Effect of different fractions of inspired oxygen on ventilator-induced lung injury during prolonged mechanical ventilation in surgery

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Research Article

Abstract

Purpose Explore the effect of different intraoperative fraction of inspiration O_2 (FiO_2) on perioperative lung injury through this trial.

Methods 102 patients undergoing lower abdominal surgery under general anesthesia were randomly divided into three groups: group A FiO_2 30% ,B(FiO_2 50%) and C(FiO_2 80%). The concentrations of surfactant protein A (SP-A) and Clara cell protein 16(CC16) in plasma, which reflect lung injuries, were also detected by ELISA at T_0 (10 minutes before anesthesia), T_1 (1 hour after intubation)and T_2 (3 hours after intubation). Lung ultrasound (LUS) was used to calculate LUS scores of all patients at T_0 and T_3 (30 minutes after extubation) to evaluate the incidence and severity of atelectasis after surgery.

Results 90 patients were enrolled in this trial. Compared with T_0, SpO_2 decreased significantly at T_3 in all three groups($P<0.05$). PaO_2/FiO_2 was higher in group A than in groups B and C at T_2 and T_3($P<0.05$). PaO_2/FiO_2 decreased with the ventilation duration in all three groups($P<0.05$). Compared with T_0, the incidence of atelectasis and LUS scores increased significantly at T_3 in the three groups ($P<0.05$).

Conclusion Intraoperative 30% FiO_2 ca nalleviate lung injury, improve oxygenation and reduce either incidence or severity of atelectasis in patients receiving prolonged mechanical ventilation(3~5h) during surgery with general anesthesia.

Trial registration: Clinicaltrials.gov ChiCTR2000029075.

1. Introduction

With the widespread of general anesthesia, perioperative ventilator-associated lung injury(VALI) has drawn much attention[1], which might increase the incidence of postoperative pulmonary complications(PPCs) and affect patients' recovery. More and more anesthesiologists attempt to improve perioperative ventilation strategies to reduce these injuries, improve perioperative pulmonary function and promote enhanced recovery after surgery (ERAS).Several widely recognized protective measures include low tidal volume (LTV), positive end-expiratory pressure (PEEP), recruitment maneuver (RM) and low FiO_2[2-7].The lung protective ventilation strategy including LTV, PEEP and RM has been proved to be beneficial to perioperative pulmonary functions in many studies[2-7].However, the settings of intraoperative FiO_2 remain controversial.

Adequate perioperative oxygen supply is crucial and hypoxia will induce serious organ dysfunction[8].Several clinical trials proposed high perioperative FiO_2 to ensure oxygen supply for organs, at the same time reduce the incidence of surgical site infections(SSIs) and postoperative nausea and vomiting, but evidences are insufficient. The World Health Organization (WHO) also recommends 80%FiO_2 during surgery to prevent SSIs[9]. On the contrary, some other researchers think it inappropriate to use high FiO_2 during surgery[10-13].M. Wenk commented that the WHO recommendation on

perioperative administration of oxygen to prevent SSIs (Strong recommendation, moderate quality of evidence) is a dangerous reductionist approach because it solely focuses on the patient's "wound", ignores all other organ systems potentially affected by hyperoxia, and may ultimately worsen patient outcomes[10]. Some studies pointed out that high FiO_2 would injure organs, especially the lungs. Mechanics of lung injury caused by high FiO_2 include oxygen toxicity and absorptive atelectasis[10-12],which in turn decrease lung compliance, increase pulmonary vascular resistance and lead to a series of postoperative pulmonary complications, ultimately affecting postoperative recovery of patients. The application of low FiO_2 reduces the incidence of atelectasis while ensuring oxygen supply[13].

Atelectasis is one of the most important complications during general anesthesia, which may be closely related to VILI and plays an important role in the occurrence of postoperative pulmonary complications[14-16]. There are many factors causing atelectasis during general anesthesia, among which prolonged high concentration of oxygen is closely related to absorptive atelectasis. LUS examination is a convenient and noninvasive method to evaluate lung ventilation. According to the method of Audrey Monastesse, atelectasis can be evaluated through LUS scores[17, 18],a semiquantitative echographic score of lung aeration.

Pulmonary surfactant (PS) is composed of phospholipid and specific binding proteins including surfactant proteins (SPs), and SP-A is the most abundant and characteristic protein, which is synthesized and secreted by type II cells and Clara cells[21].Clara cell protein 16（CC16） is the most important secretion of complete Clara cells, which is highly expressed in lung epithelial lining fluid (ELF) and has lung tissue specificity. Previous studies suggested that during mechanical ventilation, SP-A and CC16 in the alveoli will be released into the bloodif VALI occurs, and the severity of lung injuries can be judged by measuring the contents of SP-A and CC16 in plasma.

Few studies focused on the effect of perioperative FiO_2 on lung injury and patients' pulmonary function. Therefore, this study was designed to compare the effect of different FiO_2 on lung injury during prolonged mechanical ventilation through the concentrations of SP-A and CC16 in plasma, $PaO_2/FiO_2$and the incidence and severity of postoperative atelectasis detected by lung ultrasound.

2. Methods

2.1. *Study design and participants.* After approval by the Institutional Review Board, this randomized, controlled, double-blind trial was performed at the First Affiliated Hospital of Soochow University between April 2018 and April 2019. Written informed consent was also obtained from patients before surgery.The inclusion criteria were as follows: adult patients older than 18 years of age, ASA physical status I and II, body mass index(BMI) less than $28kg/m^2$ and scheduled to receive elective lower abdominal surgery lasting at least 3 h under general anesthesia. The exclusion criteria were as follows: patients with previous cardiopulmonary disease or other severe diseases, previous thoracic surgery, upper or lower airway infection, chest deformity or SpO_2 less than 90% before surgery. In addition, patients with the

following were also excluded: those with end-tidal carbon dioxide pressure(ETCO$_2$) unable to be maintained between 35 and 45 mmHg (1 mmHg=0.133 kPa) after adjusting intraoperative respiratory rate and those with intraoperative blood pressure fluctuating more than 20% around the baseline.

2.2. Sample size. Sample size was calculated by GPower3.1.9.7, selecting statistical test(ANOVA: Repeated measures, between factors) when effect size was set to 0.25, αerrprob 0.05 and power(1-βerr prob) 0.95 number of groups 3, number of measurements 3, corr among rep measures 0.Each group needed 87samplesand each sample was measured three times, therefore the sample size of each group should be at least 29. Finally, this experiment included 102 patients , 34 in each group.

2.3.Randomized. Using a computer-generated randomized software (http://www.randomization.com), the patients were randomly assigned into three groups, A (FiO$_2$ 30%), B (FiO$_2$ 50%) and C (FiO$_2$ 80%) at a ratio of 1:1:1, 34 patients in each group. Randomization sequence was generated through opaque envelopes by a trained researcher. Before induction of anesthesia, the envelopes could be opened.

2.4.Monitoring. An intravenous access was prepared before patients entering the operating room. Standard monitoring included non-invasive blood pressure, pulse oximetry, electrocardiography, ETCO$_2$ and body temperature. A catheter was placed in radial artery to monitor invasive blood pressure and arterialbloodgasanalysis.

2.5.Anesthesia. All patients were preoxygenated for at least 3 minutes with 100% oxygen by face mask, followed by induction with propofol 2mg/kg, sufentanil 0.4μg/kg and cisatracurium 0.2mg/kg and then trachea intubation. Sevoflurane, propofol, remifentanil, sufentanil and cisatracurium were used for maintenance of anesthesia.

After intubation, recruitment maneuver(RM)was performed in pressure-controlled ventilation with preallocated FiO$_2$on all patients under the guidance of lung ultrasound until no collapsed lung area could be seen. According to the latest strategy[19], by maintaining a stable airway pressure of 15cmH$_2$O, PEEP was increased within crementof 5cm H$_2$O every 5s until the peak pressure of 35~40cm H$_2$O. Airway peak pressure was maintained at 10s or 5 breaths, and then decreased. The maximum pressure limit was set as 40cmH$_2$O. Thereafter mechanical ventilation began in a volume-controlled mode with 8ml/kg of predicted body weight (PBW) tidal volume and PEEP was not used in all three groups. The inspiratory to expiratory ratio(I:E) was 1:2, and the respiratory rate(RR) was adjusted to maintain ETCO$_2$ between 35 and 45 mmHg. The FiO$_2$ was set at 30%, 50% and 80% in groups A, B and C, respectively. All above parameters were maintained until anesthesia emergence and extubation. Postoperative patients were sent to the anesthesia recovery room for further observation.

2.6.Artery blood gas analysis and oxygenation. Blood samples were collected from the radialartery for arterial blood gas analysis at four time points including 5 min before anesthesia (T$_0$), 1h after intubation(T$_1$), 3h after intubation(T$_2$) and 30 min after extubation (T$_3$). The pH, PaO$_2$ and PaCO$_2$ were recorded and PaO$_2$/FiO$_2$ was calculated.

2.7.Inflammatory factors. Blood samples at T_0, T_1 and T_2 were centrifuged for plasma to determine the concentrations of SP-A and CC16 through ELISA (SP-A and CC16 kits were provided by Shanghai ExCell Bio Company).

2.8.Lung ultrasound. Lung ultrasound examination was performed at T_0 and T_3 in all patients by the same experienced anesthesiologist who was blind to randomization, using HFL386 (SonoSite) with ultrasound probes (2~5MHz). Lung ultrasound examination was performed with the patient in supine position following the method of Audrey Monastesse[18]. The thorax was divided to 12 regions by parasternal lines, anterior axillary lines, posterior axillary lines, paravertebral lines and two nipple lines. Probes were placed at the intercostal space perpendicular to the ribs to scan all of the 12 regions of the lungs from right to left, cranial to caudal, and anterior to posterior. Atelectasis was assessed through looking for the following signs: lung sliding sign, A-lines, B-lines, subpleural consolidations, or air-bronchograms[20].Atelectasis was classified into four grades and scored from 0 to 3 (LUS score)(Table 1): 0, A-lines or lung sliding sign or 0~2 isolated B-lines; 1, ≥3 B-lines or subapleural consolidations separated by smooth pleural lines; 2, multiple coalescent B-lines or subpleural consolidations separated by thickened, irregular pleural lines; and 3,>1*2cm subpleural consolidations. Each region was scanned and the worst ultrasound images (Fig1) and 15s video clips were stored, which were separately scored and summed according to the LUS scoring standard(Table1) that reflected the severity of atelectasis[18, 20, 21].Besides, in our study, a LUS score exceeding 1 in any quadrant was considered to be atelectasis positive, and the rate of atelectasis = atelectasis positive/total.

Table1. LUS Scoring Standard

Scores	Ultrasound Images
0	A-lines or lung sliding sign or 0~2 isolated B-lines
1	≥3 B-lines or subpleural consolidations separated by smooth pleural lines
2	Multiple coalescent B-lines or subpleural consolidations separated by thickened, irregular pleural lines
3	>1*2cm subpleural consolidations or air-bronchograms

2.9.Outcome. All patients' basic characteristics were collected including age, sex, height, weight, duration of mechanical ventilation and position in surgery. Besides, SpO_2, heart rate(HR) and mean artery pressure(MAP) were recorded before anesthesia(T_0).

The primary outcomes were concentrations of SP-A and CC16and the secondary outcomes included perioperative $PaO_2/FiO_2$and LUS scores.

2.10.Statistical analysis. All data were expressed as mean±standard deviations(SDs), median (interquartile range[IQR]), or number(percentage). Continuous variables were evaluated using t-test orvariance analysis. Dichotomous outcomes were analyzed by x^2 test or Fisher's exact test. The primary

outcome was evaluated using two-way repeated-measures ANOVA. The Shapiro-Wilk test was used to determine the normality of the distribution. $P<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 17 and GraphPad Prism 8.

3. Results

From April 2018 to April 2019, eligibility assessment was performed on a total of 102 patients receiving elective lower abdominal surgery with general anesthesia in the First Affiliated Hospital of Soochow University. After removing 12 patients who failed to complete the trial due to various reasons including difficulties in lung ultrasound examination, failure to collect arterial blood, inadequate ventilation duration and no extubation after operation,90 patients were finally enrolled in the trial, 30 patients in each group. Table 2 showed the comparison of basic characteristics of patients including age, sex, BMI, duration of mechanical ventilation, ASA and surgical approach, indicating no statistical difference among the three groups($P>0.05$).

Table 2. Basic characteristics of patients in the three groups.

Basic characteristics	Groups			P
	A (n=30)	B(n=30)	C(n=30)	
Age(Year)	48.17±12.07	50.65±10.41	48.00±10.59	0.659
Sex (Man/Female)	12/18	14/16	11/19	0.800
BMI(kg/m^2)	22.78±3.07	23.50±3.14	23.39±3.37	0.646
Duration of ventilation(min)	248.73±48.43	238.13±42.52	244.07±41.18	0.649
ASA(I/II)	21/9	18/12	20/10	0.788
Surgery approach (laparoscope/laparotomy)	9/21	8/22	11/19	0.778

3.1.Primary outcome

*3.1.1. SP-A/CC16.*Table3aandFig2 showed the concentration of SP-A in the three groupsat different time points. In ANOVA analysis, the concentration of SP-A had no statistical difference among the three groups at all of the three time points($P>0.05$). Two-way repeated-measures ANOVA showed no significant effect of time by treatment interaction(F=0.05765, P=0.9938), also no significant effect of treatment(F=1.400, P=0.2485), but a significant effect of time(F=3.957, P=0.0203) on the concentration of SP-A in serum among three groups(Table3b).

The concentration of CC16 in the three groups at different time points can be seen in Table 4a. In ANOVA analysis, the concentration of CC16 had no statistical difference among the three groups at T_0 and

$T_1(P>0.05)$, but differed from each other significantly at $T_2(P<0.05)$. The concentration of CC16 was obviously lower in group A than groups B and C$(P<0.05)$, with no difference between groups B and C at $T_2(P>0.05)$. The concentration of CC16 increased with the duration of mechanical ventilation in all of the three groups$(P>0.05)$.In addition, two-way repeated-measures ANOVA showed no significant effect of time by treatment interaction$(F=1.402, P=0.2336)$,but a significant effect of time$(F=46.16, P<0.0001)$ and a significant effect of treatment$(F=4.263, P=0.0151)$ on the concentration of CC16 in serum(See Table 4b). As can be seen in Fig3, the concentration of CC16 increased with the duration of mechanical ventilation in the three groups.

Table3a . The concentration of SP-A in plasma.

Factor		Groups			P
		A(n=30)	B(n=30)	C(n=30)	
SP-A(µg/L)	T_0	16.20±2.57	16.88±3.07	16.53±2.88	0.677
	T_1	17.03±2.93	17.79±2.43	17.28±2.89	0.589
	T_2	17.54±2.75^a	18.15±3.02^a	17.43±3.21^a	0.578
P		0.170	0.212	0.463	

Note: Compared with T_0,$^a P$ 0.05

Table3b. Two-way repeated-measures ANOVA of SP-A

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1.900	4	0.475	F (4,261) = 0.05765	P=0.9938
Row Factor(time)	65.20	2	32.60	F (2,261) = 3.957	P=0.0203
Column Factor(treatment)	23.07	2	11.53	F (2, 261) = 1.400	P=0.2485
Residual	2150	261	8.239		

Table 4a .The concentration of CC16 in plasma.

Factor		Groups			P
		A(n=30)	B(n=30)	C(n=30)	
CC16(µg/L)	T_0	9.85±1.18	9.75±1.71	10.06±1.51	0.702
	T_1	10.26±1.45^a	10.47±1.46$^{\#a}$	10.66±1.32$^{\#a}$	0.535
	T_2	11.18±1.49^{ab}	12.06±1.35$^{\#ab}$	12.46±1.50$^{\#ab}$	0.001
P		0.0012	≤= 0.01	≤= 0.01	

Note: Compared with groupA, $^{\#}P$ 0.05;compared withT_0,^{a}P 0.05; compared with T_1,^{b}P 0.05

Table4b. Two-way repeated-measures ANOVA of CC16

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	11.76	4	2.940	F (4,261) = 1.402	$P=0.2336$
Row Factor(time)	193.5	2	96.75	F (2,171) = 46.16	$p≤= 0.01$
Column Factor(treatment)	18.87	2	8.937	F (2, 171) = 4.263	$P=0.0151$
Residual	547.1	261	2.096		

3.2.Secondary outcome

*3.2.1.LUS scores.*Lung ultrasound examination was performed at T_0 and T_3 to evaluated atelectasis. As can be seen in Table5, there was no significant difference in LUS scores among the three groups at T_0 and in all of the three groups, LUS scores increased at T_3 compared to T_0. In ANOVA analysis, it was shown that postoperative LUS scores (T3) had significant differences among the three groups($P<0.05$). Postoperative LUS scores(T_3) were obviously lower in group A than in groupB ($P<0.05$) and C ($P<0.05$),although there was no statistical difference between groups B and C. In terms of incidence of atelectasis, there was no difference among the three groups at T_0, however, the incidence was lower in group A(53.3%) than in groups B(80.0%) and C(86.7%) at T_3. The rate of atelectasis increased in the three groups at T_3 compared to T_0.

Table5. Lung ultrasound scores in the three groups(mean±SD).

		Groups			P
		A(n=30)	B(n=30)	C(n=30)	
LUS scores	T_0	0.13±0.43	0.17±0.46	0.17±0.53	0.913
	T_3	2.27±2.65[a]	5.10±3.43[#a]	6.40±3.36[#a]	≤ 0.01
Rate of atelectasis	T_0	10.0% 3/30	13.3% 4/30	10.0% 3/30	0.894
	T_3	53.3% 16/30 [a]	80.0% 24/30 [#a]	86.7% 26/30 [#a]	0.008

Note: Compared with group A, [#]P 0.05; compared with T_0,[a]P 0.05

3.2.2. SpO_2. SpO_2 was recorded in the three groups at different time points (Table 6). There was no difference among the three groups at all of the time points. Two-way repeated-measures ANOVA showed no significant effect of time by treatment interaction(F=0.704, P=0.6461), no significant effect of treatment(F=0.4778,P=0.6205), but significant effect of time(F=104.1, P<0.0001) in SpO_2 among the three groups(Table6b).

Table6a.SpO2 of patients at different time points.

	Groups			P
	A(n=30)	B(n=30)	C(n=30)	
T_0	98.13±1.22	98.23±1.46	98.03±1.22	0.658
T_1	99.07±0.74[a]	99.37±0.61[a]	99.20±0.71[a]	0.648
T_2	99.03±0.67[a]	99.17±0.75	99.37±0.62[a]	0.171
T_3	97.00±1.15[abc]	96.93±1.31[abc]	96.67±1.35[abc]	0.614

Note: Compared with T_0, [a]P 0.05; compared with T_1, [b]P 0.05; compared with T_2, [c]P 0.05.

Table 6b. Two-way repeated-measures ANOVA of SpO_2 in the three groups at the four time points.

ANOVA table	SS	DF	MS	F(DFn, Dfd)	P value
Interaction	4.506	6	0.7509	F(6,348)=0.704	P=0.6461
Row Factor(time)	333.0	3	111.0	F(3,348)=104.1	$p\leq 0.01$
Column Factor(treatment)	1.019	2	0.5093	F(2,348)=0.4778	P=0.6205
Residual	370.9	348	1.066		

3.2.2.PaO$_2$/FiO$_2$. PaO$_2$/FiO$_2$ in the three groups at the four time points were listed in Table 7a. ANOVA analysis showed that PaO$_2$/FiO$_2$ differed from each other in the three groups at T$_2$ and T$_3$ (P<0.05). PaO$_2$/FiO$_2$ was higher in group A than in groups B and C at T$_2$ and T$_3$ (P<0.05) and there was no difference between groups B and C. Two-way repeated-measures ANOVA showed no significant effect of time by treatment interaction(F=1.134, P=0.3418), but significant effect of treatment(F=6.871, P=0.0012), and time(F=28.20, P<0.0001) in PaO$_2$/FiO$_2$a mong the three groups(Table7b). Fig4 also showed that PaO$_2$/FiO$_2$ decreased with duration of ventilation in the three groups.

Table7a. PaO$_2$/FiO$_2$ in the three groups(mean±SD).

	Groups			P
	A(n=30)	B(n=30)	C(n=30)	
T$_0$	473.18±32.57	472.87±35.51	468.73±36.02	0.858
T$_1$	461.78±32.69^a	460.40±31.99^a	453.57±39.92^a	0.625
T$_2$	454.66±39.78^{ab}	433.73±33.49$^{\#ab}$	421.82±33.79$^{\#ab}$	0.002
T$_3$	440.63±24.92^{ab}	424.76±30.80$^{\#ab}$	417.78±29.23$^{\#ab}$	0.008
P	≤= 0.01	≤= 0.01	≤= 0.01	

Note: Compared with group A, $^{\#}P$ 0.05; compared with T$_0$, $^a P$ 0.05; compared with T$_1$,$^b P$ 0.05.

Table7b. Two-way repeated-measures ANOVA of PaO$_2$/FiO$_2$ in the three groups at the four time points.

ANOVA table	SS	DF	MS	F(DFn, Dfd)	P value
Interaction	8724	6	1454	F(6,348)=1.134	P=0.3418
Row Factor(time)	108457	3	36152	F(3,348)=28.20	p≤= 0.01
Column Factor(treatment)	17617	2	8809	F(2,348)=6.871	P=0.0012
Residual	446124	348	1282		

4. Discussion

90 patients receiving lower abdominal surgery with general anesthesia were included in this randomized double-blind controlled trial and assigned into three groups according to intraoperative FiO$_2$. In this study, we chose two-way repeated measures ANOVA to explore the interaction of two factors including treatment(FiO$_2$) and time(duration of mechanical ventilation) on lung injuries, and each sample in three groups was repeatedly measured at different times. The results showed that higher intraoperative FiO$_2$

would induce more lung injuries and atelectasis. Meanwhile, lower FiO_2 can also guarantee intraoperative oxygen supply for organs.

4.1.SPA/CC16 Researchers have been dedicated to exploring the specific inflammatory factors associated with lung injury. Currently, indicators of lung injury have not been established. SP-A and CC16 were selected in this study.

SP-A can regulate immune function and then alleviate infection and inflammation [21]. SPs have been revealed to be associated with several lung diseases in neonates, children, and adults[22-24].Injury of type   alveolar epithelial cells and tumor necrosis factors (TNFs) reduce the synthesis of SP-A. In addition, study with animal models of respiratory distress syndrome (RDS)revealed that SP-A in the lungs could enter into the blood through the damaged blood-air barrier[25].Previous studies found out that the concentration of SP-A decreased in bronchoalveolar lavage fluid(BALF) and increased in serum in patients with acute lung injury(ALI)[26, 27].SP-A can be measured to reflect the severity of lung injury[28].Studies of lung injury in neonatal rats exposed to high oxygen concentration showed that the level of SP-A was time-dependent, which increased in serum 3-10 days after exposure to 100% oxygen concentration[29]. In our study, although the concentration of SP-A in serum increased after 3h of ventilation compared with the baseline, there was no relevance between FiO_2 and SP-A, which may be owing to insufficient duration of ventilation. In addition, proteins secreted by lung epithelial cells, such as SP-A and SP-B, are present in serum in small amounts under physiological conditions, and how SPs transfer from alveoi to serum remains unclear. Previous studies have also shown that SP-A is a relatively insensitive marker of lung injury due to its relatively large molecular weight, about 650kda, making it difficult to penetrate the blood-air barrier.

Even though the concentration of CC16 in ELF is about 10000 times of that in serum, few CC16 in ELF diffuses into blood through intact blood-air barrier[30].Due to its small molecular weight, CC16 can leak from terminal bronchioles and respiratory bronchioles to systemic circulation so long as the barrier is broken[31, 32].Therefore, the level of CC16 in serum can reflect the integrity of lung epithelium and Clara cells[32, 33].CC16 has been confirmed to be relevant to lung injury[34].Similarly, it was confirmed that in the early stage of lung injury, the level of CC16 in BALF decreased gradually, while the level of CC16 in serum increased[32].What's more, studies have shown that CC16 levels tend to be stable and repeatable over time [35],so CC16 is considered a new reliable indicator of early acute lung injury[36, 37].

In our study, the concentration of CC16 in serum was lower in group A than in groups B and C after 3h of ventilation, although there was no difference between groups B and C. Two-way repeated-measures ANOVA also indicated that CC16 was influenced by FiO_2. It can be speculated that high FiO_2 would induce more lung injuries, and even 50%FiO_2 is too high for patients during mechanical ventilation. Several studies proposed that high FiO_2 increased the risk of oxygen poisoning which was detrimental to the function of some organs especially the lungs.[10, 38] In addition, as can be seen in the line charts portraying the relationship between time and the concentration of CC16, the concentration increased with the duration of mechanical ventilation. Two-way repeated-measures ANOVA also showed the relationship

between the concentration of CC16 in serum and the duration of ventilation, indicating that prolonged ventilation may increase the incidence of lung injury. The concentration of CC16 showed no difference among the three groups at T_1, suggesting that 1h of ventilation was too short to produce a recognizable difference in the concentration of CC16 in serum. Similarly, it was also believed that the absence of significant difference between groups B and C at T_2 was due to such a short duration of ventilation. It is necessary to extend the duration of ventilation to explore the correlation between lung injury and time.

Most animal experiments focused on the changes of lung injury indicators in BALF or ELF, which might be more sensitive. In this clinical study, due to the difficulty in obtaining BALF or ELF from surgical patients, we decided to determine the concentrations of SP-A and CC16 in serum to reflect lung injury, and results also showed the correlation between lung injury and the change of CC16 concentrations in serum.

4.2.Atelectasis and Lung ultrasound. Atelectasis, a consequence of a 20% reduction of the functional residual capacity during general anesthesia, may be caused by relaxation of respiratory muscles, dorsal positioning and denitrogenation during the preoxygenation period[39].It is well-known that high FiO_2 promotes absorptive atelectasis[40, 41]. Joyce *et al.* proposed that preoxygenation with a FiO_2 of 0.4 would lead to complete collapse for less than 20 min, whereas complete collapse lasted more than 2 h while breathing with a FiO_2 of 0.3 without preoxygenation[42].In this trial, we aimed at exploring the effect of different intraoperative FiO_2 on the development of atelectasis. Owing to the difficulty of chest X-ray or CT examination during surgery, LUS score was used to evaluate the degree of atelectasis. Lung ultrasonography is a safe, convenient bedside imaging modality[18],and has been proved a valuable tool for the diagnosis of several pulmonary diseases such as pneumothorax (sensitivity 91%, specificity 98%) [43],community-acquired pneumonia (sensitivity 94%, specificity 96%)[44],and pulmonary edema (sensitivity 91%, specificity 94%)[45].Previous studies have proved that it is feasible to use pulmonary ultrasonography in all stages of the perioperative period to track perioperative atelectasis and detect respiratory complications. Therefore, we hypothesized it practicable to use lung ultrasound to test the degree of atelectasis in this study, so as to compare the effect of different FiO_2 on atelectasis[18, 46].

Our study used the LUS score to evaluated atelectasis, which is convenient, noninvasive and cheap. It indicated that high intraoperative FiO_2 induced more absorptive atelectasis.

It was found out in this study that postoperative LUS scores and rates of atelectasis were lower in the 30% FiO_2 group than in the 50% and 80% FiO_2 groups, suggesting that higher intraoperative FiO_2 might induce more atelectasis.

The first lung ultrasound examination was performed when patients breathed spontaneously before anesthesia. There was no significant difference in the basic LUS scores among the three groups before surgery. In order to reduce atelectasis caused by 100% preoxygenation, all patients were given lung recruitment maneuvers after endotracheal intubation. The second pulmonary ultrasound examination was performed after extubation, which represented the state of lungs after mechanical ventilation.

Therefore, the effect of different FiO_2 on atelectasis during mechanical ventilation could be discussed considering the differences in LUS scores at this time.

4.3.Oxygenation. Lung injury arising from various causes, which induces disturbance of air exchange, would impair oxygenation. The results in our study showed that there was no significant difference in PaO_2/FiO_2 among the three groups at baseline but the 30% FiO_2 group showed higher PaO_2/FiO_2 than the 50% and 80% FiO_2 groups after prolonged mechanical ventilation, suggesting that the reduction of FiO_2 did not impair oxygen supply on the contrary, it improved oxygenation. In our study, with the prolonged duration of ventilation, PaO_2/FiO_2 decreased gradually, and the decrease was more obvious in the high FiO_2 group. 3h after mechanical ventilation, significant difference was also observed in PaO_2/FiO_2 among the three groups.

Lin*et al* found a significant negative correlation between plasma CC16 level and PaO_2/FiO_2[47],which was consistent with our results that 3h after ventilation, the high FiO_2 group had lower PaO_2/FiO_2 and higher concentration of CC16 in serum. In addition, as for the decrease of PaO_2/FiO_2 in patients with high FiO_2 at T_2 and T_3, the author considered it relevant to absorptive atelectasis caused by intraoperative high FiO_2 during long-term mechanical ventilation, as was observed in our trial, patients receiving high FiO_2 had more atelectasis after surgery.

It is worth mentioning that, our study showed that patients ventilated with 30% FiO_2 during surgery had higher PaO_2/FiO_2, lower LUS scores, lower rates of atelectasis, and lower expression of CC16 after 3~5h mechanical ventilation than those receiving 50% and 80% FiO_2, but there was no difference between 50% and 80%FiO_2. Our team supposed that 50% FiO_2 is already so high for patients that even 80% FiO_2 would not cause more lung injuries. Besides, the differences in effect between 50% and 80% FiO_2 on lung injury and pulmonary functions may take longer to show up because our study demonstrated that the severity of lung injury was related to time. In addition, recruitment maneuvers performed with different FiO_2 also produce different effects, which may interfere with the final results[20].

5. Conclusion

In summary, 30% FiO_2 can reduce the severity of lung injury and atelectasis, improve oxygenation in patients with long-term mechanical ventilation during surgery with general anesthesia. Therefore, the sample size of this study was small, but the basic characteristics and ventilation duration among the three groups were comparable. In this study, postoperative SSIs of patients were not followed up and the relationship between FiO_2 and the incidence of SSIs could not be investigated. Intraoperative high FiO_2 was proved to be relevant to lung injury and absorptive atelectasis. Lung ultrasound was used to detect perioperative atelectasis, which is not accurate enough to distinguish small differences in ultrasound images. Comparisons were made longitudinally before and after for the same individuals since horizontal comparisons between different individuals are less reliable. No difference was found

between the 50% and 80% FiO_2 groups in our study. The duration of mechanical ventilation may be extended to further explore the relationship between lung injury and time or FiO_2.

Abbreviations

ALI: acute lung injury

BALF: bronchoalveolar lavage fluid

BMI: body mass index

CC16: Clara cell protein 16

ELF: epithelial lining fluid

ERAS: enhanced recovery after surgery

$ETCO_2$: end-tidal carbon dioxide pressure

HR: heart rate

IQR: interquartile range

LTV: low tidal volume

MAP: mean artery pressure

PBW: predicted body weight

PEEP: positive end-expiratory pressure

PPCs: postoperative pulmonary complications

PS: Pulmonary surfactant

RDS: respiratory distress syndrome

RM: recruitment maneuver

RR: respiratory rate

SDs: standard deviations

SPs: surfactant proteins

SSIs: surgical site infections

TNFs: tumor necrosis factors

VALI: ventilator-associated lung injury

WHO: World Health Organization

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Suzhou University (2019-039-1). The statement on ethics approval and consent was uploaded as supplementary material.

Consent for publication

Not applicable.

Availability of data and material

The data used to support the findings of this study are available from the corresponding author upon request. The location of original data:

https://pan.baidu.com/s/19MtqHOQ82HE7GGyAzpTFYQ?pwd=7h1m

Authors' Contributions

JF, YQ, XC, XM, FJ, SL and XL participated in the study design, analyzed the data, and wrote this report. JF, XC and SL prepared experimental materials and carried out the experiments. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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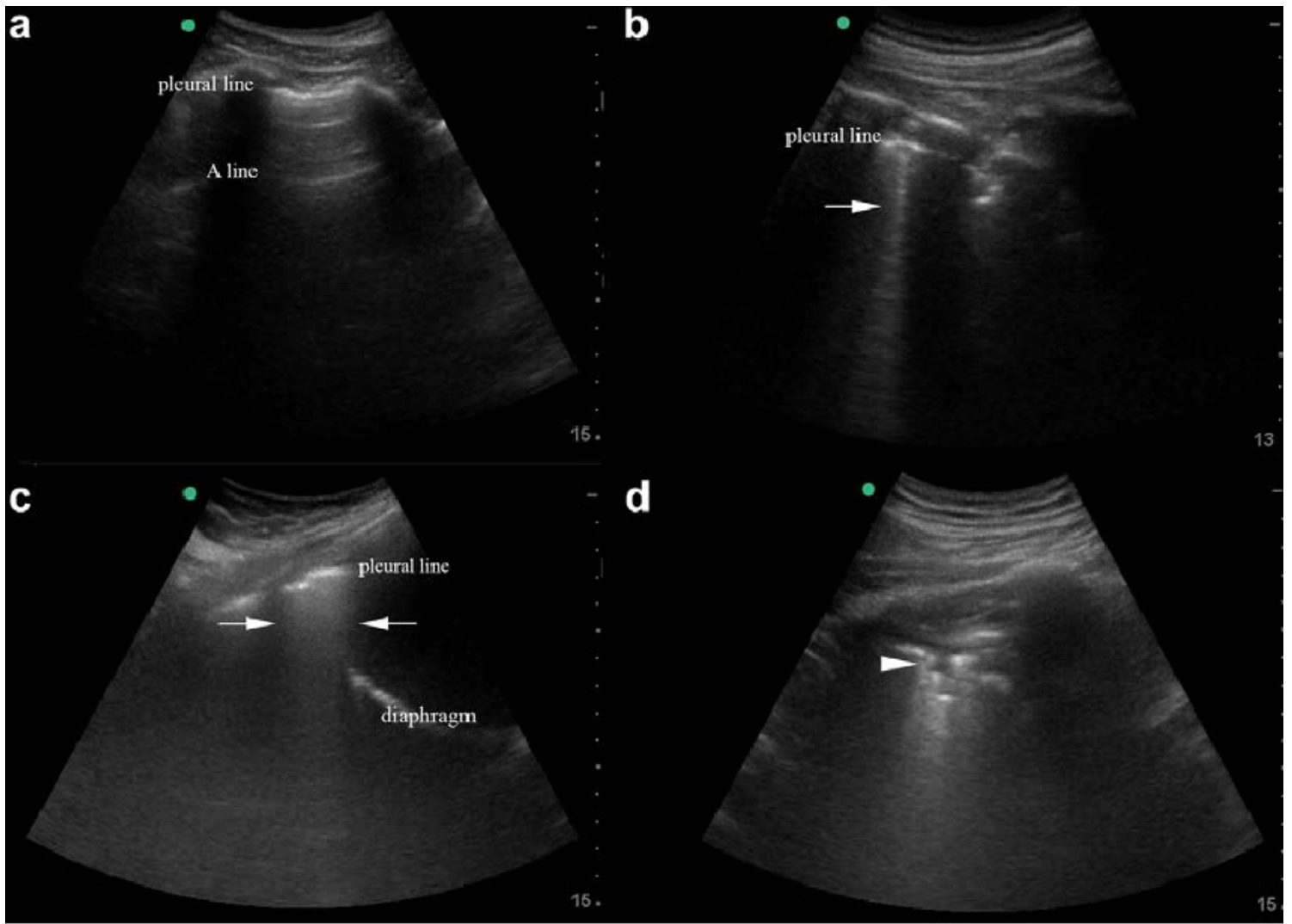
Figures

Figure 1

Ultrasound images

Fig1a. Smooth pleural line and clear A-line; Fig1b.≥3 B-lines and subpleural consolidations separated by smooth pleural lines; Fig1c. Multiple coalescent B-lines or subpleural consolidations separated by thickened, irregular pleural lines; Fig1d.>1*2cm subpleural consolidations and air-bronchograms.

Figure 2
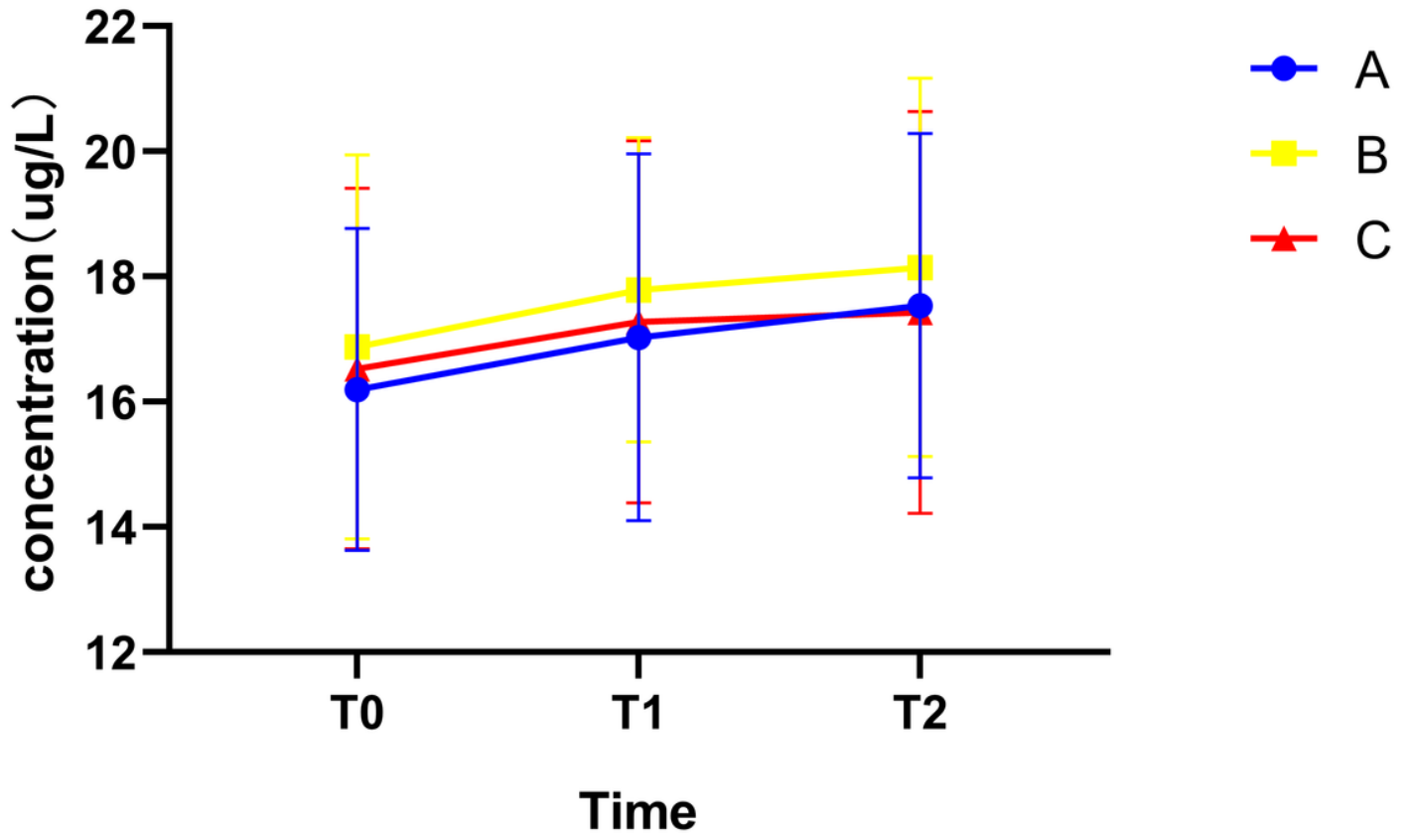
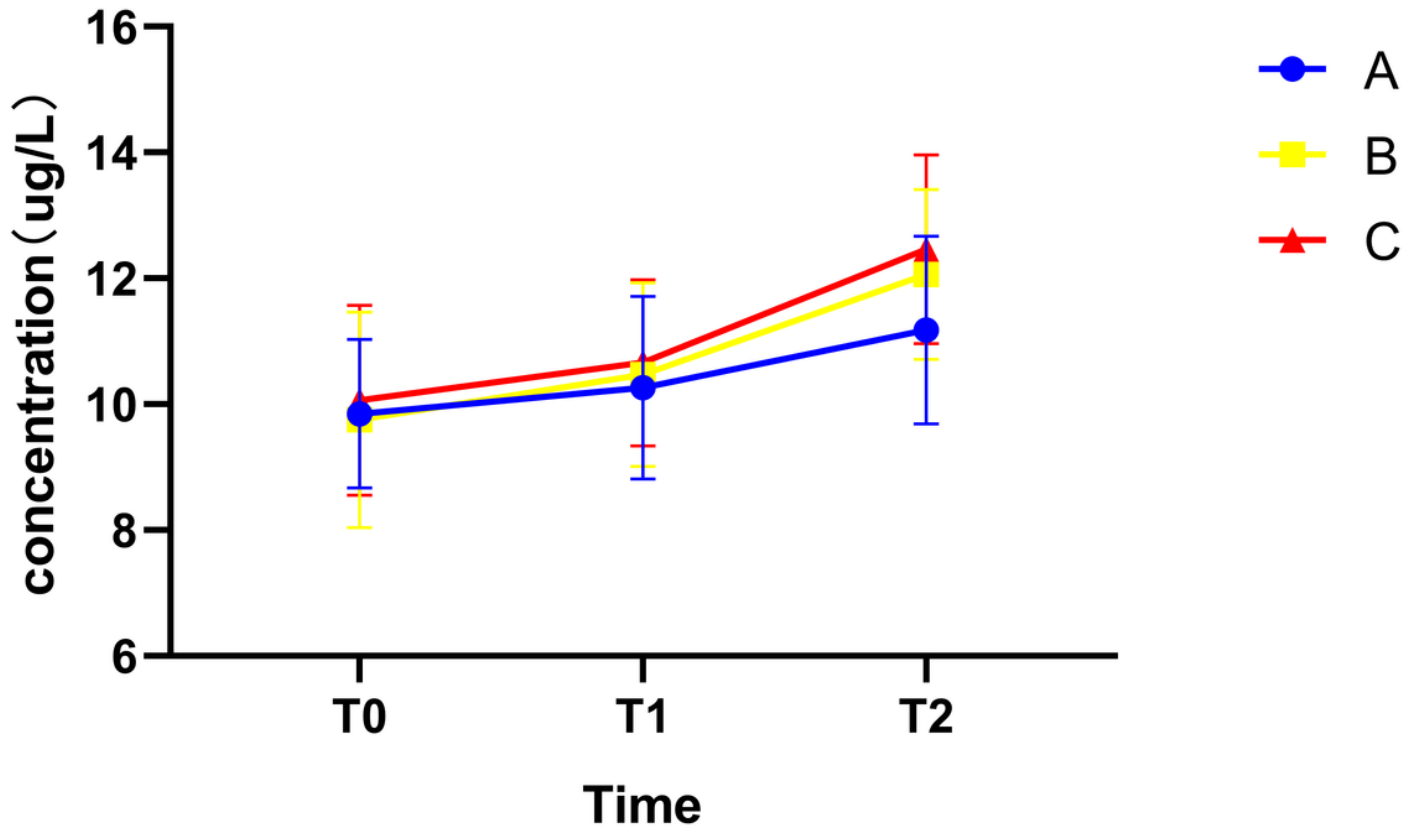Concentration of SP-A in serum in thethree groups at different time points.

CC16

Figure 3

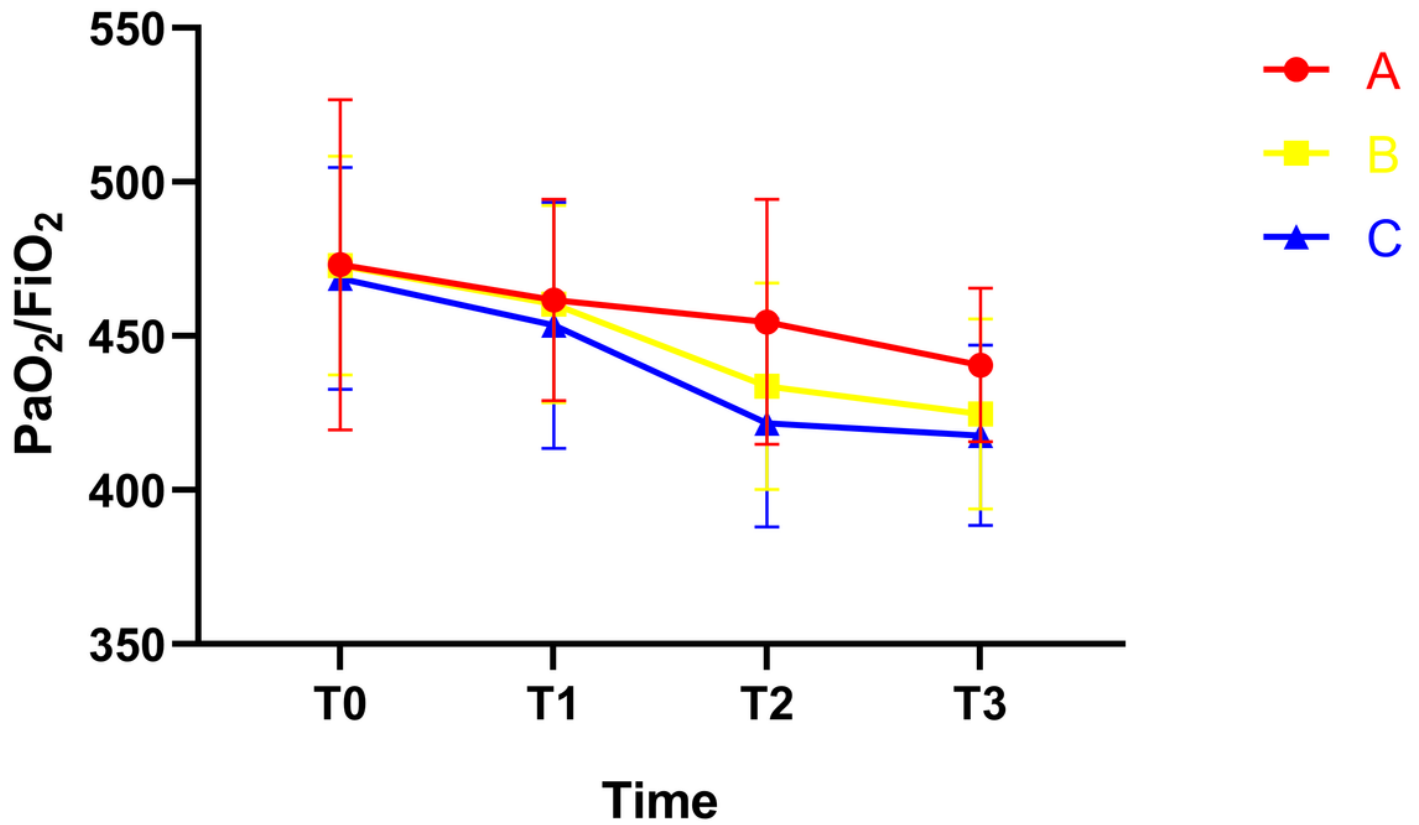Concentration of CC16 in serum in the three groups at different time points.

Figure 4

PaO_2/FiO_2 in the three groups at the four time points.

Supplementary Files

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