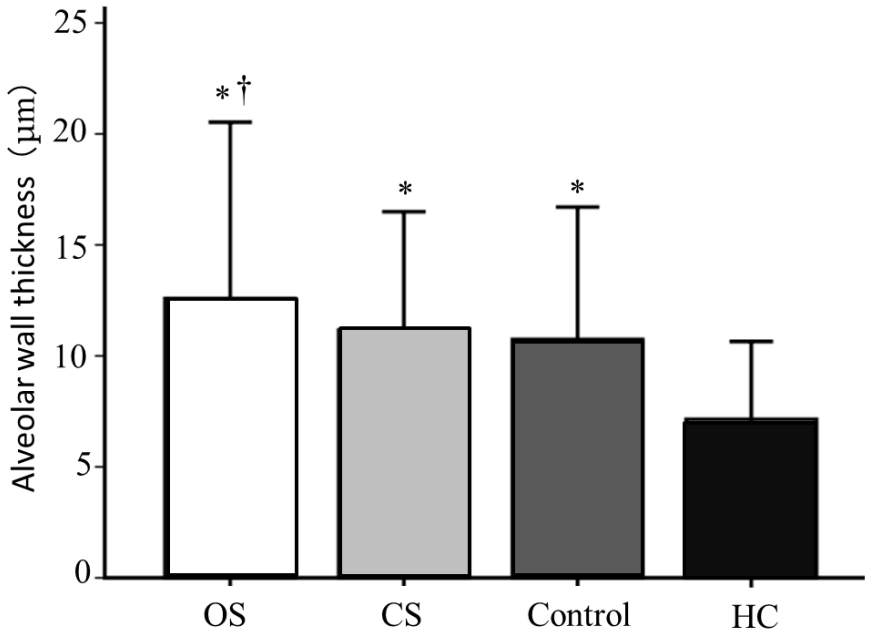

Figure 7. Alveolar wall thickness.

Alveolar wall thickness by histological analysis. Data are shown as means ± standard deviation (SD). OS, open endotracheal suctioning; CS, closed endotracheal suctioning; Control, control group with lung injury, but without endotracheal suctioning; HC, healthy control group with 6 hour ventilation, but without lung injury and endotracheal suctioning. *$p < 0.05$ compared with HC group. $^{\dagger}p < 0.05$ compared with CS and control group.

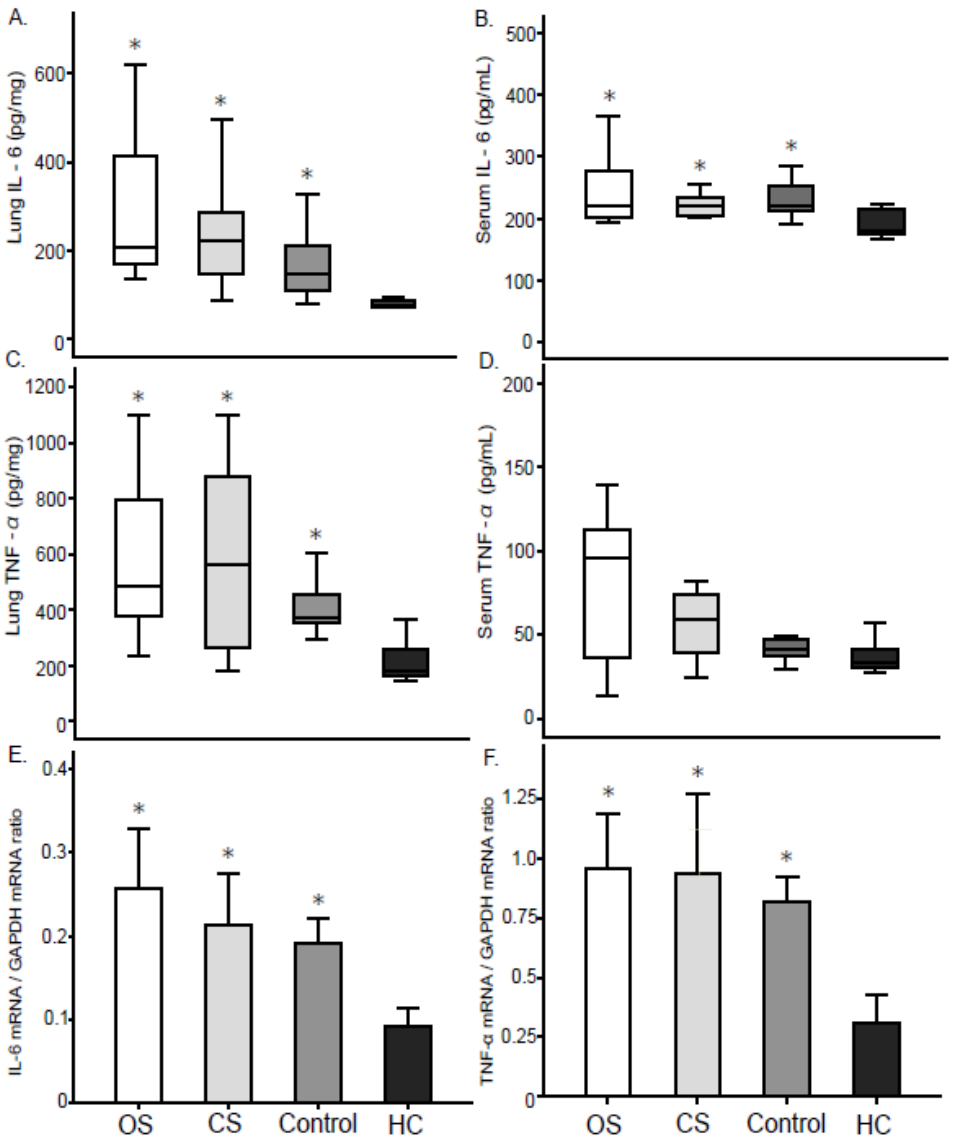

Figure 8. Expression level of potential inflammatory cytokines, as revealed by ELISA and Real Time PCR.

Serum and pulmonary levels of interleukin (IL)-6 and tumor necrosis factor (TNF) –α at the end of the study by ELISA (Figure. 3A-D). The ends of the boxes indicate the 25th and 75th percentiles and the lines in the bars indicate the median value. The 10th and 90th percentiles were indicated with whiskers. The mRNA

expression of IL-6 and TNF-α at the end of the study by Real Time PCR (Figure. 6E-F). The ends of the boxes indicate mean and the lines in the bars indicate standard deviation. OS, open endotracheal suctioning; CS, closed endotracheal suctioning; Control, control group with lung injury, but without endotracheal suctioning; HC, healthy control group with 6 hour ventilation, but without lung injury and endotracheal suctioning. *$p < 0.05$, compared with healthy control (HC) group.

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