RESEARCH ARTICLE

Risk factors for ventilator-induced-lung injury develop three to five times faster after a single episode of lung injury

Elizabeth C. Rohrs[1,2,3], Thiago G. Bassi[3,4], Michelle Nicholas[2,3], Jessica Wittmann[2], Marlena Ornowska[3], Karl C. Fernandez[2,3], Matt Gani[4], Steven C. Reynolds[1,2,3]

EC Rohrs, K Bevan, TG Bassi, M Nicholas, J Wittmann, M Ornowska, K C Fernandez, M Gani, SC Reynolds. Risk factors for ventilator-induced-lung injury develop three to five times faster after a single episode of lung injury. Can J Respir Ther 2023;59:103–110. doi: 10.29390/cjrt-2022-075.

Introduction: Mechanical ventilator breaths provided to deeply sedated patients have an abnormal volume distribution, encouraging alveolar collapse in dependent regions and promoting alveolar overdistention in non-dependent regions. Collapse and overdistention both start with the first breath and worsen over time, driving ventilator-induced lung injury (VILI). This is exacerbated when the lung is already injured or has increased heterogeneity. Our study investigated the impact of a single episode of lung injury on lung mechanics and the risk factors for ventilator-induced injury, compared with non-injured lungs.

Methods: Two groups of pigs were sedated and ventilated using lung-protective volume-controlled mode at 8 mL/kg, positive end-expiratory pressure (PEEP) 5 cmH_2O, with respiratory rate and FiO2 set to maintain normal blood gas values. Animals in one group were ventilated for 50 h (50-Hour MV group, n=10). Animals in the second group had lung injury induced using oleic acid and were ventilated for 12 h post-injury (LI MV group, n=6). Both groups were compared with a never-ventilated control group (NV, n=6). Lung mechanics and injury were measured using electrical impedance tomography, esophageal pressure monitoring and tissue histology.

Results: End-expiratory lung-volume loss was greater in the 50-Hour MV group ($P<0.05$). Plateau pressure, driving pressure and lung injury score were higher in the LI MV group, ($P<0.05$).

Conclusion: Risk factors for VILI developed three- to five-times faster in the group with injured lungs, demonstrating that a single lung-injury episode substantially increased the risk of VILI, compared with normal lungs, despite using a lung-protective mechanical ventilation protocol.

Key Words: *acute lung injury; mechanical ventilation; ventilator-induced lung injury*

INTRODUCTION

The efficiency of gas exchange performed by lung tissue depends on the number of alveoli that remain functional, the thickness of the alveolar-capillary membrane, and a functional and perfused capillary bed [1]. Normal alveoli are homogenously inflated, interdependent, and have little change in alveolar volume during normal tidal breathing [2]. Lung injury compromises these structures, causing alveoli to become dysfunctional, impairing gas exchange, and necessitates the use of mechanical ventilation to support a patient through their critical illness [1]. In normal tidal breathing, alveolar expansion occurs gradually as the pleural pressure gradient increases, with a 4%–6% change in alveolar volume for each breath cycle, thus causing very little dynamic strain [2, 3]. Surfactant, the mechanical support of interconnected alveolar microanatomy, and non-diffusible nitrogen stabilize the alveolus [4]. Alveolar dynamics change when the lung tissue is injured, increasing alveolar instability, and lowering the threshold for the occurrence of ventilator-induced lung injury (VILI) [5].

Alveolar dynamics in injured lungs are more heterogeneous and unstable than in normal lungs [6]. Instability leads to increased alveolar collapse and reopening during the ventilator breath cycle [7]. This is due to the increased closing capacity of alveoli in the presence of increased pulmonary edema, such as in patients with acute respiratory distress syndrome (ARDS), in addition to the loss of lung stabilizers [8]. Pulmonary edema deactivates surfactant, causing lung tissue to collapse at atmospheric pressure [9]. This rapid collapse leads to significant alveolar strain in adjacent alveoli, the main driver of VILI [2]. Overdistension occurs adjacent to collapsed or unstable alveoli as the shared walls are stretched, creating an up to fourfold increase in stress and strain [10]. The primary mechanism of VILI is the alteration of normal dynamic alveolar physiology resulting in excessive alveolar strain with each breath [2, 4]. This may also occur when a normal lung is ventilated over time, becoming heterogenous due to alveolar collapse from gravity and pressure exerted by the chest wall and abdomen in a sedated patient lying supine and immobile for periods of time [11, 12]. Once lung tissue is injured, the threshold for further injury is lower and requires less energy; thus, ventilator-induced lung injury is more prevalent in lungs with an existing injury [9, 13–15].

The physiological differences between a normal lung and a lung that has been injured highlight the differing speed and severity potentials for the occurrence of VILI. The present study investigated the alterations that occur, along with the different rates of change, in the lung physiology of injured compared with non-injured lungs. It evaluated the effects on the potential risk for VILI in a normal-lung model ventilated with lung-protective ventilation for 50 h compared with an injured-lung

[1]*Advancing Innovation in Medicine Institute, New Westminster, BC, Canada*
[2]*Fraser Health Authority, Royal Columbian Hospital, New Westminster, BC, Canada*
[3]*Simon Fraser University, Burnaby, BC, Canada*
[4]*Lungpacer Medical USA Inc., Exton, PA, USA*
Correspondence: Elizabeth Rohrs, Advancing Innovation in Medicine Institute, 330 East Columbia Street, New Westminster, BC V3L 3W7. E-mail: lizrohrs@gmail.com

Published online at https://www.cjrt.ca on 11 April 2023

model ventilated with the same lung-protective ventilation for 12 h post-oleic-acid-induced lung injury. The knowledge gained from the present study could not ethically be obtained at the bedside in human patients and enhances our understanding of the impact that lung injury has on the physiological interactions between lung tissue and positive-pressure mechanical ventilation. This is vital to informing clinical strategies and approaches for the use of positive-pressure mechanical ventilation in the care of critically ill patients.

The study hypothesis was that an injured-lung model, ventilated for only 12 h post-injury, will demonstrate risk factors for VILI at similar levels as a normal-lung model after 50 h of mechanical ventilation.

METHODS

This was a preclinical, non-randomized, quantitative study in large, adolescent pigs that were sedated and ventilated under ICU conditions. The present study was approved by the Simon Fraser University (1247K-17) and the University of British Columbia (A20-0245) Animal Care Committees and adhered to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Twenty-two (22) female, adolescent, Yorkshire pigs were separated into three groups, two of which were mechanically ventilated. These groups of animals were the control groups of a series of linked studies investigating a novel method of lung protective ventilation and the duration of experiments and number of animals were dictated by those specific study protocols [16, 17]. All animals in the mechanically ventilated groups were intubated and sedated with intravenous anesthesia to ablate respiratory drive. Animals were ventilated in the supine position using volume-control ventilation (8 mL/kg, PEEP 5 cmH_2O, respiratory rate and FiO_2 set to achieve normal arterial blood gases). The 50-Hour MV animals (n=10) were mechanically ventilated for 50 h post-intubation and did not receive any lung injury. The LI MV animals (n=6) had lung injury induced by cis-9-Octadecenoic acid (oleic acid) administered via a pulmonary artery catheter, a validated preclinical ARDS model [18, 19]. Oleic acid (0.1 mL – 0.4 mL) was gently mixed with 2 mL of the animal's own fresh arterial blood, and injected into the distal port of the pulmonary artery catheter every 2 min until $PaO_2/FiO_2 \leq 200$ was achieved [19]. Animals were then ventilated using the same volume-control protocol for 12 h post-injury, with only changes to respiratory rate and FiO_2 permitted during this time. Tidal volume and PEEP remained constant. A third group of non-intubated animals that have been previously published by our group were used as a normal-lung histology control group, the never-ventilated (NV) group (n=6) [16, 17, 20]. These animals were lightly sedated using inhaled anesthetic delivered via a mask for approximately 30 min, and remained spontaneously breathing and were never intubated or mechanically ventilated. Animals in all groups were euthanized at study termination for tissue samples.

LUNG MECHANICS, DYNAMICS, AND FUNCTION MEASUREMENTS

Distribution of tidal volume, change in end-expiratory lung volume and lung mechanics were measured using electrical impedance tomography (EIT; Dräger PulmoVista 500) [16, 17]. Respiratory mechanics and ventilation pressures were measured hourly (Fluxmed). PaO_2/FiO_2 was calculated from arterial blood gases.

Lung homogeneity and injury

Alveolar expansion at the time of tissue fixation was used to evaluate alveolar homogeneity and was assessed by measuring alveolar chord length [21]. Lung tissue injury was assessed by modifying a standardized and validated histology score as previously published [21].

Statistical analysis and power calculation

A power calculation with a standard alpha of 0.01 and a beta of 0.90, based on the PaO_2/FiO_2 ratio changes seen in the experiment by Grasso et al. [22] with negative-pressure ventilation in lung-injured rabbits (350 mmHg vs. 75 mmHg, SD 75) yielded two animals per arm. A minimum of six animals per group was selected, to improve statistical power. An additional four animals were included in the 50-Hour MV group to replace tissue samples lost following a freezer failure, and due to procedural requirements (having to study pairs of pigs for each study). Statistical analysis was performed using GraphPad Prism 9.2.0 (GraphPad, USA). Measurements are reported as median interquartile range (IQR) and compared using non-parametric Kruskal-Wallis one-way ANOVA or Mann-Whitney U test. Post-hoc Dunn's test of Multiple Comparisons was performed if the Kruskal-Wallis test showed significance. Secondary analysis of the rates of change over different time periods was conducted with multiple regression using least-squares method.

RESULTS

Group comparison

Weights were significantly different and higher in the LI MV group versus the NV group, P=0.006 (Table 1). Animal fluid balance, Midazolam dose and Ketamine dose were not significantly different between the two ventilated groups. Propofol dose was significantly higher in the 50-Hour MV group versus the LI MV group, P=0.001. Fentanyl dose showed a tendency toward a significant difference and was higher in the 50-Hour MV group versus the LI MV group, P=0.053. Respiratory rate, arterial pH and PaO_2 were not significantly different between the groups. $PaCO_2$ was significantly lower in the 50-Hour MV group versus the LI MV group, P<0.001. FiO_2 was also significantly lower in the 50-Hour MV group versus the LI MV group, P<0.001.

Distribution of ventilation

Tidal volume was distributed more to the dorsal lung regions in the NV group compared with the groups receiving mechanical ventilation, P<0.001 (Table 2, Figure 1).

Change in end-expiratory lung volume

The 50-Hour MV group had a decrease of 1203 mL (985–1554) in end-expiratory lung volume over the study duration versus 899 mL (554–1107) in the LI MV group, P=0.056 (Table 2, Figure 2). The rate of change-over-time (total volume lost/number of hours in study) was significantly different at 24.1 mL/h for the 50-Hour MV group versus 64.2 mL/h for the LI MV group, P=0.002 (see Figure 2A).

Ventilation pressures

Plateau pressure was significantly higher in the LI MV group versus the 50-Hour MV group, both at baseline (P=0.020) and at study end (P<0.001) (Table 2, Figure 2). The change-over-time (Δ P) was not significantly different between the two groups although had a trend toward a greater change in the LI MV group, P=0.062. The rate of change-over-time (Δ P/total study time) was significantly different, with 0.08 cmH_2O/h change in the 50-Hour MV group compared with 0.43 cmH_2O/h change in the LI MV group, P<0.001 (see Figure 2B).

Driving pressure was significantly higher in the LI MV group versus the 50-Hour MV group, both at baseline (P=0.020) and at study end (P<0.001). The change-over-time (Δ P) was significantly greater in the LI MV group, P=0.0367. The rate of change-over-time (Δ P/total study time) was significantly different, with 0.08 cmH_2O/h in the 50-Hour MV group versus 0.43 cmH_2O/h in the LI MV group, P<0.001 (see Figure 2C). The rate of change-over-time for driving pressure was the same as for plateau pressure, as all animals had the same PEEP (5 cmH_2O).

Respiratory system static compliance

Static compliance was not statistically different at baseline between the two ventilated groups (Table 2, Figure 2). Static compliance was statistically different and lower in the LI MV group versus the 50-Hour MV group at study-end, P=0.007. The change-over-time (Δ C) was not significantly different between the two ventilated groups. The rate of change-over-time (ΔC/total study time) was significantly different, with 0.3 cmH_2O/h in the 50-Hour MV group versus 1.0 cmH_2O/h in the LI MV group, P<0.001 (see Figure 2D).

TABLE 1

Group comparisons

Group comparisons		NV	50-Hour MV	LI MV			Dunn's multiple comparisons test		
Parameter	Units		Median (IQR)		Kruskal-Wallis Test (P-value)	Mann-Whitney U test (P-value)	NV vs. 50-Hour MV	NV vs. LI MV	50-Hour MV vs. LI MV
Weight	kg	54 (52–56)	57 (54–64)	64 (62–69)	0.002	-	ns	0.006	ns
Fluid balance	mL/kg/h	n/a	1.0 (0.4–1.4)	1.3 (1.2–1.4)	-	ns	-	-	-
Midazolam	mg/h	n/a	53 (44–80)	70 (68–74)	-	ns	-	-	-
Fentanyl	µg/h	n/a	481 (393–804)	390 (360–410)	-	ns (0.053)	-	-	-
Propofol	µg/kg/min	n/a	109 (67–119)	50 (48–55)	-	0.001	-	-	-
Ketamine	µg	n/a	1356 (856–3425)	1300 (1200–2050)	-	ns	-	-	-
pH	-	n/a	7.43 (7.42–7.44)	7.44 (7.42–7.47)	-	ns	-	-	-
PaO_2	mmHg	n/a	128 (121–144)	141 (92–156)	-	ns	-	-	-
$PaCO_2$	mmHg	n/a	46 (44–48)	52 (48–55)	-	<0.001	-	-	-
RR	-	n/a	21 (21–22)	20 (20–21)	-	ns	-	-	-
FiO_2	-	n/a	0.30 (0.30–0.30)	0.40 (0.37–0.42)	-	<0.001	-	-	-

IQR, Interquartile Range; RR, Respiratory rate; NV, Never ventilated; LI, Lung-injured; MV, mechanically ventilated; MV, Mechanically ventilated.

TABLE 2

Outcomes

Lung mechanics, dynamics, and function			Group							
Parameter	Time/ location	Units	NV	50-Hour MV	LI MV	Kruskal-Wallis Test (P-value)	Mann-Whitney U test (P-value)	NV vs. 50-Hour MV	NV vs. LI MV	50-Hour MV vs. LI MV
			Median (IQR)							
Distribution of Tidal Volume	Dorsal	%	61 (49–70)	37 (32–45)	43 (39–46)	<0.001	-	0.001	ns (0.054)	ns
	Ventral	%	39 (30–51)	63 (55–68)	57 (54–61)	<0.001		0.001	ns (0.054)	ns
Lung injury score		-	0.20 (0.18–0.21)	0.20 (0.16–0.29)	0.47 (0.41–0.49)	<0.001		ns	0.007	0.015
								Regression analysis (P-value)		
Decrease in end-expiratory lung volume		mL	-	1203 (985–1554)	899 (554–1107)	-	ns (0.056)	-	-	0.002
Plateau pressure	Baseline	cmH_2O		15 (14–17)	18 (17–18)		0.020			<0.001
	Study-end	cmH_2O		19 (18–21)	24 (23–27)		<0.001			
Driving pressure	Baseline	cmH_2O		10 (9–12)	13 (12–13)		0.020			<0.001
	Study-end	cmH_2O		14 (13–16)	19 (18–22)		<0.001			
Static compliance	Baseline	mL/cmH_2O		44 (38–48)	40 (36–42		ns			<0.001
	Study-end	mL/cmH_2O		30 (29–36)	27 (22–28)		0.007			
PaO_2/FiO_2	Baseline	mL/cmH_2O		521 (455–552)	512 (467–543)		ns			ns
	Study-end	mL/cmH_2O		403 (358–444)	364 (327–376)		ns			

IQR, Interquartile Range; NV, Never ventilated; LI, Lung-injured; MV, mechanically ventilated; MV, Mechanically ventilated.

PaO2/FiO2

PaO_2/FiO_2 was not statistically different between the two ventilated groups at baseline or at study-end (Table 2). The rate of change-over-time was also not significantly different.

Lung injury score

The LI MV group had the highest lung injury score and was significantly different from both the NV group (P=0.007) and the 50-Hour MV group (P=0.015) (Table 2).

Alveolar homogeneity

Alveolar chord length was not statistically different between sample locations within the NV group (Table 3, Figure 3). The 50-Hour MV and LI MV groups both had statistically different alveolar chord length measurements for all sample locations (P<0.001) within each group.

Adverse events

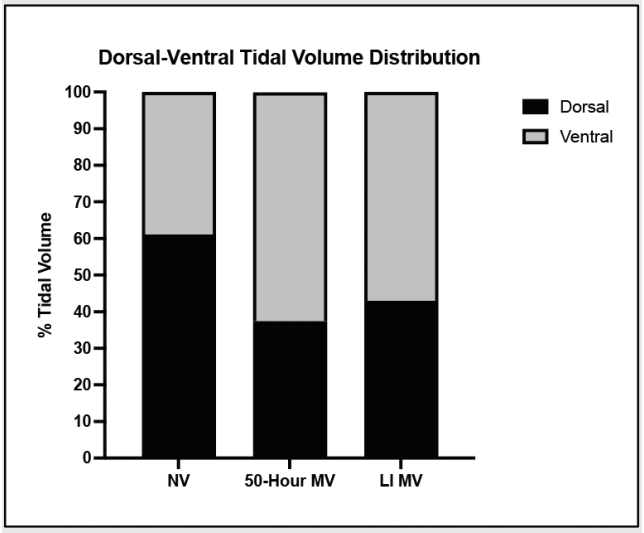
Pressure and flow waveforms recorded at the termination of the ventilator circuit were manually reviewed, and no evidence of ventilator asynchrony such as reverse triggering or breath stacking was observed. There were no adverse hemodynamic events and no dangerous cardiac arrythmias recorded. There was no evidence of significant spontaneous breathing efforts in the two groups receiving mechanical ventilation.

DISCUSSION

The present study describes the different rates of atelectasis formation in healthy lungs compared with injured lungs exposed to sedation and mechanical ventilation. Both ventilated groups had a significant amount of atelectasis formation, loss of compliance, increase in ventilation pressures, and alveolar heterogeneity, all risk factors for the development of VILI. The injured-lung group demonstrated an almost three times greater rate of alveolar collapse, a three times greater decrease in lung compliance and a five times greater rate of driving pressure increase. This group also had the highest lung injury scores. Not only does this demonstrate that both healthy and injured lungs are at risk of VILI despite being managed with a lung-protective mechanical ventilation protocol, but also that lung injury makes the risks for VILI development substantially greater. Many ICU patients require mechanical ventilation for longer than 50 h, and a single event of lung injury accelerates atelectasis formation and decreases the time to develop VILI in these patients. The Lung SAFE study reports that 23.4% of mechanically ventilated ICU patients develop ARDS, and this occurs in the first 48 h after initiation of mechanical ventilation [23]. Better methods of delivering

FIGURE 1

Dorsal-ventral distribution of tidal volume as measured by EIT



mechanical ventilation are needed to address the impact and burden of VILI in ventilated, critically ill patients.

A higher percentage of tidal volume was distributed to the dorsal lung regions in the spontaneously breathing, NV group, while the ventilated groups had more tidal volume delivered to the ventral lung regions. This shows the difference in the pleural pressure gradients between normal spontaneous breathing, where the diaphragm is contracted, and positive-pressure ventilation in the absence of diaphragm contraction, and was expected based on current literature [24–27]. Inspiration in a spontaneously breathing subject relies on a pleural pressure gradient generated from diaphragm contraction that starts at the upper airway and terminates at the alveoli [27]. The resulting distribution of tidal volume is determined by this pleural pressure gradient and the elastic properties of the receiving alveoli [25]. The gradient is lowest in the non-dependent regions of the lung because of gravity and the shape of the chest wall, resulting in the dependent regions receiving a larger volume change for a unit of change in pleural pressure [25–27]. In the absence of diaphragm contraction, the intrapleural pressure gradient needs to be provided by positive airway pressure provided by the mechanical ventilator. This changes the nature of the pleural pressure gradient, so that the dependent regions have the lowest gradient and the non-dependent regions receive a larger volume change for a unit of change in pleural pressure [27, 28]. Therefore, less tidal volume is distributed to dependent regions in the absence of respiratory muscle contraction, contributing to alveolar collapse (atelectasis) and more is distributed to non-dependent regions,

FIGURE 2

(A) Change in end-expiratory lung volume over time. (B) Plateau pressure over time. (C) Driving pressure over time. (D) Respiratory system static compliance over time

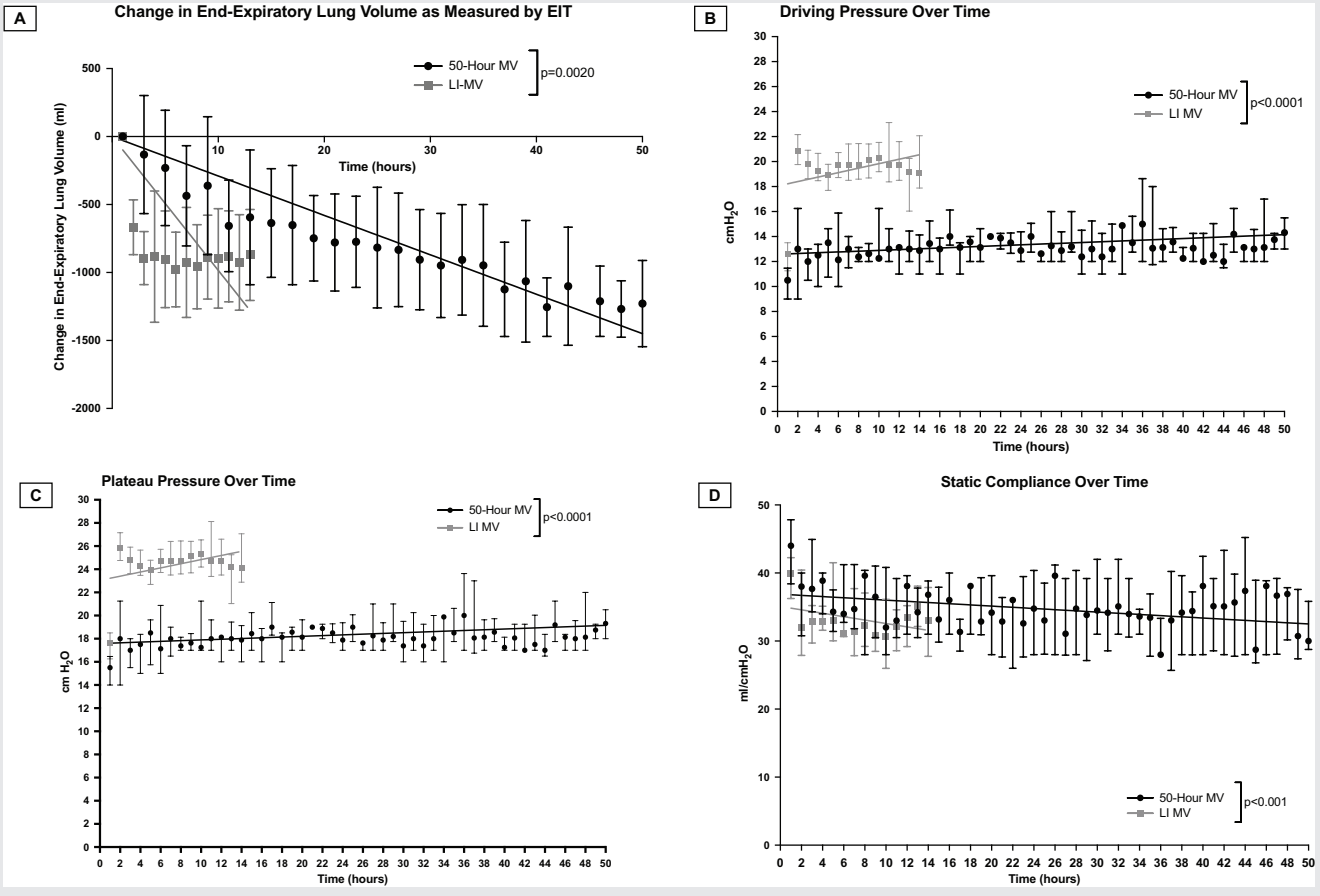


TABLE 3

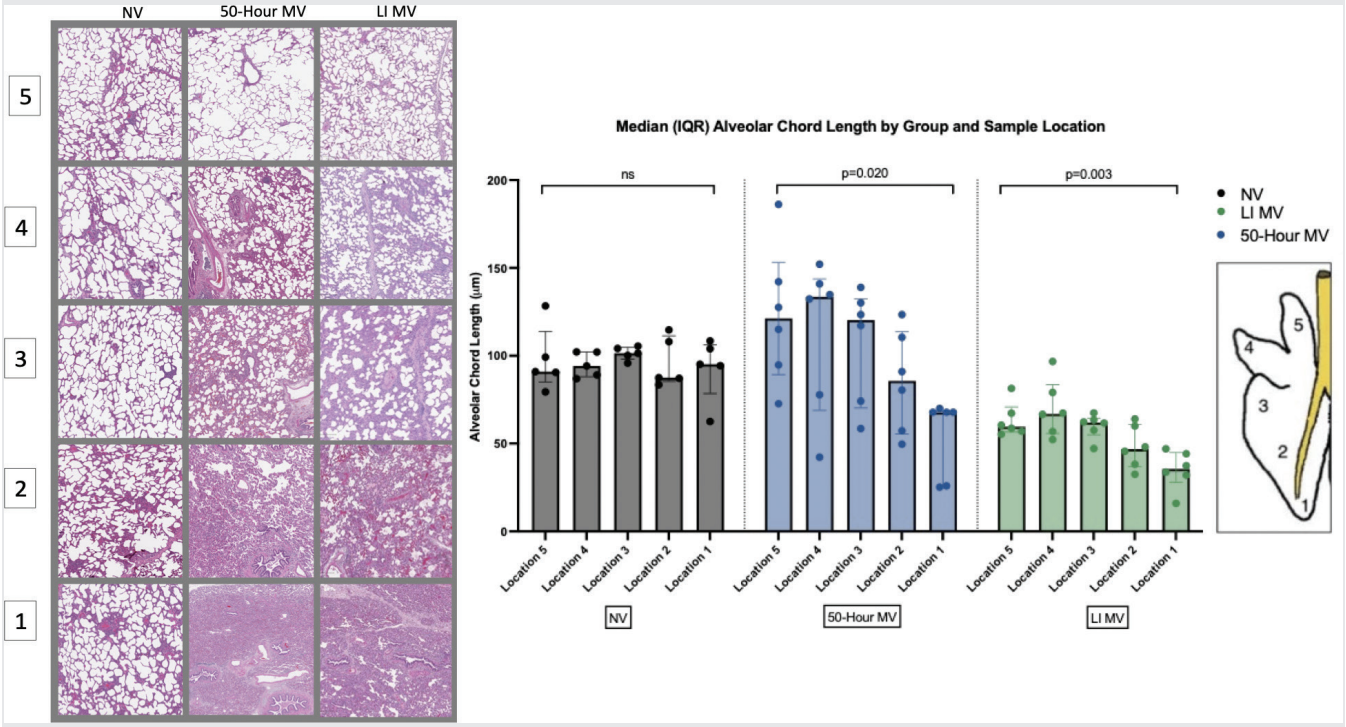
Alveolar homogeneity

		NV	50-Hour MV	LI MV
Alveolar chord length measurement	**Units**		**Median (IQR)**	
Sample location 5	µm	91 (88–107)	121 (89–153)	60 (57–71)
Sample location 4	µm	94 (89–102	133 (69–143)	67 (56–84)
Sample location 3	µm	101 (99–105)	120 (70–132)	62 (55–64)
Sample location 2	µm	88 (86–110)	86 (55–114)	47 (37–61)
Sample location 1	µm	95 (86–105)	68 (26–70)	36 (28–45)
Kruskal-Wallis Test (P-value)		ns	0.020	0.003
Dunn's multiple comparisons test (P-value)				
Location 5 vs. 4		-	ns	ns
Location 5 vs. 3		-	ns	ns
Location 5 vs. 2		-	ns	ns
Location 5 vs. 1		-	0.039	0.032
Location 4 vs. 3		-	ns	ns
Location 4 vs. 2		-	ns	0.023
Location 4 vs. 1		-	0.048	0.005
Location 3 vs. 2		-	ns	ns
Location 3 vs. 1		-	ns	0.031
Location 2 vs. 1		-	ns	ns

IQR, Interquartile Range; NV, Never ventilated; LI, Lung-injured; MV, mechanically ventilated; MV, Mechanically ventilated.

FIGURE 3

Alveolar chord length and examples of H&E-stained lung tissue from all groups



contributing to alveolar overdistension and injury [27, 28]. This is a significant driver of VILI, and both ventilated groups had more tidal volume distributed to the ventral, non-dependent lung zones. This increases their potential for dependent alveolar collapse and non-dependent alveolar overdistention.

Both the ventilated groups lost a significant amount of end-expiratory lung volume at study-end despite different durations of experiment. The LI MV group lost volume at a rate almost three times greater than the 50-Hour MV group. This demonstrates that ARDS-related lung injury accelerates the formation of atelectasis. Atelectasis forms due to compression of lung tissue by other parts of the body, loss of surfactant and/or absorption of alveolar gas behind a closed airway [29, 30]. Recent studies suggest that the application of anesthesia, without paralysis, results in a cephalad movement of the posterior/dorsal diaphragm region and caudal movement of the anterior/ventral regions [30, 31]. Atelectasis leads to decreased lung compliance, decreased gas exchange capacity and increased pulmonary vascular resistance [32]. The loss of lung volume by the formation of atelectasis increases transpulmonary

pressure, decreases compliance and results in fewer open alveoli between which to distribute tidal volume [33]. More distending pressure is then required to stretch these alveoli to deliver tidal volume, exposing them to increased stress, which leads to strain and injury [33, 34].

Plateau pressure was higher in the LI MV group at baseline and study-end, and the rate of change-over-time was also five times higher in this group. In addition, driving pressure was higher in the LI MV group at baseline and study-end. The rate of change-over-time was also five times greater. The pressure applied directly to lung tissue (stress) is coupled with a change in lung volume relative to resting volume (strain) [35]. The risk of damage depends on the stress and strain development in the individual microstructures of the lungs, and the distribution of this stress and strain determines the extent of regional VILI risks [14, 35, 36]. Plateau pressure is a static measurement quantifying the force per unit area applied to the alveolus at the extreme of the tidal cycle, which is peak- or end-inspiration [10, 14, 35, 37–39]. Plateau pressure and PEEP individually do not directly reflect the associated volume change, rate of volume expansion, or resulting strain [14]. Their difference, driving pressure, describes these factors and is a good indicator of strain and risk of developing VILI [14, 35]. The increase in strain over the study period was approximately five times larger in the group with induced lung injury. Amato et al. have reported mortality increases with every increase in respiratory system driving pressure of approximately 7 cmH_2O, and recommend that driving pressure be limited to less than 15 cmH_2O [39]. Driving pressure increased 6 cmH_2O (5–9) in the LI MV group, despite their management with lung-protective ventilation, and was 19 cmH_2O at study-end. The 50-Hour MV group had a study-end driving pressure of 14 cmH_2O, close to the maximum recommended value. This highlights the impact that lung injury has on the time required to develop an increased risk for VILI, and demonstrates the impact that days of mechanical ventilation, and associated morality risks, will have on ICU patients with and without lung injury.

PaO_2/FiO_2, an indicator of pulmonary shunt, is well correlated with atelectasis [40–42]. A large decline in PaO_2/FiO_2 was not expected in the 50-Hour MV group as these animals had normal, healthy lungs and were ventilated with a lung-protective ventilation strategy. It was interesting to observe this group still had a decline of PaO_2/FiO_2 of greater than 20% in 50 h. There was also no statistical difference between the study-end measurements, showing that 50 h of lung-protective mechanical ventilation resulted in the same amount of lung impairment at study-end as did injury of the lungs followed by 12 h of ventilation. The amount of atelectasis formation in each group supports the similar study-end PaO_2/FiO_2 values.

Alveolar chord length was not different between sample locations in the NV group, showing that normal, healthy lung tissue has a homogenous alveolar expansion throughout the various lung regions. Both ventilated groups showed a wide range of alveolar expansion, with the smallest measurements in the most dependent sample locations, and the largest measurements in non-dependent locations. VILI originates from repeated alveolar collapse and expansion leading to alveolar overdistention, all of which are exacerbated by alveolar heterogeneity and instability [43–46]. Mead et al. determined that there is an increase in stress in alveoli adjacent to collapsed or atelectatic alveoli. The magnitude of this stress increase depends on the number of collapsed alveoli, their location and surface tension [10]. Tidal volume delivered to lungs with regional heterogeneity will not distribute evenly across lung parenchyma but will be preferentially distributed to areas of higher compliance, resulting in non-uniform stretch [47–49]. This abnormal stretch of the alveolar epithelial cells causes injury, altering surfactant secretion and permeability, further worsening heterogeneity [50–52]. The increased stress in these alveoli concentrates regional strain to a level that can be up to four times that of global lung strain [10]. The two ventilated groups were both at risk for increased strain-related VILI due to the amount of heterogeneity present in tissue samples.

The modified lung-injury score was more than two times higher in the LI MV group than the 50-Hour MV group, demonstrating the presence of lung tissue injury in these animals. Neutrophil recruitment into the alveolar spaces and interstitium is a hallmark of acute lung injury and forms the basis of this scoring system [21]. It has also been shown that apoptotic epithelium contributes to the recruitment of neutrophils in early acute lung injury [54]. This lung injury score was designed to reduce interobserver variability by using a simple scheme that relies on coarse gradations of severity for each finding [21]. The lack of difference between the 50-Hour MV group and the NV group was not unexpected, as both groups had healthy, normal lungs, and mechanical ventilation in the 50-Hour MV group was provided according to a protective ventilation protocol. However, this scoring system requires any neutrophil counts greater than five to be given the same score of 2, regardless of whether there were six neutrophils or 25, for example. This may explain why there is very little difference seen between these two groups using this score. There would be a benefit to reporting the average neutrophil count in each group; however, this information was not recorded and thus could not be included. This is a future consideration for further analysis.

Oleic acid infused into the pulmonary artery is an accepted method of inducing lung injury in a large-animal model [18]. Oleic acid-induced lung injury is characterized by necrosis and microvascular thrombi, followed by a repair phase where type 2 cells proliferate and fibrosis forms in the subpleural regions [55]. This creates a multifocal and heterogenous injury [56]. Alveolar permeability is significantly increased, altering gas exchange and decreasing lung efficiency [18]. The advantage of using this model of ARDS is that it reproduces an early, reversible, and heterogeneous inflammatory lung injury that is typical of clinical ARDS, and changes in permeability along with gas-exchange impairments and lung-mechanics changes occur [18]. It is extremely reproducible between subjects and is reported to be an excellent model in which to study ventilatory strategies, and was therefore the chosen model for the present study [18].

While the LI MV group subjects were heavier than the other groups, their bodies were proportionally larger and not just heavier, as the length-to-weight ratio was the same in the two ventilated groups, and tidal volume was normalized to 8 mL/kg, thus should be of a similar magnitude between all groups. Arterial blood gas values of pH and PaO_2, and respiratory rate were not different between the MV groups. $PaCO_2$ was higher in the LI MV group for a respiratory rate similar to the 50-Hour MV group. This was an expected finding as gas exchange is more impaired in the presence of ARDS and these patients usually require more intense mechanical ventilation to maintain adequate arterial blood gas values [57]. Animal fluid balance and all sedative drug doses except Propofol were not different between the two ventilated groups. Significantly less Propofol was used in the LI MV group. This was likely due to the duration of the experiments, as the animals develop a tolerance to the sedative effects of Propofol over time and require a higher dose to achieve the same effect. The pigs in the LI MV group were also sourced from a new farmer and were of a different lineage, albeit the same strain, from the 50-Hour MV group and perhaps this had some influence on the sensitivity to Propofol. The depth of anesthesia was monitored using Bispectral Index (BIS) monitoring and all animals were sedated to a common BIS value goal. Thus, the level of sedation achieved, despite the differing dose of drug used, was similar between these groups.

Study limitations

The present study has certain limitations, as it uses a preclinical model and only a small number of subjects. This data cannot be ethically obtained at the bedside and this model is traditionally used as a human surrogate. While many ICU patients do not fit this model, the patients who require extended sedation and mechanical ventilation, and are the most at risk for VIILI, do fit this model. The goal of the present study was to investigate the effects of mechanical ventilation, both on normal and injured lungs of sedated subjects who are immobile for long periods of time. This applies to heavily sedated, critically ill patients receiving controlled mechanical ventilation. The present study was limited in its ability to measure transpulmonary driving pressure, as we did not obtain a full data set due to delayed equipment acquisition. Critically ill patients who are affected by VILI are typically ventilated for more than 50 h; however, a longer duration of these experiments would have been technically difficult.

CONCLUSION

Atelectasis, decreased compliance, and increased alveolar heterogeneity developed in both the 50-Hour MV group with normal lungs, and the LI MV group with injured lungs, despite different durations of mechanical ventilation. These changes required increased driving pressure to provide adequate mechanical ventilation, and placed both groups at great risk of developing VILI. The duration of mechanical ventilation used in the present study was adequate to show evidence of VILI in each group. Critically ill patients managed with mechanical ventilation often require longer durations of mechanical ventilation than used in the present study, and this highlights the potential for mechanical ventilation to exacerbate lung injury and compels the investigation of new ways to reduce VILI in these patients. The present study enhances our understanding of the physiological effects of mechanical ventilation to inform clinical strategies and approaches that minimize the risk of lung injury in our critically ill patients.

DISCLOSURES

Ethics approval and consent to participate

The present study was approved by the Simon Fraser University (1247K-17) and the University of British Columbia (A20-0245) Animal Care Committees and adhered to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Availability of data and materials:

All data is available on request.

Competing interests

MG and TGB are employees of Lungpacer Medical Inc. SCR is a shareholder and has interest in patents with Lungpacer Medical Inc. All other authors have received consulting fees from Lungpacer Medical Inc. Elizabeth Rohrs is Editor-in-Chief of the *CJRT*, but was blinded to the decision-making process.

Funding

Lungpacer Medical USA, Inc supported the study with respect to funds for the animal experiments and providing monitoring equipment. Royal Columbian Hospital Foundation provided funds to support research staff. TB Vets Foundation supports Dr Reynolds academic seat at Simon Fraser University. BC Lung Association provided grant funds for the animal experiments.

Authors' contributions

ECR and SCR were responsible for hypothesis generation. ECR, SCR, and TGB were responsible for the conception of the present study. ECR, SCR, TGB, MG and MN contributed to study design and data interpretation. ECR, TGB, MN, KCF, MO, JW and SCR executed the study. ECR, TGB, JW, MO, MG, SCR and MN conducted data analysis. ECR and SCR were responsible for writing the article. All authors have agreed with the final version of the manuscript before submission.

Acknowledgements

The authors would like to acknowledge the following people for their contribution to this published work: Doug Evans, Suzette Willems, Kate Orchard, Stephanie Smith, Jessica Rabang, Pamela Zurek, and Carli Peters.

REFERENCES

1. Gehr P, Bachofen M, Weibel ER. The normal human lung: Ultrastructure and morphometric estimation of diffusion capacity. Respir Physiol 1978;32(2):121–40. doi: 10.1016/0034-5687(78)90104-4
2. Nieman GF, Satalin J, Kollisch-Singule M, et al. Physiology in medicine: Understanding dynamic alveolar physiology to minimize ventilator-induced lung injury. J Appl Physiol (1985) 2017;122(6):1516–22. doi: 10.1152/japplphysiol.00123.2017
3. Tabuchi A, Mertens M, Kuppe H, Pries AR, Kuebler WM. Intravital microscopy of the murine pulmonary microcirculation. J Appl Physiol (1985) 2008;104(2):338–46.
4. Nieman GF, Andrews P, Satalin J, et al. Acute lung injury: How to stabilize a broken lung. Crit Care 2018;22(1):136. doi: 10.1186/s13054-018-2051-8
5. Dos Santos CC, Slutsky AS. Invited review: Mechanisms of ventilator-induced lung injury: A perspective. J Appl Physiol (1985) 2000;89(4):1645–55. doi: 10.1152/jappl.2000.89.4.1645
6. Matuszak J, Tabuchi A, Kuebler WM. Ventilation and perfusion at the alveolar level: Insights from lung intravital microscopy. Front Physiol 2020;11:291. doi: 10.3389/fphys.2020.00291
7. Bates JHT, Smith BJ. Ventilator-induced lung injury and lung mechanics. Ann Transl Med 2018;6(19):378. doi: 10.21037/atm.2018.06.29
8. Albert RK. The role of ventilation-induced surfactant dysfunction and atelectasis in causing acute respiratory distress syndrome. Am J Respir Crit Care Med 2012;185(7):702–8. doi: 10.1164/rccm.201109-1667PP
9. Hoegl S, Burns N, Angulo M, et al. Capturing the multifactorial nature of ARDS – "Two-hit" approach to model murine acute lung injury. Physiol Rep. 2018;6(6):e13648. doi: 10.14814/phy2.13648
10. Mead J, Takishima T, Leith D. Stress distribution in lungs: A model of pulmonary elasticity. J Appl Physiol. 1970;28(5):596–608. doi: 10.1152/jappl.1970.28.5.596
11. Hedenstierna G, Rothen HU. Atelectasis formation during anesthesia: Causes and measures to prevent it. J Clin Monit Comput. 2000;16(5–6):329–335. doi: 10.1023/a:1011491231934
12. Gunnarsson L, Strandberg A, Brismar B, Tokics L, Lundquist H, Hedenstierna G. Atelectasis and gas exchange impairment during enflurane/nitrous oxide anaesthesia. Acta Anaesthesiol Scand. 1989;33(8):629–37. doi: 10.1111/j.1399-6576.1989.tb02981.x
13. Beitler JR, Malhotra A, Thompson BT. Ventilator-induced lung injury. Clin Chest Med 2016;37(4):633–46.
14. Marini JJ, Rocco PRM, Gattinoni L. Static and dynamic contributors to ventilator-induced lung injury in clinical practice. pressure, energy, and power. Am J Respir Crit Care Med 2020;201(7):767–74. doi: 10.1164/rccm.201908-1545CI
15. Caironi P, Langer T, Carlesso E, Protti A, Gattinoni L. Time to generate ventilator-induced lung injury among mammals with healthy lungs: A unifying hypothesis. Intensive Care Med. 2011;37(12):1913–20. doi: 10.1007/s00134-011-2388-9
16. Rohrs EC, Bassi TG, Fernandez KC, et al. Diaphragm neurostimulation during mechanical ventilation reduces atelectasis and transpulmonary plateau pressure, preserving lung homogeneity and PaO_2/FiO_2. J Appl Physiol. 2021;131(1):290–301. doi: 10.1152/japplphysiol.00119.2021
17. Rohrs EC, Bassi TG, Nicholas M, et al. Negative-pressure-assisted ventilation lowers driving pressure and mechanical power in an ARDS model. J Appl Physiol 2022;133(6):1237–49. doi: 10.1152/japplphysiol.00486.2022
18. Matute-Bello G, Frevert CW, Martin TR. Animal models of acute lung injury. Am J Physiol Lung Cell Mol Physiol. 2008;295(3):379. doi: 10.1152/ajplung.00010.2008
19. Kamuf J, Garcia-Bardon A, Ziebart A, et al. Oleic acid-injection in pigs as a model for acute respiratory distress syndrome. J Vis Exp 2018;(140):57783.
20. Bassi TG, Rohrs EC, Fernandez KC, et al. Transvenous diaphragm neurostimulation mitigates ventilation-associated brain injury. Am J Respir Crit Care Med 2021;204(12):1391–1402. doi: 10.1164/rccm.202101-0076OC
21. Matute-Bello G, Downey G, Moore BB, et al. An official American thoracic society workshop report: Features and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol 2011;44(5):725–738. doi: 10.1165/rcmb.2009-0210ST
22. Grasso F, Engelberts D, Helm E, et al. Negative-pressure ventilation: Better oxygenation and less lung injury. Am J Respir Crit Care Med 2008;177(4):412–8.
23. Bellani G, Laffey JG, Pham Tà, Fan E, On behalf of the LUNG SAFE Investigators and the ESICM, Trials Group. The LUNG SAFE study: A presentation of the prevalence of ARDS according to the berlin definition! Crit Care 2016;20(1):268. doi: 10.1186/s13054-016-1443-x
24. Milic-Emili J, Henderson JA, Dolovich MB, Trop D, Kaneko K. Regional distribution of inspired gas in the lung. J Appl Physiol 1966;21(3):749–59. doi: 10.1152/jappl.1966.21.3.749
25. Glaister DH, Schroter RC, Sudlow MF, Milic-Emili J. Transpulmonary pressure gradient and ventilation distribution in excised lungs. Respir Physiol 1973;17(3):365–385. doi: 10.1016/0034-5687(73)90010-8
26. Glazier JB, Hughes JM, Maloney JE, West JB. Vertical gradient of alveolar size in lungs of dogs frozen intact. J Appl Physiol 1967;23(5):694–705. doi: 10.1152/jappl.1967.23.5.694
27. Lai-Fook SJ, Rodarte JR. Pleural pressure distribution and its relationship to lung volume and interstitial pressure. J Appl Physiol (1985) 1991;70(3):967–78. doi: 10.1152/jappl.1991.70.3.967

28. Reber A, Engberg G, Sporre B, et al. Volumetric analysis of aeration in the lungs during general anaesthesia. Br J Anaesth 1996;76(6):760–66.
29. Strandberg A, Tokics L, Brismar B, Lundquist H, Hedenstierna G. Atelectasis during anaesthesia and in the postoperative period. Acta Anaesthesiol Scand 1986;30(2):154–8. doi: 10.1111/j.1399-6576.1986.tb02387.x
30. Reber A, Nylund U, Hedenstierna G. Position and shape of the diaphragm: Implications for atelectasis formation. Anaesthesia 1998;53(11):1054–61. doi: 10.1046/j.1365-2044.1998.00569.x
31. Puybasset L, Cluzel P, Chao N, Slutsky A, Coriat P, Rouby J. A computed tomography scan assessment of regional lung volume in acute lung injury. Am J Respir Crit Care Med 1998;158(5):1644–1655. doi: 10.1164/ajrccm.158.5.9802003
32. Randtke MA, Andrews BP, Mach WJ. Pathophysiology and prevention of intraoperative atelectasis: A review of the literature. J PeriAnesthesia Nurs. 2015;30(6):516–27.
33. Ray K, Bodenham A, Paramasivam E. Pulmonary atelectasis in anaesthesia and critical care. Contin Educ Anaesth Crit Care Pain 2013;14(5):236–45. doi: 10.1093/bjaceaccp/mkt064
34. Chiumello D, Carlesso E, Cadringher P, et al. Lung stress and strain during mechanical ventilation for acute respiratory distress syndrome. Am J Respir Crit Care Med 2008;178(4):346–55. doi: 10.1164/rccm.200710-1589OC
35. Faffe DS, Zin WA. Lung parenchymal mechanics in health and disease. Physiol Rev 2009;89(3):759–775. doi: 10.1152/physrev.00019.2007
36. Fu Z, Costello ML, Tsukimoto K, et al. High lung volume increases stress failure in pulmonary capillaries. J Appl Physiol (1985) 1992;73(1):123–33. doi: 10.1152/jappl.1992.73.1.123
37. Birukov KG, Birukova AA, Dudek SM, et al. Shear stress-mediated cytoskeletal remodeling and cortactin translocation in pulmonary endothelial cells. Am J Respir Cell Mol Biol 2002;26(4):453–464. doi: 10.1165/ajrcmb.26.4.4725
38. Vlahakis NE, Hubmayr RD. Cellular stress failure in ventilator-injured lungs. Am J Respir Crit Care Med 2005;171(12):1328–42.
39. Amato MBP, Meade MO, Slutsky AS, et al. Driving pressure and survival in the acute respiratory distress syndrome. N Engl J Med 2015;372(8):747–755. doi: 10.1056/NEJMsa1410639
40. Feiner JR, Weiskopf RB. Evaluating pulmonary function: An assessment of PaO_2/FiO_2. Crit Care Med 2017;45(1):e40–8. doi: 10.1097/CCM.0000000000002017
41. Radermacher P, Maggiore SM, Mercat A. Fifty years of research in ARDS. Gas exchange in acute respiratory distress syndrome. Am J Respir Crit Care Med 2017;196(8):964–84. doi: 10.1164/rccm.201610-2156SO
42. Szakmany T, Heigl P, Molnar Z. Correlation between extravascular lung water and oxygenation in ALI/ARDS patients in septic shock: Possible role in the development of atelectasis? Anaesth Intensive Care 2004;32(2):196–201.
43. Bilek AM, Dee KC, Gaver DP,3rd. Mechanisms of surface-tension-induced epithelial cell damage in a model of pulmonary airway reopening. J Appl Physiol (1985) 2003;94(2):770–83.
44. Kay SS, Bilek AM, Dee KC, Gaver DP,3rd. Pressure gradient, not exposure duration, determines the extent of epithelial cell damage in a model of pulmonary airway reopening. J Appl Physiol (1985) 2004;97(1):269–76.
45. Oeckler RA, Hubmayr RD. Alveolar microstrain and the dark side of the lung. Crit Care 2007;11(6):177.
46. Protti A, Andreis DT, Monti M, et al. Lung stress and strain during mechanical ventilation: Any difference between statics and dynamics? Crit Care Med 2013;41(4):1046–55. doi: 10.1097/CCM.0b013e31827417a6
47. Zhao Z, Pulletz S, Frerichs I, Müller-Lisse U, Möller K. The EIT-based global inhomogeneity index is highly correlated with regional lung opening in patients with acute respiratory distress syndrome. BMC Res Notes 2014;7:82. doi: 10.1186/1756-0500-7-82
48. Güldner A, Braune A, Ball L, et al. Comparative effects of volutrauma and atelectrauma on lung inflammation in experimental acute respiratory distress syndrome. Crit Care Med 2016;44(9):e854–65. doi: 10.1097/CCM.0000000000001721
49. Cressoni M, Cadringher P, Chiurazzi C, et al. Lung inhomogeneity in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 2014;189(2):149–158. doi: 10.1164/rccm.201308-1567OC
50. Tschumperlin DJ, Margulies SS. Equibiaxial deformation-induced injury of alveolar epithelial cells in vitro. Am J Physiol 1998;275(6):1173. doi: 10.1152/ajplung.1998.275.6.L1173
51. Tschumperlin DJ, Oswari J, Margulies AS. Deformation-induced injury of alveolar epithelial cells. Effect of frequency, duration, and amplitude. Am J Respir Crit Care Med 2000;162(2 Pt 1):357–62. doi: 10.1164/ajrccm.162.2.9807003
52. Wirtz HR, Dobbs LG. Calcium mobilization and exocytosis after one mechanical stretch of lung epithelial cells. Science 1990;250(4985):1266–9. doi: 10.1126/science.2173861
53. Cohen TS, Cavanaugh KJ, Margulies SS. Frequency and peak stretch magnitude affect alveolar epithelial permeability. Eur Respir J 2008;32(4):854–61. doi: 10.1183/09031936.00141007
54. Grommes J, Soehnlein O. Contribution of neutrophils to acute lung injury. Mol Med 2011;17(3–4):293–307. doi: 10.2119/molmed.2010.00138
55. Derks CM, Jacobovitz-Derks D. Embolic pneumopathy induced by oleic acid. A systematic morphologic study. Am J Pathol 1977;87(1):143–58.
56. Motohiro A, Furukawa T, Yasumoto K, Inokuchi K. Mechanisms involved in acute lung edema induced in dogs by oleic acid. Eur Surg Res 1986;18(1):50–57. doi: 10.1159/000128505.
57. Bellani G, Laffey JG, Pham T, et al. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. JAMA 2016;315(8):788–800. doi: 10.1001/jama.2016.0291