Clinical Research

Effects of lung recruitment maneuver using mechanical ventilator in preterm infant microcirculation: a clinical trial

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pISSN: 0853-1773 • eISSN: 2252-8083
https://doi.org/10.13181/mji.oa.247472
Med J Indones. 2025;34:21–9

Received: February 27, 2024
Accepted: December 10, 2024

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ABSTRACT

BACKGROUND Preterm infants often require continuous positive airway pressure due to immature respiratory tracts. Bronchopulmonary dysplasia (BPD) manifests as prolonged oxygen dependence until 28 days of age and is classified into mild, moderate, or severe forms. The lung recruitment maneuver (LRM) aims to reopen collapsed alveoli, enhancing oxygenation during mechanical ventilation using the assist control volume guarantee mode (MV-AC/VG). This study aimed to evaluate the impact of LRM on alveolar and endothelial injuries, neonatal microcirculation, and its relation to BPD reduction or mortality in preterm infants.

METHODS This study was conducted from March 2021 to April 2022 at Cipto Mangunkusumo and Bunda Menteng Hospitals, Jakarta. The participants are <32 weeks infants with severe respiratory distress syndrome requiring MV-AC/VG, divided into LRM and control groups (n = 55 each). The alveolar injury was assessed using plasma surfactant protein-D (SP-D), endothelial injury by flow cytometry for endothelial microparticles ($CD\text{-}31^+/CD\text{-}42^-$), and neonatal microcirculation via transcutaneous-artery CO_2 gap ($TcPCO_2$-$PaCO_2$) and transcutaneous O_2 index ($TcPO_2/PaO_2$) measurements at 1 and 72 hours post-ventilation.

RESULTS LRM did not negatively affect preterm infants (24–32 weeks) undergoing invasive mechanical ventilation. At 72 hours, no significant differences were observed in alveolar (SP-D) and endothelial injury ($CD\text{-}31^+/CD\text{-}42^-$), nor in BPD reduction or mortality by 36 weeks.

CONCLUSIONS LRM is a beneficial intervention for enhancing respiratory support and microcirculation in preterm infants. Among survivors, LRM reduced the time to achieve the lowest FiO_2 (60.0 *versus* 435.0 hours, $p<0.0001$), shortened respiratory support duration (25.0 *versus* 36.83 days, $p = 0.044$), and improved TcO_2 index (1.00 *versus* 1.00, $p = 0.009$).

KEYWORDS bronchopulmonary dysplasia, endothelial cell, mechanical ventilation, platelet endothelial cell adhesion molecule-1, pulmonary surfactant-associated protein D

Bronchopulmonary dysplasia (BPD) is a significant morbidity associated with preterm birth. In infants with a gestational age of <32 weeks, BPD is defined as requiring respiratory support for >28 days, with severity classified based on the fraction of inspired oxygen (FiO_2): severe (FiO_2 >30%), moderate (FiO_2 >21–<30%), and mild (FiO_2 = 21%).[1,2] Survival outcomes for infants with BPD were followed up until they reached 36 weeks of corrected age or were discharged from the hospital. Brener Dik et al[3] found an incidence of BPD of 22% among patients managed with the assist control volume guarantee (AC/VG) ventilator mode.

Iskandar et al[4] showed a 12% prevalence of BPD among infants aged <32 weeks with moderate respiratory distress (Downes score, 4–5) who failed nasal continuous positive airway pressure and required mechanical ventilation. Additionally, Kaban et al[5] reported a 42.8% incidence of BPD in infants aged 25–32 weeks receiving oxygen therapy with FiO_2 30% and 40.5% in those with FiO_2 50%. No studies have been conducted on lung recruitment maneuvers (LRMs) in Indonesia. However, preliminary trials by Castoldi et al[6] suggest that LRM can be safely performed in infants managed with conventional ventilator modes, potentially reducing the incidence of BPD by 20% in those both at <27 weeks of gestation compared to standard therapy.

Invasive mechanical ventilation (IMV) can lead to lung injury within a short time, resulting in inflammation and surfactant dysfunction. LRM may safely reopen collapsed alveoli by gradually increasing the positive end-expiratory pressure (PEEP) along the pressure-volume curve. Castoldi et al[6] successfully implemented pilot studies using the mechanical ventilation using the assist control volume guarantee mode (MV-AC/VG) mode. The relationship between BPD and ventilator-induced lung injury (VILI) is significant.[7,8] Alveolar damage can trigger an upregulation of surfactant protein-D (SP-D) production, causing the surfactant to translocate into the circulation.[9] Dahmer et al[10] reported that increased SP-D plasma levels in pediatric patients with acute respiratory distress syndrome (RDS) receiving mechanical ventilation were associated with increased morbidity, mortality, and duration of ventilator support. Endothelial microparticle CD-31^+, known as platelet–endothelial cell adhesion molecule-1 (PECAM-1), is an adhesion molecule located at the junctions of adjacent endothelial cells.[11] The CD-$42b^-$ receptor is involved in interactions between endothelial and thrombocyte, serving as a receptor for von Willebrand factor. An increase in plasma CD-31^+/CD-42^- is a sign of endothelial injury and dysfunction.[12,13]

High tidal volume mechanical ventilation can disrupt endothelial junctions, releasing CD-31^+ into circulation.[14] The more quickly the newborn's lungs inflate, the better the pulmonary and systemic blood flow, while alveolar inflation reduces pulmonary vascular resistance (PVR). As infants begin to breathe, arterial oxygen levels increase, thereby enhancing cardiac contractility and output.[15,16] Adequate oxygen delivery is essential to meet cellular oxygen demand.[17] When mismatches occur, the microcirculation responds first, often prioritizing vital organs, such as the brain, heart, and adrenal gland, over the skin, which can lead to decreased skin oxygenation and increased carbon dioxide levels in the skin.[18] Anaerobic metabolism in skin cells produces lactic acidosis and decreases strong ion difference (SID).[6,19] This study aimed to investigate the correlation between LRM and reduction in BPD or mortality, and its effects on alveolar and endothelial injuries and microcirculation in preterm infants using the MV-AC/VG mode.

METHODS

Research design and subject participants

This study is a phase II clinical trial utilizing the randomized controlled trial methods with double-blinding, registered under the clinical trial number: NCT04555889. Only the research team knew which participants received the LRM experimental intervention. We planned a minimum sample size of 110 patients. The inclusion criteria included infants with gestational age between 24–32 weeks, severe RDS, birth weight >600 g, and requiring MV-AC/VG mode within <48 hours of age. The diagnosis of BPD in these infants required observation of whether respiratory support was utilized up to 28 days of chronological age, after which it was categorized as mild, moderate, or severe.

This study was conducted from March 2021 to April 2022 at Cipto Mangunkusumo and Bunda Hospitals, Jakarta. Of the 166 patients eligible for our study, 56 were excluded due to equipment unavailability, metabolic abnormalities at birth, or dropout. A total of 110 patients were randomly assigned to either the LRM group or the control group, with 55 patients in each group (Figure 1).

Study intervention and preparation

Upon identifying the participants and obtaining approval from their guardians, LRM was performed by an assisting researcher. The procedure involved intubating the infant and using MV-AC/VG mode with Dräger Babylog® VN-500 (Drägerwerk AG & Co. KGaA, Germany) with an initial PEEP setting of 5 cmH_2O, a tidal volume expansion target of 5 ml/KgBW, a backup rate of 50 breaths/min, and an inspiration time of 0.4 sec. The PEEP was increased by 0.2 cmH_2O every 3 min while monitoring pre-ductal oxygen saturation (SpO_2)

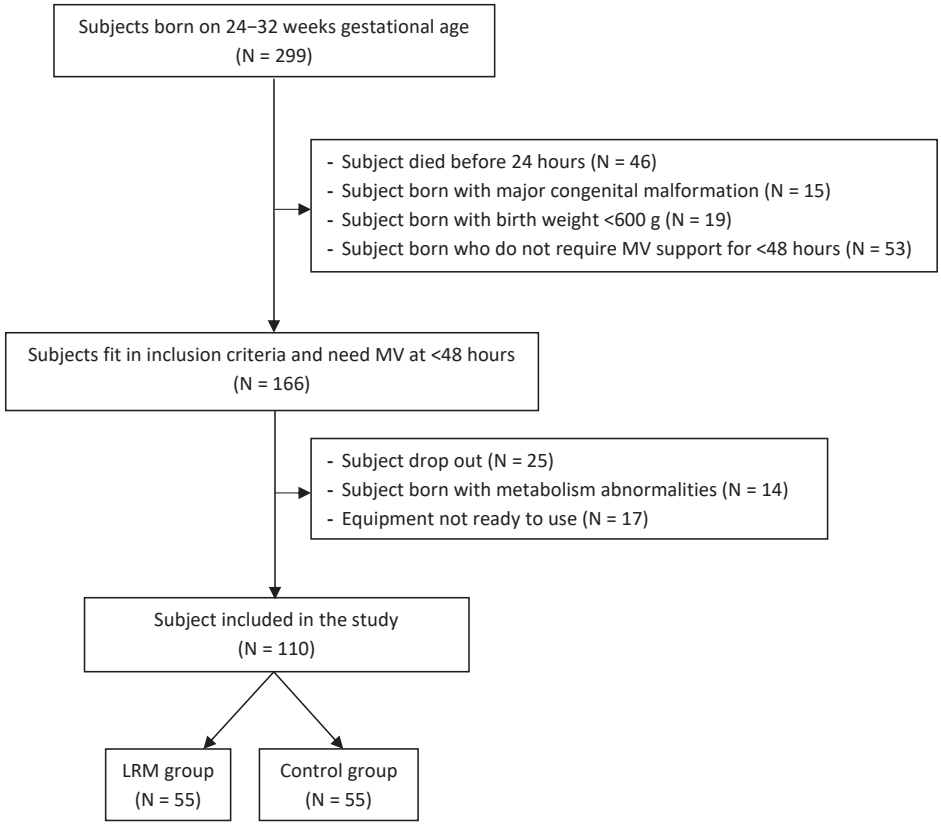

Figure 1. Participant flow diagram. LRM=lung recruitment maneuver; MV=mechanical ventilation

using Masimo Rad-5® (9196) (Masimo Corporation, Switzerland) neonatal pulse oximeter. If SpO_2 did not increase, PEEP gradually increased further. FiO_2 was reduced by 5% when SpO_2 was >95%. PEEP did not increase further (opening point) if FiO_2 was <21%, if two consecutive PEEP increments failed to lower FiO_2 further, or if infants began to desaturate (SpO_2 <85%). Following the opening point, PEEP was gradually decreased by 0.2 cmH_2O every 3 min. We stopped decreasing the PEEP any further if desaturation occurred or if a higher FiO_2 was needed to maintain SpO_2 >90%. Finally, PEEP was set at the opening point for 3 min, followed by a reduction of 0.2 cmH_2O above the closing point (optimal point). The participants in the control group did not undergo LRM.

Blood samples were collected from both groups to measure several secondary outcomes: SP-D levels assessed using the enzyme-linked immunosorbent assay method; endothelial microparticle ($CD\text{-}31^+/CD\text{-}42^-$) measured by flow cytometry; transcutaneous CO_2 gaps ($TcPCO_2$-$PaCO_2$); and transcutaneous O_2 index ($TcPO_2/PaO_2$) using transcutaneous Sentec® digital monitoring SW-V08.03 (Sentec AG, Switzerland); and arterial blood gas and SID using the Siemens RAPIDPoint® 500e blood gas analyzer machine (Siemens Healthineers, Germany).

Time and subject measurement

Variables in this study were categorized into independent and dependent variables. The independent variables included infants receiving mechanical ventilation with LRM, while the dependent variables included BPD survival rate, the effect of LRM on SP-D, $CD\text{-}31^+/CD\text{-}42^-$(PECAM), and microcirculation.

Over the 13-month study period, dependent variables were measured at two points: within 1 hour of intubation and mechanical ventilation initiation, and again at 72 hours of age. Data were analyzed using intention-to-treat (ITT) and per-protocol analyses. In the ITT analysis, participants who died before 28 days of age were considered as having BPD. Per-protocol analysis was conducted for other secondary outcomes. Population characteristics are described using descriptive statistics presented in the tables and text. Normally distributed data were analyzed using mean and standard deviation, while non-normally distributed data were assessed using median and interquartile range. The sample sizes for endothelial microparticle variables were calculated based on the formula for the difference in means between the two unmatched groups. For BPD occurrence, 50 participants were included; for SP-D level, 51 participants; for endothelial microparticle variable, 28

participants; and for the difference between $TcPCO_2$ and $PaCO_2$, 55 participants. We selected a maximum sample size of 55 infants per group to ensure significant findings across all outcomes. To anticipate potential dropouts, the sample size was increased to 61 for each group, with a total of 122 infants. Nominal data proportions were analyzed using the chi-square test or Fisher's exact test. Normally distributed numerical data were analyzed using an independent *t*-test, and non-normally distributed data were analyzed using the Mann–Whitney *U* test.

Outcome

All subjects were monitored for side effects of the LRM and underwent clinical and hemodynamic assessments. We followed BPD survival outcomes until 36 weeks of corrected age or discharge from the hospital, using the BPD classification system (mild, moderate, and severe). Secondary outcomes were measured twice, at 1 hour of age and 72 hours of age, to observe participants' responses to the LRM. The effect of the LRM on microcirculation was assessed after collecting blood samples from all subjects. The samples were grouped according to the time of collection (1 hour of age or 72 hours of age) and stored at –80°C.

RESULTS

Characteristics of subjects

The incidence of deliveries at 24–32 weeks of gestational age in Cipto Mangunkusumo and Bunda Menteng Hospitals was 19%. Among these, approximately 55% had severe RDS and required IMV in AC/VG mode. This study indicated that only 32% of the participants received a complete dose of antenatal steroids. Additionally, 33% of the participants were born to mothers with premature rupture of membranes lasting >18 hours, and 74% of the participants were delivered via cesarean section. The median APGAR score at 1 min was 4. Approximately 86% of the participants required intubation in the delivery room, all of which contributed to the need for IMV. More detailed demographic data can be seen in Table 1.

Ventilation parameter related to LRM

There was no statistically significant difference in the initial FiO_2 or FiO_2 at 72 hours after mechanical

Table 1. Characteristics of subjects

Characteristics	LRM (n = 55)	Control (n = 55)	*p*
Sex, n (%)			1.00*
Male	31 (56)	31 (56)	
Female	24 (44)	24 (44)	
Hypertension in pregnancy (mother), n (%)	11 (20)	13 (24)	0.760*
DM in pregnancy (mother), n (%)*	3 (5)	3 (5)	1.00*
Clinical chorioamnionitis, n (%)	1 (2)	0 (0)	1.00*
PROM >18 hours, n (%)	19 (35)	17 (31)	0.515*
Mode of delivery, n (%)			0.279*
Normal delivery	12 (22)	17 (31)	
Cesarean section	43 (78)	38 (69)	
Multiple pregnancy, (mother) n (%)	9 (16)	5 (9)	0.252*
Antenatal steroid use, (mother) n (%)			0.202*
Never	20 (36)	19 (35)	
Incomplete	14 (25)	22 (40)	
Complete	21 (38)	14 (25)	
Birth weight (g), median (IQR)/mean (SD)	1,010 (890–1,325)	1,120.7 (331.3)	0.729†
Gestational age (weeks), median (IQR)	29 (28–3)	28 (27–3)	0.210‡
Weight <1,000 g, n (%)			0.252*
Gestational age <28 weeks	14 (25)	20 (36)	
Gestational age ≥28–<32 weeks	41 (75)	35 (64)	
SGA, n (%)			**0.022***
Yes	11 (20)	3 (5)	
No	44 (80)	52 (95)	
APGAR score, median (IQR)			
APGAR 1st min	4 (2–8)	4 (1–8)	0.413†
APGAR 5th min	8 (4–9)	8 (4–9)	0.838†
Resuscitation, n (%)			0.219*
Chest compression/ intubation	50 (91)	45 (82)	
CPAP/NIPPV	5 (9)	10 (18)	
Radiologic diagnosis, n (%)*			0.081*
RDS 1–2	36 (65)	39 (71)	
RDS 3–4	7 (13)	5 (9)	
Pneumonia or others	12 (22)	11 (20)	

CPAP=continuous positive airway pressure; DM=diabetes mellitus; IQR=interquartile range; LRM=lung recruitment maneuver; NIPPV=non invasive positive pressure ventilation; PROM=premature rupture of membrane; RDS=respiratory distress syndrome; SD=standard deviation; SGA=small for gestational age
*Chi-square test; †Fisher's test; ‡Mann–Whitney *U* test
Others are atelectasis and pneumothorax

Table 2. LRM vs. control outcome

Variables	1 hour		*p*	72 hours		*p**
	LRM (n = 55)	Control (n = 55)		LRM (n = 52)	Control (n = 50)	
SP-D (ng/dl), median (IQR)	10.90 (4.17–21.11)	11.12 (6.48–19.06)	0.793	6.94 (0.24–68.81)	5.72 (2.28–15.61)	0.923
CD–31$^+$/CD–42b$^-$, median (IQR)	252.72 (143.18–393.42)	243.00 (132.54–587.25)	0.718	326.38 (228.47–486.00)	356.54 (147.03–598.38)	0.901
$TcPCO_2$-$PaCO_2$ (mmHg), median (IQR)	0.20 (−2.20–4.20)	0.90 (−1.00–5.30)	0.379	1.05 (0–3.25)	1.45 (−0.02–6.10)	0.322
$TcPO_2$ index (mmHg), median (IQR)	1.00 (0.99–1.00)	1.00 (0.99–1.00)	0.596	1.00 (1.00–1.02)	1.00 (0.99–1.00)	**0.009**
SID (mEq/l), median (IQR)	33.34 (25.02–39.99)	33.64 (26.69–37.64)	0.881	33.77 (30.23–41.32)	29.70 (18.03–38.26)	0.112

IQR=interquartile range; LRM=lung recruitment maneuver; SID=strong ion difference; SP-D=surfactant protein-D; $TcPCO_2$-$PaCO_2$=gap between transcutaneous partial pressure of carbon dioxide ($TcPCO_2$) and arterial partial pressure of carbon dioxide ($PaCO_2$); $TcPCO_2$=transcutaneous partial pressure of oxygen
*Mann–Whitney test

Table 3. Outcome at 36 weeks corrected age

Variables	LRM (n = 55)	Control (n = 55)	95% CI	*p*
Death, n (%)	26 (47)	27 (49)	0.141–0.295	0.216*
Survival with BPD, n (%)				
Mild to moderate BPD	6 (11)	13 (24)		
Severe BPD	6 (11)	3 (5)		
Survival without BPD, n (%)	17 (31)	12 (22)		
Time to reach lowest FiO_2 (min), median (IQR)	60.0 (54.0–75.0)	435.0 (375.0–495.0)	0–0.027	**<0.0001**†
Age of extubate (days), median (IQR)	4.00 (2–7)	4.00 (2–7.8)	0.684–0.843	0.733*
Length of use MV (days), median (IQR)	25.0 (19.00–37.00)	36.83 (11–19)	0.012–0.097	**0.044***

BPD=bronchopulmonary dysplasia; CI=confidence interval; FiO_2=fraction of inspired oxygen; IQR=interquartile range; LRM=lung recruitment maneuver; MV=mechanical ventilation
*Chi-square test; †Mann–Whitney testo

ventilation between the LRM and control groups (36.0 [35.0; 40.0] *versus* 38.0 [35.0; 40.0], p = 0.599; 21.0 [21.0; 25.0] *versus* 21.0 [2.0; 22.0], p = 0.151). This study observed statistically significant PEEP values. The opening PEEP was 9.44 (2.0) *versus* 5.0, p = 0.0001; the closing PEEP was 4.66 (0.66) *versus* 5.0, p = 0.0001; and the optimal PEEP was 5.44 (0.64) *versus* 5.0, p<0.001.

LRM outcome

Comparative analyses of various physiological parameters between the LRM and control groups at 1 hour and 72 hours after the intervention are presented in Table 2. Statistical analysis of the $TcPO_2$ index at 72 hours shows that the LRM group has a median of 1.00 (1.00–1.02), while the control group has a median of 1.00 (0.99–1.00). The *p*-value was 0.009, suggesting a statistically significant difference. For other variables, including SP-D, CD–31$^+$/CD–42b$^-$, TcPCO2-PaCO2, and SID, there are no significant differences between the LRM and control groups at either 1 hour or 72 hours.

Outcome at 36 weeks corrected age

LRM significantly reduced the time to reach the lowest FiO_2 (p<0.0001, 95% confidence interval [CI]: 0–0.027) and decreased the length of respiratory support (p = 0.044, 95% CI: 0.012–0.097) (Table 3). The results of our study showed that BPD and mortality

Table 4. Outcome at 1 vs. 72 hours after mechanical ventilator was initiated

Variables	LRM		*p*	Control		*p*
	1 hour (n = 55)	72 hours (n = 52)		1 hour (n = 55)	72 hours (n = 50)	
SP-D (ng/dl), median (IQR)	10.9 (4.17–21.11)	7.36 (1.51–15.30)	**<0.0001***	11.12 (6.482–19.062)	5.99 (2.338–15.381)	**<0.0001***
CD–31$^+$/CD–42b$^-$, median (IQR)	252.72 (143.18–393.42)	328.76 (229.45–489.62)	**0.049**†	243.00 (132.545–587.250)	356.40 (141.750–562.736)	0.978†
$TcPCO_2$-$PaCO_2$ (mmHg), median (IQR)	0.20 (−2.20–4.20)	1.05 (0–3.25)	0.831†	0.90 (−1.00–5.300)	1.45 (−0.025–6.100)	0.313†
$TcPO_2$ index (mmHg), median (IQR)	1.00 (0.997–1.004)	1.00 (1.00–1.021)	0.894†	1.00 (0.993–1.0022)	1.00 (0.997–1.0001)	**0.047**†
SID (mEq/l), median (IQR)	32.33 (23.960–38.750)	32.84 (28.995–40.285)	0.569†	32.70 (25.490–36.800)	29.70 (18.035–38.262)	**0.047**†

IQR=interquartile range; LRM=lung recruitment maneuver; SID=strong ion difference; SP-D=surfactant protein-D; $TcPCO_2$-$PaCO_2$=gap between transcutaneous partial pressure of carbon dioxide ($TcPCO_2$) and arterial partial pressure of carbon dioxide ($PaCO_2$); $TcPCO_2$=transcutaneous partial pressure of oxygen
*Independent *t*-test; †Mann–Whitney test

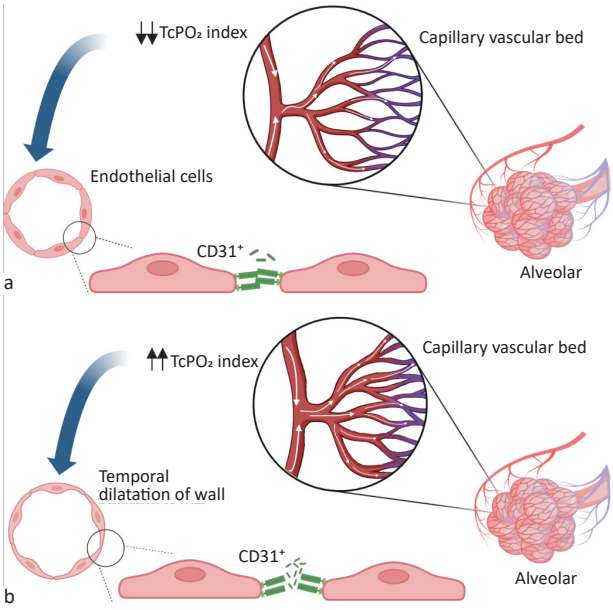

Figure 2. Effect of LRM in microcirculation and endothelial microparticle CD-31$^+$/CD-42$^-$ in control group (a) and LRM group (b). LRM=lung recruitment maneuver; $TcPO_2$=transcutaneous partial pressure of oxygen

were not statistically significant and that the number of subjects experiencing BPD (mild-to-moderate or severe) or mortality did not differ between the LRM and control groups.

Outcome at 1 hour *versus* 72 hours after mechanical ventilator was initiated

Table 4 shows the outcomes of intubation. Both the LRM and control groups showed a statistically significant decrease in SP-D levels from 1 hour to 72 hours (p<0.0001). However, the CD–31$^+$/CD–42b$^-$ marker only showed a statistically significant increase in the LRM group, with p = 0.049. The $TcPO_2$ index in the control group showed a statistically significant increase, with p = 0.047. Similarly, the SID in the control group showed a statistically significant decrease with p = 0.047.

DISCUSSION

Based on the sample subjects, many infants born at <32 weeks of gestation age, with a birth weight <1,000 g, incomplete antenatal steroid administration, and born with a 1 min APGAR score of 4, presented with respiratory distress. Factors including gestational age, birth weight, delivery method, and the history of newborn resuscitation were related to the use of IMV.[20,21] Antenatal steroids could upregulate endogenous surfactant production and reduce the severity of RDS.[22] Risk factors for asphyxia[23] are also present in our sample subject, shown by the median APGAR score at 1 min, which was 4.

BPD remains a significant concern in preterm infants born with respiratory distress, particularly those requiring mechanical ventilation and surfactant therapy. Currently, no perfect criteria exist for diagnosing BPD. Conditions other than lung injury, such as apnea, prematurity, laryngomalacia, and necrotic enterocolitis, could provide respiratory support for more than 28 days. This raises the possibility that our criteria for diagnosing BPD may not be sensitive enough

to detect chronic lung injury effectively. In our study, no significant difference existed in BPD survival among participants at 36 weeks of corrected age, consistent with the findings of Castoldi et al[6] and Wu et al.[15] We have some logical explanations for this finding. First, the combination of positive pressure ventilation (PPV) with T-piece resuscitator, early surfactant instillation, and IMV in AC/VG mode creates lung functional residual capacity (FRC) in the control group similar to that in the LRM group, thus minimalizing lung injury in both groups.[24–26] Additionally, while LRM accelerates lung expansion and creates lung FRC quickly, lung FRC is still created gradually in the control group despite having a slower pace. We suspect that the VILI will be similar if the optimal lung FRC is achieved, regardless of the time taken. However, other factors unrelated to VILI, such as micronutrient deficiency, oxidative stress, and sepsis, can also contribute to chronic lung disease. To achieve optimal lung FRC, PEEP should be maintained at 4.6 and 9.6 cmH_2O. LRM reduces the time required to reach the optimal lung FRC. If LRM cannot be performed for any reason, a PEEP of 5.4 cmH_2O will be enough to make optimal lung FRC in 72 hours. A previous animal study concluded that PPV with PEEP resulted in gas exchange (tidal volume) occurring during 100% of the breathing period, compared to only 30–50% without PEEP.[23,24] Although it was not as fast as LRM, all participants were administered PEEP upon intubation, ensuring optimal gas exchange in both groups.

LRM aims to open collapsed alveoli, potentially reducing lung injury and inflammation. The inflammatory process resulting from lung injury induces alveolar type II cells to produce more SP-D.[9] Animal studies have shown an increase in plasma messenger ribonucleic acid expression of SP-D and a decrease in its level in the alveoli.[27] Mechanical ventilation increases gene expression and SP-D production.[28] Although not statistically significant, SP-D plasma levels in the LRM group at 72 hours tended to be higher than those in the control group. This may be due to a higher proportion of small for gestational age (SGA) infants in the LRM group. Briana et al[29] found that SP-D levels in SGA preterm infants were higher than those in infants appropriate for gestational age preterm (18.16 ng/ml; 95% CI: 6.86–29.4; p = 0.002). SGA preterm infants experience more intrauterine stress, leading to increased cortisol production by their adrenal glands, which in turn induce type II pneumocyte cells to express more SP-D.[24] Similar findings were observed in an animal study conducted by Fandiño et al,[30] where more integrity disruption of the air-fluid barrier was found in preterm SGA alveoli.

The microvasculature of the lung endothelium is often exposed to shear stress and cyclic stretch, which vary according to breathing patterns and heart rate, ranging from 10 to 50 dyn/cm^2 in pulmonary arteries to 50–20 dyn/cm^2 in pulmonary microvasculature.[31] Dagenais et al[32] showed that stretching in the alveolar-endothelial barrier triggers a wave of Ca^{2+} influx through transient receptor potential voltage 4, resulting in abnormalities in intracellular signaling activity. Although not statistically significant, the median CD-31$^+$ level in the control group was lower than in the LRP group 1 hour after ventilation. Conversely, the opposite trend was observed at 72 hours. These findings aligned with Dodson et al,[33] who found that lambs experiencing intrauterine growth restriction exhibited decreased nuclear factor kappa-light-chain-enhancer of activated B cell signal expression in their pulmonary arterioles, leading to endothelial dysfunction.

In our study, within 72 hours of mechanical ventilation, a significant increase in CD-31$^+$ levels was observed in the LRM group but not in the control group. This finding can be explained as follows: LRM increases lung compliance faster by increasing alveolar diameter, resulting in reduced PVR and increased arterial pulmonary blood flow. Consequently, endothelial shear and stretch stresses are increased. This phenomenon is consistent with the findings of Vitvitsky et al,[34] who reported that premature pigs with ligated right pulmonary arteries experienced a loss of vasodilation ability in their left pulmonary arteries due to endothelial dysfunction.

Several studies have noted that comparing the $TcPO_2$ index and the difference in $TcPCO_2$ to arterial gas ($TcPCO_2$ gap) is highly dependent on skin circulation disorders. Vallée et al[35] performed transcutaneous ear blood gas measurements and found that $TcPCO_2$ gaps were higher in patients experiencing shock than in healthy participants (14.8 [12.6] *versus* 6 [2.7] mmHg; p<0.0001). Notably, $TcPCO_2$-$PaCO_2$ values >16 mmHg correlated with poorer outcomes and increased mortality. Mari et al[36] attempted to measure the $TcPO_2$ index across various circulatory disorders and reported values of 0.79 (12) mmHg under normal conditions (cardiac index >2.2 l/min/m^2), 0.48 (0.07) mmHg in moderate circulatory disorders (cardiac index 1.5–2.2 l/min/m^2), and 0.12 (0.12) mmHg during severe shock (cardiac index <1.5 l/min/m^2).

This study found no significant difference in the transcutaneous partial pressure of carbon dioxide ($TcCO_2$) gap between the LRM and control groups. However, 72 hours after mechanical ventilation initiation showed a significant difference, where the control group exhibited a decrease in the $TcCO_2$ gap, whereas the LRM group revealed an increase. These findings suggest that microcirculation improved in the LRM group but reduced in the control group. Several factors may explain these findings: (a) LRM rapidly increases lung FRC, enhancing oxygen uptake from the alveoli and raising oxygen levels in arterial blood; (b) the LRM group revealed dilation of microcirculation blood vessels, while the control group revealed a decrease (Figure 2); and (c) despite the decrease in microcirculation of the control group at 72 hours, capillary function remained sufficient to transport CO_2 products to be excreted by the lungs. This adequacy was reflected in the absence of an overall difference in the $TcCO_2$ gap between the groups.

Meza et al[37] observed a decrease in the ability of arteriolar endothelial vasodilation in CD-31$^+$/CD-42$^-$ gene deletion mice when given increased endothelial stretch from fluid shear stress due to a decrease in endogenous nitric oxide (NO) production. This finding aligns with our study, where CD-31$^+$/CD-42$^-$ microparticle levels were higher in the LRM group than in the control group at 72 hours. We hypothesize that LRM is associated with increased exhaled NO levels, enhanced skin arteriolar vasodilatation, and physiological shear stress due to increased microcirculation.

Based on these findings, this study attempted to develop a new theory regarding LRM in premature infants. The pulmonary recruitment maneuver quickly creates lung FRC, increases cardiac output, and improves microcirculation, thereby enhancing the tensile and tearing forces of the microvascular endothelium. In response, endothelial cells produce more CD-31$^+$ adhesion molecules to maintain the endothelial wall integrity. Notably, the stretching forces exerted on the endothelial wall are insufficient to disrupt the endothelial junction.[38,39] For more details, see Figure 2.

Based on our findings, we propose a mechanism related to LRM in preterm infants (aged <32 weeks gestation) receiving mechanical ventilation in AC/VG mode. LMR increases oxygen delivery to peripheral organs, which may increase skin microcirculation. Higher microcirculation may elevate endothelial strain as fluid shear stress. To maintain vascular integrity, endothelial cells express more adhesion molecule CD-31$^+$, releasing CD-31$^+$ into circulation.

However, this study has certain limitations: (1) the possibility of subjective bias, as it was a randomized clinical trial rather than a double-blind study. We attempted to minimize this by ensuring that no researchers intervened in the clinical management of the participants, leaving responsibility to the attending physicians; (2) early targeted treatment management, which involves administering paracetamol or ibuprofen promptly upon significant echocardiographic evidence of patent ductus arteriosus within first <24 hours of life, might have influenced the speed of ductal closure in both groups; (3) despite needing 120 participants for the minimum sample size, only 110 were successfully recruited, potentially diminishing the statistical power of the findings; and (4) eight patients died before the age of 3 days, preventing the collection objective measurements at 72 hours post-initiation of respiratory support devices.

In conclusion, this study examined the impact of LRM on respiratory outcomes in preterm infants born at <32 weeks of gestational age, many of whom were at high risk due to low birth weight, low APGAR scores, and incomplete antenatal steroid administration. LRM was associated with improved lung function and microcirculation, as shown by the increased levels of CD-31$^+$ adhesion molecules, which may help maintain endothelial integrity under mechanical ventilation. While both LRM and standard treatments achieved lung FRC, LRM was faster, potentially reducing lung injury and inflammation. Additionally, LRM may enhance oxygenation and NO levels, further supporting microcirculation and endothelial health. These findings suggest that LRM could benefit preterm infants by enhancing lung expansion, microcirculation, and endothelial resilience. Further research is recommended to confirm these findings and refine LRM techniques to better support the respiratory health of preterm infants.

Conflict of Interest

The authors affirm no conflict of interest in this study.

Acknowledgment

We would like to thank all the patients and their families who participated in this study and placed their trust in our research team. We are also grateful to the clinical staff and healthcare professionals who assisted in patient recruitment, data collection, and laboratory analysis.

Funding Sources
None.

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