

The Effects of Protective Ventilation on the Production of Endogenous Melatonin and Prognosis in Patients Undergoing Esophageal Cancer Surgery: A Prospective Randomized Double-Blind Controlled Study

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Research article

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Abstract

Background: Exogenous melatonin exerts a similar effect to protective ventilation on attenuating ventilator-induced lung injury (VILI) by inhibiting NLRP3 inflammasome activation in mouse model. However, the effect of protective ventilation on the production of endogenous melatonin and prognosis in patients undergoing esophageal cancer surgery remains unknown. In this study, we aimed to reveal the effects of protective ventilation on the production of endogenous melatonin, interleukin (IL)-1 β , IL-18 and major complications in patients undergoing esophageal cancer surgery.

Methods: Eight-eight patients were randomized to receive “conventional” ventilation ($V_t=10$ mL/kg) or lung protective ventilation [$V_t=5$ mL/kg along with 5 cm of H₂O positive end-expiratory pressure (PEEP)]. IL-1 β , IL-18 and melatonin levels in bronchoalveolar lavage fluid (BALF) and serum were measured. Respiratory variables and outcomes were evaluated.

Results: Lung protective ventilation decreased the peak airway pressure (P_{peak}), plateau airway pressure (P_{plat}) and driving pressure (ΔP) compared with the “conventional” ventilation group. Lung protective ventilation inhibited polymorphonuclear (PMN) cells invasion into the BALF ($P=0.000$). Likewise, lung protective ventilation suppressed alveolar and serum IL-1 β and IL-18 secretion after mechanical ventilation. Furthermore, lung protective ventilation resulted in a decrease in the inhibition of endogenous MT production compared to “conventional” ventilation ($P=0.000$). In addition, lung protective ventilation reduced the incidence of postoperative pulmonary complications ($P=0.04$) and the rate of major postoperative complications ($P=0.023$).

Conclusions: Taken together, lung protective ventilation for esophageal cancer surgery suppressed the secretion of IL-1 β , IL-18 and restored the endogenous melatonin level. Meanwhile, lung protective ventilation improved postoperative outcomes after esophageal cancer surgery.

Trial registration: The Chinese Clinical Trial Registry, ChiCTR1900026190. Registered 25 September 2019, <http://www.chictr.org.cn/edit.aspx?pid=34677&htm=4>

Background

One-lung ventilation (OLV) is required for esophageal cancer and can contribute to the surgical field [1]. However, inappropriate ventilation modes may cause or augment acute lung injury, which is known as ventilator-induced lung injury (VILI) [2]. Lung protective ventilation [low tidal volume + positive end-expiratory pressure (PEEP)] was shown to achieve good clinical effects and protect against VILI [3, 4]. Furthermore, clinical studies have demonstrated that lung protective ventilation induced an immune response with lower concentrations of inflammatory mediators than that of “conventional” ventilation [5]. Therefore, further studies of the effect of lung protective ventilation on the pulmonary immune response are essential to prevent VILI.

Increasing studies have shown that OLV may lead to proinflammatory cytokine release and inflammatory signaling pathway activation [5–8]. Overdistension in ventilated lungs followed by compression of alveolar vessels initiates a robust release of proinflammatory cytokines, such as interleukin (IL)-6, IL-8 and tumor necrosis factor (TNF)- α , in bronchoalveolar lavage fluid (BALF) [5, 9]. These proinflammatory cytokines are important chemotactic factors for polymorphonuclear (PMN) cells [10]. Excessive PMN cell aggregation will amplify the inflammatory cascade. Furthermore, a recent study showed that in mouse alveolar macrophages, Nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome activation contributes to the development of VILI [11].

Melatonin (N-acetyl-5-methoxytryptamine, MT), which is mainly secreted in the pineal gland, has well-documented anti-inflammatory and immunomodulatory functions [12, 13]. Early preliminary studies have shown that exogenous MT ameliorates VILI by increasing the anti-inflammatory response [14]. Recently, Zhang et al. demonstrated that exogenous MT inhibited NLRP3 inflammasome activation in mice with acute lung injury [15]. However, researchers have not determined whether lung protective ventilation affects NLRP3 inflammasome-related inflammatory cytokine and endogenous melatonin production in patients.

Our study aimed to investigate the effects of lung protective ventilation on NLRP3 inflammasome-related inflammatory cytokine and endogenous MT secretion in patients undergoing video-assisted thoracoscopic esophagectomy (VATS). In addition, the effect of lung protective ventilation on postoperative complications was also investigated.

Materials And Methods

Study Design

Patients scheduled for elective VATS at the First Affiliated Hospital of Anhui Medical University (Anhui, China) were included in the study. The study protocol had received prior approval from the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (No. 20190385), and this trial was registered in the Chinese Clinical Trial Registry (No. ChiCTR1900026190). Before participation in the study, all patients provided informed consent.

Study Population

Patients with esophageal cancer who were treated at our hospital were considered for enrollment. The inclusion criteria were as follows: American Society of Anesthesiologists (ASA) physical status I–II, requirement for OLV during operation, and aged 45–77 years. Exclusion criteria were preexisting hypoxemia, diagnosed major obstructive or restrictive pulmonary disease [preoperative forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) <70% of the predicted value], pulmonary infection before surgery, body mass index (BMI) of less than 20 or more than 35, and use of immune modulators.

Randomization and Blinding

The randomized numbers were generated by a research coordinator using block sizes on a 1:1 ratio. This ensured that each group had an equal number of subjects. Then, the research coordinator sealed the numbers in opaque envelopes. Before mechanical ventilation, the anesthesia assistant opened the envelopes, set the breathing parameters and covered the breathing parameters using opaque paper. The anesthesia assistant did not participate in the next study. One anesthesiologist collected the specimens, and another anesthesiologist recorded the breathing parameters. Both physicians were blinded to the allocation.

Study Protocol

Standard monitoring devices were applied after admission to the operating room. Before induction of anesthesia, an artery catheter was inserted into the left radial artery. Anesthesia induction was performed with 2.0-2.5 mg/kg propofol, 0.02-0.06 mg/kg midazolam, 0.4-0.6 µg/kg sufentanil and 0.6-0.9 mg/kg rocuronium. 3 min after assisted breathing, a double-lumen endotracheal tube (Broncho-Cath® 35 F or 37 F; Covidien, Ireland) was inserted into the left main bronchus. Anesthesia was maintained with 50–100 µg/kg/min of propofol, 0.1–1 µg/kg/min of remifentanil and 5.0–10.0 µg/kg/min of rocuronium to maintain the proper depth of anesthesia (BIS 40–60). A forced-air warming system (3M Company, Shanghai, China) was used to keep the patients warm.

After intubation, the patients were randomly divided into 2 groups. In the control group (group A), patients received volume controlled mechanical ventilation with a tidal volume of 10 mL/kg of ideal body weight (IBW). In the lung protective ventilation group (group B), lung protective ventilation with a low tidal volume ($V_t=5$ mL/kg IBW) and 5 cm H₂O PEEP was chosen. After 15 minutes, all patients were turned to the left lateral position and OLV was initiated. During OLV, the ventilation mode was not changed except the plateau airway pressure (P_{plat}) exceeded 30 cmH₂O. If the P_{plat} exceeded 30 cmH₂O, the tidal volume was decreased and this patient discontinued the experiment. With both two-lung ventilation (TLV) and OLV, mechanical ventilation was performed with an inspiratory to expiratory ratio of 1:2, and an appropriate respiratory rate to maintain an end-tidal CO₂ (ETCO₂) below 45 mmHg. All surgeries were always performed at 8:30 in the morning.

Observational Indexes

Peak airway pressure (P_{peak}), P_{plat}, respiratory rate and blood gas analyses were evaluated at two stages: during TLV before surgery and 30 minutes after OLV. Furthermore, the driving pressure (ΔP) was recorded. ΔP was defined and calculated as follows: $\Delta P=P_{plat}-PEEP$ [16].

The major postoperative complications were pulmonary complications and nonpulmonary complications. The pulmonary complications included pulmonary infection, acute lung injury or acute

respiratory distress syndrome and reintubation or invasive mechanical ventilation. Nonpulmonary complications included anastomotic fistula, incision infection, ICU stay and death before hospital discharge.

Bronchoalveolar lavage was performed after induction of general anesthesia (baseline) and at the end of the surgical procedures. BALF was aspirated from the lung after instillation of 20 mL of sterile isotonic saline. Then, the recovered BALF was centrifuged at 700 g for 10 minutes at 4°C, and the supernatant was stored at -80°C. The cell pellets were resuspended in ice-cold sterile isotonic saline for staining and counting.

Blood samples were obtained during TLV after induction of general anesthesia (baseline) and at the end of the surgical procedures. Five milliliters of arterial blood samples was centrifuged at 800 g for 5 minutes. The upper serum phase was separated and stored at -80°C.

MT, IL-18 and IL-1 β concentrations in the serum and BALF were determined using commercial ELISA kits (Cusabio, Wuhan, China). We performed the assays according to the manufacturer's instructions. The limitations for MT, IL-18 and IL-1 β were 0.1 pg/mL, 7.8 pg/mL and 2.2 pg/mL, respectively.

Statistical Analysis

According to previous studies, the cell numbers in the BALF increased by more than 30% after OLV [5], which required 12 patients per group with $\alpha=0.05$ and $\beta=0.02$; thus, we aimed to enroll 88 patients to allow for dropouts. The sample size was calculated using PASS 11.0 software.

Data are presented as the mean \pm SD or number of patients (proportion, %). The independent-samples t-test or paired-samples t-test were used to analyze normally distributed data. Non-normally distributed data were analyzed by chi-square tests or Fisher's exact test. All statistical analyses were performed with SPSS 19, and a P value of < 0.05 was considered significant.

Results

Baseline Parameters of Patients

88 patients were included and assessed. Four patients did not meet the criteria, and 84 were included in this study. However, two patients withdrew for technical reasons, and the other patient were excluded for higher Pplat. Finally, 81 patients completed the study (Fig. 1). The patient characteristics and preoperative details showed no significant differences between the two groups (Table 1).

Table 1
Baseline parameters of patients

	Group A (n = 40)	Group B (n = 41)	P
Male/Female (n)	36/4	35/6	0.529
Age (y)	63.25 ± 6.91	64.46 ± 6.95	0.433
BMI (kg/m ²)	23.93 ± 2.20	24.16 ± 1.89	0.614
Oxygenation index (mm Hg)	409.78 ± 21.91	406.11 ± 19.65	0.431
PaCO ₂ (mm Hg)	40.05 ± 3.26	40.29 ± 2.99	0.728
SpO ₂ (%)	98.63 ± 1.08	98.73 ± 1.12	0.663
FEV ₁ (%)	86.58 ± 6.12	85.64 ± 6.31	0.500
FVC(%)	91.40 ± 9.31	88.65 ± 6.39	0.127
Operative time (min)	291.60 ± 35.41	293.46 ± 40.97	0.827
OLV time (min)	110.25 ± 24.17	118.15 ± 25.06	0.153
<p>Data were presented as numbers or the mean ± SD. Group A: the patients chose volume controlled mechanical ventilation with a routine tidal volume (V_t = 10 mL/kg) as control; Group B: the patients chose lung protective ventilation with a low tidal volume (V_t = 5 mL/kg) and 5 cm H₂O PEEP. BMI: body mass index; PaO₂: arterial oxygen tension; FiO₂: fraction of inspired oxygen; PaCO₂: arterial carbon dioxide tension; FEV₁: forced expiratory volume; FVC: forced vital capacity; OLV: one-lung ventilation; SpO₂, oxygen saturation.</p>			

Changes In Respiratory Parameters

The respiratory and gas exchange variables are presented in Table 2. The P_{peak}, P_{plat} and ΔP were significantly decreased in the protective ventilation group. While the respiratory rate increased substantially compared with that in the control group. Additionally, the oxygenation index in the protective ventilation group was higher than that in the control group at 30 minutes after OLV (P = 0.006).

Table 2
Respiratory variables and Oxygenation Index During TLV Before Surgery (At baseline) and During OLV (After 30 min)

	Group A (n = 40)	Group B (n = 41)	P
Peak pressure (cm H₂O)			
At baseline	14.85 ± 2.38	13.41 ± 1.53	0.002 ^a
30 min after OLV	26.63 ± 1.93	22.22 ± 2.12	< 10 ^{-3a}
Plateau pressure (cm H₂O)			
At baseline	11.78 ± 2.19	10.49 ± 1.52	0.003 ^a
30 min after OLV	22.90 ± 2.13	19.00 ± 1.99	< 10 ^{-3a}
Driving pressure (cm H₂O)			
At baseline	11.78 ± 2.19	5.49 ± 1.52	< 10 ^{-3a}
30 min after OLV	22.90 ± 2.13	14.00 ± 1.99	< 10 ^{-3a}
Oxygenation index (mmHg)			
At baseline	406.42 ± 30.38	407.57 ± 26.72	0.857
30 min after OLV	314.70 ± 26.02	332.57 ± 30.52	0.006 ^a
Respiratory rate (bpm)			
At baseline	10.408 ± 1.61	14.39 ± 1.24	< 10 ^{-3a}
30 min after OLV	13.35 ± 1.08	17.54 ± 1.03	< 10 ^{-3a}
Data were presented as the mean and SD. Group A: the patients chose volume controlled mechanical ventilation with a routine tidal volume (Vt = 10 mL/kg) as control; Group B: the patients chose lung protective ventilation with a low tidal volume (Vt = 5 mL/kg) and 5 cm H ₂ O PEEP. OLV: one-lung ventilation; TLV: two-lung ventilation. a Compared Group A with Group B, P < 0.05			

Changes In The Number Of Cells In The Balf

The cells in the BALF were counted after Wright-Giemsa staining. The number of total cells (in groups A and B, P = 0.000) and PMN cells (in groups A and B, P = 0.000) in the BALF were substantially increased after mechanical ventilation (Fig. 2). However, in the group treated with the lung-protective strategy, the total cells (P = 0.000) and PMN cells (P = 0.000) in the BALF were significantly reduced compared to the control group (Fig. 2).

Changes in IL-1 β and IL-18 Levels in the BALF and Serum

Commercial ELISA kits were used to detect the levels of both IL-1 β and IL-18 in the BALF and serum. The IL-1 β and IL-18 levels in the BALF and serum showed an increasing trend after mechanical ventilation (Fig. 3A, B, D and E). However, lung protective ventilation resulted in a significant decrease in the BALF and serum IL-1 β and IL-18 concentrations compared to the control group (Fig. 3A, B, D and E).

Changes In Mt Levels In The Balf And Serum

Endogenous MT levels in both the BALF and serum were also detected. In contrast to the IL-18 and IL-1 β levels, the BALF and serum MT levels were significantly decreased in both groups after mechanical ventilation (Fig. 3C, F). Additionally, lower BALF and serum MT concentrations were observed in the control group than in the lung protective ventilation group (Fig. 3C and F).

The Incidence Of Complications

Pulmonary complications occurred in 2/41(4.88%) patients in the protective ventilation group and 8/40 (20%) patients in the control group (P = 0.04). 2 (4.88%) patient in the protective ventilation group developed a nonpulmonary complication compared with 4 (10%) patients in the control group (P = 0.382). The rate of major postoperative complications was 9.76% and 30% in the protective ventilation group and control group, respectively (P = 0.023). The incidence of major postoperative complications was lower in the lung protection group than in the control group (Table 3).

Table 3
Outcomes analysis

	Group A (n = 40)	Group B (n = 41)	P
Incidence of Complications (%)	12(30%)	4(9.76%)	0.023 ^a
Pulmonary complications	8(20%)	2(4.88%)	0.040 ^a
Pulmonary infection	4	1	0.160
ALI/ARDS	2	1	0.544
Reintubation	2	0	0.150
Nonpulmonary complications	4(10%)	2(4.88%)	0.382
Anastomotic fistula	1	1	0.986
Incision infection	1	1	0.986
ICU stay	2	0	0.150
Hospital death	0	0	1.0
Date were presented as numbers and percentage. ALI: acute lung injury; ARDS: acute respiratory distress syndrome. Group A: the patients chose volume controlled mechanical ventilation with a routine tidal volume (Vt = 10 mL/kg) as control; Group B: the patients chose lung protective ventilation with a low tidal volume (Vt = 5 mL/kg) and 5 cm H ₂ O PEEP. a Compared Group A with Group B, P < 0.05			

Discussion

As shown in the present study, lung protective ventilation improved respiratory variables, including Ppeak, Pplat and ΔP . Lung protective ventilation not only inhibited PMN cell invasion but also suppressed IL-1 β and IL-18 secretion. Lung protective ventilation resulted in a decrease in the inhibition of endogenous MT production compared to “conventional” ventilation. In addition, lung protective ventilation decreased the incidence of pulmonary complications and major postoperative complications.

OLV is an established procedure performed during VATS. However, clinical studies have shown that the extended use of OLV is an independent risk factor for postoperative pulmonary dysfunction [17]. Excessive stretching or repeated opening of lung tissues is an important cause of VILI during OLV [18]. A lung-protective strategy using low Vt along with PEEP during OLV was confirmed to improve postoperative pulmonary dysfunction [6]. In our study, the lung-protective strategy notably decreased Ppeak and Pplat, indicating that the shear force was reduced by the lung-protective strategy. Meanwhile, we also observed a substantial decrease in ΔP with the lung-protective strategy, which suggested that the lung-protective strategy was associated with a reduced incidence of postoperative pulmonary complications [16]. Indeed, postoperative pulmonary complications occurred less frequently in the lung protective ventilation group in our study.

Increased mechanical strain further activating the inflammatory response is a key event during the development of VILI [5]. The results from previous and recent studies have shown that IL-1 β is a special proinflammatory cytokine that promotes VILI in animal models and patients [19–22]. Regulation and inhibition of IL-1 β can finally achieve organ protection because blockade of the IL-1 receptor has been demonstrated to inhibit neutrophil sequestration and edema formation in VILI [23]. In our study, mechanical ventilation clearly increased the alveolar and serum concentration of IL-1 β and the alveolar PMN cell counts in the BALF. However, lung protective ventilation blocked the elevated IL-1 β level and PMN cell infiltration. Most interestingly, we observed a dramatic increase in both the alveolar and serum concentrations of IL-18 after OLV, while lung protective ventilation resulted in a profound reduction in IL-18 levels. IL-1 β and IL-18 were confirmed to be products of NLRP3 inflammasome activation [24]. Furthermore, current studies have demonstrated that NLRP3 inflammasome activation plays a key role in the pathogenesis of VILI in a mouse model [25, 26]. Therefore, lung protective ventilation may inhibit inflammatory responses by inhibiting the activation of the NLRP3 inflammasome. For the first time, we showed that mechanical ventilation may activate the NLRP3 inflammasome, and lung protective ventilation seems to inhibit the NLRP3 inflammasome activation in patients.

In recent years, the anti-inflammatory effects of both exogenous and endogenous MT have been observed in many conditions [27, 28]. Paula et al. demonstrated that the exogenous addition of MT protected against VILI through decreasing the levels of inflammatory cytokines in a mouse model [14]. Further research confirmed that exogenous replenishment of MT alleviated lipopolysaccharide-induced acute lung injury by inhibiting NLRP3 inflammasome activation [15]. However, researchers have not determined whether VILI affects the production of endogenous MT. Therefore, we hypothesized that endogenous MT may play a pivotal role in the pathogenesis of VILI. As expected, mechanical ventilation substantially reduced the levels of endogenous MT in patient serum and BALF. Surprisingly, pulmonary protective ventilation significantly inhibited the reduction of endogenous MT. Accordingly, our results suggested that endogenous MT may be involved in the pathogenesis of VILI, and pulmonary protective ventilation may attenuate VILI by restoring the level of endogenous MT in patients.

As described above, lung protective ventilation not only improved respiratory parameters but also suppressed NLRP3 inflammasome-related inflammatory cytokine secretion and restored the level of endogenous MT: which are likely to be required to improve outcomes during esophageal surgery. Indeed, lung protective ventilation not only reduced the incidence of pulmonary complications but also decreased the rate of major postoperative complications in our study, consistent with the results reported by Marret [29].

This study has some limitations. First, the sizes of the samples were small, which may lead to bias. Second, based on our data, we were unable to conclusively determine the relationship between inflammasome-related inflammatory cytokines and endogenous MT. Therefore, the crosstalk between endogenous MT and the NLRP3 inflammasome in VILI requires further animal experiments.

Conclusions

In conclusion, pulmonary protective ventilation improved outcomes by decreasing the rate of pulmonary complications and major postoperative complications. These effects may be attributed to the ability of pulmonary protective ventilation to suppress NLRP3 inflammasome-related inflammatory cytokine secretion and restore the level of endogenous MT in patients undergoing VATS.

Abbreviations

OLV

one-lung ventilation

VILI

ventilator-induced lung injury

PEEP

positive end-expiratory pressure

IL

interleukin

TNF

tumor necrosis factor

BALF

bronchoalveolar lavage fluid

PMN

polymorphonuclear

NLRP3

Nucleotide-binding domain and leucine-rich repeat protein 3

MT

melatonin

FEV

forced expiratory volume

FVC

forced vital capacity

BMI

body mass index

TLV

two-lung ventilation

IBW

ideal body weight

ETCO₂

end-tidal pressure of carbon dioxide

P_{peak}

peak airway pressure

P_{plat}

plateau airway pressure

ΔP

driving pressure

Declarations

Ethics approval and consent to participate

The study protocol had received prior approval by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (No. 20190385). In the study, all patients signed written informed consent.

Consent for publication

All authors have consented to publication of the manuscript.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interests.

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Authors' Contributions

LXW, JL and YTH collected the data, and drafted the manuscript, they contributed equally as co-first authors; YZ performed the statistical analysis; QYS and HYZ revised the manuscript critically for important intellectual content. All authors were responsible for the conception and design of the trial, and approved the final manuscript.

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Figures

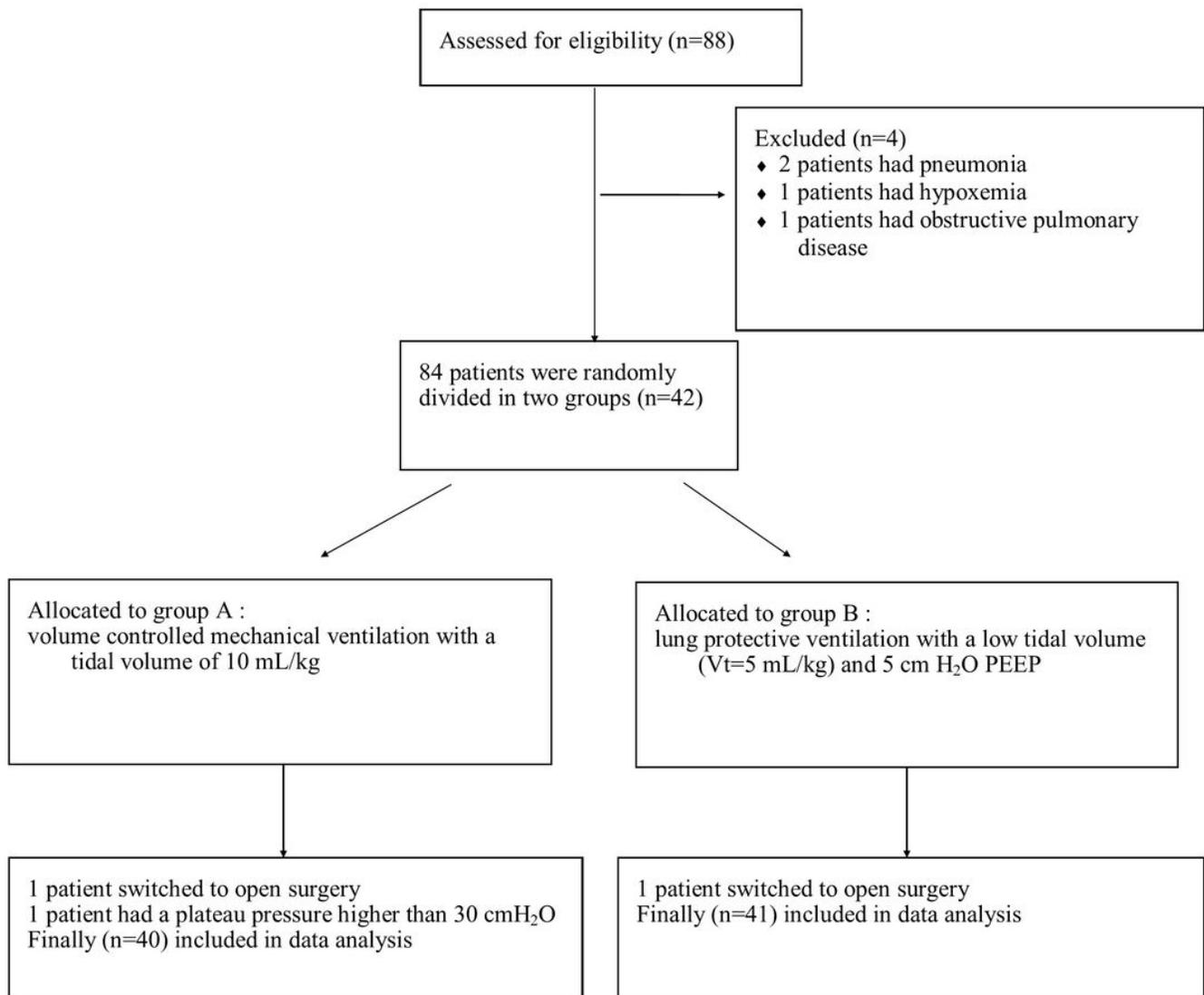


Figure 1

Consort flow chart that outline patients assignment and treatment protocols. Group A: Volume controlled mechanical ventilation with a tidal volume of 10 mL/kg was used; Group B: lung protective ventilation with a low tidal volume ($V_t=5$ mL/kg IBW) and 5 cm H₂O PEEP was chosen.

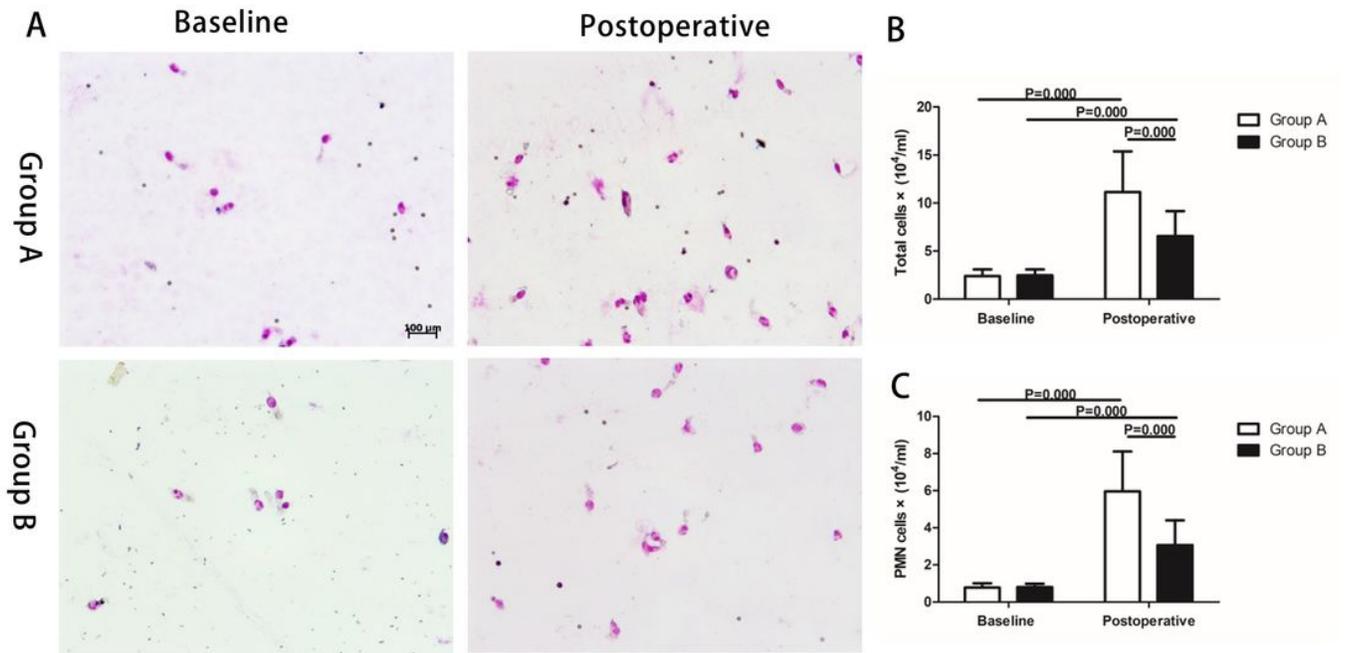


Figure 2

Effect of lung protective ventilation on polymorphonuclear (PMN) cells in BALF. (A) Representative Wright-Giemsa stained smear of BALF from different groups (magnification $\times 20$). Data shown represent changes in the total number of cells (B), PMN cells (C) in the BALF. Data are expressed as the mean \pm SD of 30 patients per group.

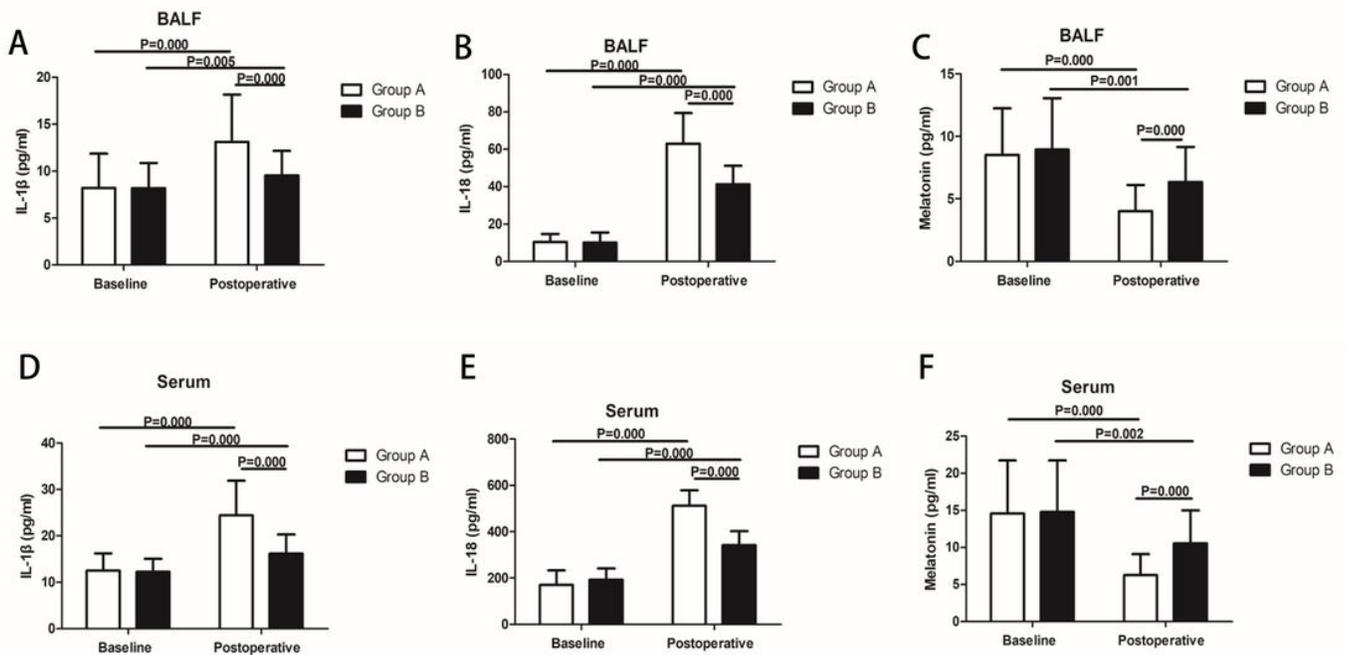


Figure 3

Effect of lung protective ventilation on IL-1 β , IL-18 and endogenous melatonin production in BALF and serum. (A-C) Productions of IL-1 β , IL-18 and endogenous melatonin in the BALF. (D-F) Productions of IL-1 β , IL-18 and endogenous melatonin in the serum. Data are expressed as the mean \pm SD of 30 patients per group.

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