Effect of low dose naloxone on the immune system function of a patient undergoing video-assisted thoracoscopic resection of lung cancer with sufentanil controlled analgesia — a randomized controlled trial

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Abstract

Background: Perioperative immune function plays an important role in the prognosis of patients. Several studies have indicated that low-dose opioid receptor blockers can improve immune function.

Methods: Seventy patients undergoing video-assisted thoracoscopic resection of the lung cancer were randomly assigned to either the naloxone group (n=35) or the non-naloxone group (n=35) for postoperative analgesia during the first 48 hours after the operation. Both groups received sufentanil and palonosetron via postoperative analgesia pump, while 0.05μg·kg -1 ·h -1 naloxone was added in naloxone group. The primary outcomes were the level of opioid growth factor （OGF） and immune function assessed by natural killer cells and CD4 + /CD8 + . Second outcomes were assessed by the score of postoperative pain, postoperative rescue analgesia dose, postoperative nausea and vomiting (PONV).

Results: The level of OGF in the naloxone group was significantly increased at 24 hours (p 0.001) and 48 hours after the operation (P <0.01). The natural killer cells (P <0.05) and CD4+/CD8+ (P <0.01) in the naloxone group increased significantly at 48 hours after the operation. The rest VAS score was better with naloxone at 12 and 24 hours after operation（ P <0.05）, and the coughing VAS score was better with naloxone at 48 hours after the operation（ P <0.05). The consumption of postoperative rescue analgesics in the naloxone group was lower (0.00（0.00-0.00）vs 25.00（0.00-62.50）， P <0.05). Postoperative nausea score at 24 hours after operation decreased in naloxone group（0.00 (0.00-0.00) vs 1.00 (0.00-1.00), P < 0.01).

Conclusion: Infusion of 0.05μg·kg -1 ·h -1 naloxone for patients undergoing sufentanil-controlled analgesia for postoperative pain can significantly increase the level of OGF, natural killer cells, and CD4 + /CD8+ 。And also reduce the postoperative pain intensity, request for rescue analgesics, and opioid-related side effects.

Trial registration: ChiCTR1900021043 on January 26, 2019.

Background

Cancer has become a major public health concern all over the world, among which lung cancer is a prominent problem. Surgical resection is the principal treatment for tumors [1,2]. Recurrence and metastasis of tumors are the main causes of death in patients with lung cancer [3]. The perioperative period is a dangerous window for tumor recurrence and metastasis. Immunosuppression plays a significantly important role in the development of tumors [4]. Improvement of postoperative immune function is vitally important for patients. In addition, appropriate postoperative pain control, and effective management of postoperative nausea and vomiting (PONV) lead to several benefits, including earlier restoration of mobility, shorter hospital stays, lower hospital costs and increase in patient comfort and satisfaction.

Opioid receptor antagonists such as naloxone are widely used in the clinical setting to treat opioid-induced respiratory depression and drug addiction. Regulation of endogenous opioids by opioid receptor antagonists may explain the role of opioid peptide-opioid receptor interactions in many biological processes and diseases [5]. One of the functions of endogenous opioids is the regulation of cell growth [6]. Studies have shown that one of the endogenous opioids called opioid growth factor (OGF, chemically termed [MET^5]-Enkephalins) enhances the immune function by increasing the activity of natural killer cells (NK cell), T-cells and the level of interleukin−2 [7–9].

Gans et al. were among the first to report that morphine requirement was significantly less in patients receiving low-dose naloxone, a finding suggesting that low-dose naloxone enhanced analgesia [10]. Moreover, several studies have shown that low-dose naloxone might enhance analgesia and reduce opioid-related adverse effects, such as nausea and vomiting and pruritus [11–13]. Studies showed that low-dose naloxone may enhance analgesic effect through increasing the release of endogenous opioids and up-regulating opioid receptor [14–16]. Some studies further suggested that low-dose naloxone may improve the analgesic effect by releasing enkephalin [17]. However, a survey of the literature shows that little is known about the effects of low dose naloxone on the immune system function of a patient undergoing video-assisted thoracoscopic resection of lung cancer with sufentanil-controlled analgesia. This study aimed to explore the effects of low dose infusion of naloxone 0.05μg·kg^{-1}·h^{-1} on a patient undergoing video-assisted thoracoscopic resection of lung cancer with sufentanil-controlled analgesia.

Methods

This randomized controlled trial was conducted after the approval of the Ethics Committee of the First Affiliated Hospital of the Dalian Medical University on January 24, 2019 (protocol number: PJ-KY−2018–141(X)). Written informed consent was obtained from patients after providing them with adequate explanation regarding the aims of this study. The trial was registered at the Chinese Clinical Trial Registry before patients' enrolment (www.chictr.org.cn, number□ChiCTR1900021043) on January 26, 2019, with Lin Yun as principal investigator. The trial completed a pilot study of 20 patients to calculate the sample size of this trial. The pilot study was performed from February 1, 2019, to February 16, 2019, and the patient data were included in this trial. We enrolled 70 patients aged 18 to 65 with American Society of Anesthesiology physical status □ to □ undergoing video-assisted thoracoscopic resection of the lung cancer. Patients with severe cardiopulmonary, liver or kidney diseases□allergy to naloxone□opioid addiction or drug abuse□and vertigo were excluded.

Upon arrival in the operation room, standard monitoring was determined. Anesthesia was induced with midazolam, sufentanil, cisatracurium, and propofol, subsequently intubation with double lumen tube and location by fiber bronchoscope. Ventilator parameters were adjusted to maintain pulse saturation of oxygen□SpO_2) 95%−100% and end-tidal carbon dioxide between 35 and 40 mmHg. Anesthesia was maintained with propofol, remifentanil and cisatracurium and the depth of anesthesia were maintained at a bispectral index value of 40 to 60. The postoperative analgesic pump was used at the end of the

operation. Sufentanil 0.04μg·kg^{-1}·h^{-1} (calculated at 48 hours), palonosetron 0.5 mg and saline diluted to 100 mL were used in a non-naloxone group, while 0.05μg·kg^{-1}·h^{-1} naloxone (calculated at 48 hours) was added in naloxone group based on the non-naloxone group. PCA was set to administer a bolus dose of 2mL with a lockout interval of 20 minutes and a background infusion rate of 2mL/h. Patients were randomly allocated into 2 groups (1:1 allocation ratio) by a sequence generated from a pseudorandom number seed. Because other non-opioid drugs may have different effects on immune function, postoperative rescue analgesia was chosen to perform intramuscular injection with Sauteralgyl in both groups. All patients in both groups were instructed on how to use the PCA device and on how to use the visual analogue scale (VAS) to rate the intensity of the pain at rest or coughing on a scale from 0 to 10 (with 0 denoting the lowest level of intensity of the symptom and 10, the worst imaginable intensity).

The primary outcome of the study was the level of OGF and postoperative immune function assessed by NK cells and $CD4^+/CD8^+$. Second outcomes were assessed by the score of postoperative pain, nausea, vomiting, analgesic dose, inflammatory responses measured by white blood cell (WBC) count, neutrophil percentage, respiratory depression, and hospital stay. Immune function and inflammatory responses were measured before the surgery, and 24 and 48 hours after surgery. 1, 6, 12, 24 and 48 hours after the operation, both groups of patients rated the intensity of their pain with VAS and respiratory depression. Both groups of patients rated the scale of nausea and vomiting and the dose of Sauteralgyl at 24 and 48 hours after operation and hospital stay. Respiratory depression: respiratory rate ≤8/min or SpO_2 90%. Nausea score: 0 = no nausea, 1 = no nausea at rest, slightly nausea at exercise, 2 = intermittent nausea at rest, 3 = persistent nausea at rest, and severe nausea at exercise. Vomiting score: 0 = no vomiting, 1 = mild vomiting (1–2 times per day), 2 = moderate vomiting (3–5 times per day), 3 = severe vomiting (6 times or more per day).

T lymphocyte subsets and Natural killer cells Assay

Venous blood sample was taken before the surgery, and 24 and 48 hours after surgery. Moreover flow cytometry (BD Company USA) was applied to assess the changes in peripheral blood T lymphocyte subsets ($CD3^+$ $CD4^+$ $CD8^+$ and $CD4^+$ $CD8^+$) and NK cells

OGF Assay

Venous blood sample was taken before the surgery, 24 and 48 hours after surgery. Interleukin–2 was measured in serum using a commercial ELISA kit (MEK (Methionine-Enkephalin) ELISA kit; Elabscience.).

Statistical Analysis

The primary aim of this study was to determine the differences in the level of OGF, NK cells and $CD4^+/CD8^+$ and the secondary outcomes including a score of postoperative pain, nausea and vomiting, Postoperative rescue analgesia dose, WBC count, Neutrophil Percentage, respiratory depression and hospital stay in naloxone and control groups. Results were expressed as means ± SD, medians with interquartile range, or numbers and percentages of participants as appropriate. The demographics and intraoperative situations were compared by Student *t* test or χ^2 test. Fisher's exact test was used for small sample sizes (expected frequencies < 5). The level of OGF, NK cells, $CD4^+/CD8^+$, WBC count, Neutrophil percentage and hospital stay analyzed with a one-way ANOVA between the two groups, and non-normally distributed variables were analyzed with the Mann-Whitney U test. *P* values <0.05 were considered significant. Statistical analysis was performed using SPSS version 22.0.

A pilot study was performed prior to patient recruitment to estimate an appropriate sample size. The pilot study included 20 subjects, 10 in each arm. We calculated the primary outcome of the study assessed by NK cells. The sample size of 32 participants each group provided α = 0.05, 80% power, and an allocation ratio = 1.0. Accounting for loss of data, each group needed 35 patients. The sample calculation was performed with PASS version 11.0.

Results

Of the 81 patients assessed for eligibility, 70 patients were enrolled and randomly assigned to the groups, and 69 patients completed the study (Figure 1). Data from one patient was excluded from the analysis due to early discharge home (the next day after the operation). There were no significant differences in patient characteristics (Figure 1).

The level of OGF in the naloxone group was significantly higher at 24 hours（*p*＜0.001）and 48 hours after the operation (*P*<0.01) in Figure 2. NK cells (*P*<0.05) （Table 2） and CD4+/CD8+ (*P*<0.01) （Table 3） in patients from the naloxone group significantly increased at 48 hours after the operation. There was no significant difference in the NK cells（Table 2） and $CD4^+/CD8+$（Table 3） at separate time points. The rest VAS score was better with naloxone at 12 and 24 hours after the operation（*P*<0.05）（Figue 3）. The coughing VAS score was also better with naloxone at 48 hours after the operation (*P*<0.05) （Figue 3）.There was no significant difference at separate time points in Figure 3.The rescue postoperative analgesics dose injected in patients from the naloxone group was 0.00（0.00–0.00）mg lower compared with 25.00（0.00–62.50）mg injected in patients from the non-naloxone group (*P*<0.05) （Table 4）.

Table 4 showed that postoperative nausea score significantly decreased in patients from the naloxone group（0.00 (0.00–0.00) vs. 1.00(0.00–1.00), *P* < 0.01） 24 hours after the operation. There was no significant difference in nausea and vomiting scored between the different time points（Table 4）. There was no significant difference in the postoperative hospital stay (P＞0.05）in Table 4. The data showed no significant difference in the postoperative inflammatory responses assessed by WBC count and the percentage of neutrophil between the two groups (*P*＞0.05) in Table 5.

Discussion

In this study, we found that 0.05 μg·kg^{-1}·h^{-1} naloxone for patients with sufentanil-controlled analgesia could increase the levels of OGF, NK cells and CD4$^+$/CD8$^+$.Studies have shown that OGF can increase NK cells activity and T cells proliferation [8,18,19]. NK cells which could directly kill tumor cells are essential immuno-regulation cellsand they are the primary defensive line in the body [20–22]. The decline of NK cell activity can lead to the occurrence and development of tumors [23, 24] and the vitality of the NK cell is of great significance in judging clinical prognosis. Our results showed that low-dose naloxone may inhibit tumors by increasing the level of NK cells regulated by OGF.

T cell subsets play a major role in cellular immunity. The number of CD3$^+$ T cells represents the overall cellular immune status of the body. CD4$^+$ is a T helper cell with the function of immune regulation.CD8$^+$ is a cytotoxic T lymphocyte, which can remove cancer cells.CD4$^+$/CD8$^+$ can reflect the immune status of the body [25]. In physiological state, CD4$^+$/CD8$^+$ is relatively constant. The decrease of CD4$^+$/CD8$^+$ indicates the decrease of immune function and the severity of disease or poor prognosis. The results showed that CD4$^+$/CD8$^+$ in naloxone group was higher 48 hours after the operation, suggesting that low dose naloxone may enhance cellular immunity and anti-tumor effect. The presence of pain may affect immune function [26]. Low-dose naloxone may enhance immune function by decreasing pain intensity, but whether the increase of CD4$^+$/CD8$^+$ is related to OGF is uncertain.

Many experiments have been carried out to evaluate the effects of Low-dose Naloxone on postoperative analgesia and opioid-related side effects. The analgesic and adverse effects of opioids are dose-dependent. The dose of naloxone administration in the report provided highly variable ranging from 0.008μg·kg^{-1}·h^{-1} to 0.57 μg·kg^{-1}·h^{-1}[27]. The reason why 0.05μg·kg^{-1}·h^{-1} naloxone was chosen was that patient-controlled intravenous analgesia (PCIA) with this dose in YAO's experiment confirmed that low-dose naloxone increased the analgesic effect by increasing the level of endogenous opioid peptides [16]. The data showed that the rest pain score decreased significantly at 12 and 24 hours after surgery and coughing pain score decreased significantly at 48 hours after surgery, and the rescue analgesic dose after surgery was lower in the naloxone group, indicating that low-dose naloxone could enhance the analgesic effect of sufentanil and reduce the dose of analgesics. And the score of nausea decreased significantly on the first day after the operation. The mechanism of the effect of low-dose naloxone on analgesic efficacy and opioid-related side effects is not clear. In addition to releasing enkephalins [28], it is believed that the functions of the μ-opioid receptor excitatory G-protein complexes (GS) are antagonized by naloxone at a low dose, triggering improvement of analgesic effect and reduction in adverse effects such as nausea and vomiting [17]. Some studies also indicated that low-dose of naloxone could reduce neuropathic pain by lowering the levels of inflammatory factors [29]. Our study found that the level of OGF increased significantly two days after the operation, suggesting that the mechanism of low dose naloxone enhancing the analgesic effect of sufentanil, reducing opioids consumption and PONV may be related to the level of endogenous OGF.

We noticed the basic Studies had shown that the regimen of short-term exposure to naltrexone appeared to leading to enhanced interaction of the up-regulated OGF [28]. Blockade of opioid peptides from opioid receptors for a short period each day (4–6 h), using a daily administration of low-dose naltrexone (LDH), provides an 18–20h window wherein the elevated levels of endogenous opioids and opioid receptors can interact to elicit a response [28]. However, in this study, we used 0.05μg·kg^{-1}·h^{-1} naloxone continuous infusion along with sufentanil PCIA for about 48 hours, and the level of OGF increased significantly within 48 hours. There may be two possibilities for this difference, one of which may be related to the difference of half-time of naloxone and naltrexone. Both naltrexone and naloxone are opioid receptor antagonists and have no intrinsic activity, but the duration of naltrexone blockade is about 3–4 times longer than that of naloxone. The other one may be associated with different dose. According to the potency relationship between naltrexone and naloxone, low dose naloxone (4.5 mg) [30] in the report was far more than 0.05 μg·kg^{-1}·h^{-1} naloxone we used in this study. Although we used continuous naloxone infusion for 48 hours, the shorter blockade duration and lower dose might not block all opioid receptors. These may be the reason why the elevated levels of OGF can still interact with its receptor to elicit a response. The mechanism of the increase in the level of OGF is that low-dose naloxone may cause excessive release of endogenous opioids through blockade of presynaptic auto-inhibition of enkephalin release [17].

There are two limitations in this study. First, the duration of immune function detected was limited to 48hours after the operation, and we did not further observe changes of immune indexes. The experimental data showed that there was no significant change in the immune function first day after the operation. The NK cell and CD4+/CD8+ in naloxone group began to be higher on the second day after the operation. If we continue to test NK cells and T cells, we can explore the extent and duration of using low dose naloxone to improve immune function with PCA after the operation. Second, the long-term prognosis of the patients was not observed. Studies have shown that OGF can not only enhance immune function but also directly inhibit tumors. OGF activates the Rb pathway by up-regulating p16 and/p21, which are cyclin-dependent inhibitory kinases, with delayed cell replication and ultimate cell number resulting [31]. Thus, low-dose opioid receptor antagonists-mediated modulation of the OGF-OGFr axis appears to account for the depressed DNA synthesis and proliferation of cancer cells [28]. OGF may inhibit the recurrence and metastasis of tumors after resection. Since no further follow-up of the patients' OGF level and the incidence of postoperative complications and recurrence or metastasis after surgery between the groups, it was not observed whether low-dose naloxone could directly affect the occurrence and development of tumors through OGF-OGFr.

Conclusion

In conclusion, 0.05 μg·kg^{-1}·h^{-1} naloxone increased the levels of NK cells and CD4^{+}/CD8^{+} and the analgesic effect after thoracoscopic resection of lung cancer on PCIA, and reduced analgesics dose and PONV after the operation. The enhancement of immune function and the analgesic effect of sufentanil and reduction of PONV may be related to the increased level of endogenous OGF.

Abbreviations

OGF Opioid Growth Factor

PONV Postoperative nausea,and vomiting

VAS Visual analogue scale

NK cells Natural killer cells

CD Clusters of Differentiation

SpO_2 Pulse saturation of oxygen

PCA Patient Controlled Analgesia

PCIA Patient-controlled intravenous analgesia

WBC White blood cell

T cells T lymphocytes

GS G-protein complexes

LDH Low-dose naltrexone

ASA American Society of Anesthesiologists

Declarations

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Authors' contributions

LY designed the study, drafted and wrote the manuscript. LY and GFF implemented the trial and contributed samples collection.MZ and WY collected the data and did statistical analysis. WQP revised the manuscript critically and final approval of the manuscript. All authors gave intellectual input to the study and approved the final version of the manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

This clinical trial was approved by Ethics Committee of the First Affiliated Hospital of the Dalian Medical University on January 24, 2019 (protocol number: PJ-KY–2018–141(X)). All the participants provided written informed consent following principles of the Helsinki Declaration.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

TABLE 1. Patient Characteristics

	Naloxone（n=35）	Non-naloxone （n=34）	*P* Value
Age（y）	55.46±8.65	55.46±8.65	0.378
Gender（N（%）			0.900
Male	17（48.57）	16（47.06）	
Female	18（51.43）	18（52.94）	
Body mass index(kg/m$^{2)}$	25.42±4.19	24.52±3.67	0.351
ASA physical status（N (%)			1.000
Ⅰ	4（11.43）	4（11.76）	
Ⅱ	31（88.57）	30（88.24）	
Type of operation, N (%)			0.777
Video-assisted thoracoscopic pulmonary lobectomy	25（71.43）	26（76.47）	
Video-assisted thoracoscopic pulmonary lobectomy	9（25.71）	8（23.93）	
Video-assisted thoracoscopic pulmonary segmentary	1（2.86）	0（0.00）	
Duration of operation（min）	150.00 （120.00-180.00）	180.00 （120.00-180.00）	0.339
Fluid intake(mL)	1000.00（1000.00-1000.00）	1000.00（1000.00-1000.00）	0.854
Blood loss(mL)	113.71 ±38.278	106.76 ±36.987	0.446
Type of cancer, N (%)			0.780
Carcinoma in situ	8（22.85）	6（17.65）	
Microinvasive adenocarcinoma	14（40.00）	11（32.35）	
infiltrating adenocarcinoma	12（34.29）	14（41.18）	
Mucinous adenocarcinoma	1（2.86）	2（5.88）	
Moderately differentiated adenocarcinoma	0（0.00）	1（2.94）	
Lymphatic metastasis			0.614
Yes	1（2.86）	2（5.88）	
No	34(97.14)	32(94.12)	

ASA：American Society of Anesthesiologists

TABLE 2. Changes in NK Cells After Surgery

		Naloxone（n=35）	Non-naloxone （n=34）	*P* Value
NK cells（%）	Before surgery	16.14±5.75	16.63±6.40	0.519
	24h after operation	14.13±6.28	14.53±5.85	0.918
	48h after operation	15.97±5.44	13.06±5.47$^{\#}$	0.030

NK cells = Natural Killer Cells. $^{\#}P$＜0.05 versus “before surgery” for each group

TABLE 3. Changes in T cells After Surgery

		Naloxone （n=35）	Non-naloxone （n=34）	*P* Value
$CD3^+$T cells（%）	Before surgery	56.94±9.01	56.95±8.98	0.997
	24h after operation	46.22±12.67$^{\#\#\#}$	41.48±9.99$^{\#\#\#}$	0.089
	48h after operation	56.17±8.96	53.36±10.58	0.237
$CD4^+$T cells（%）	Before surgery	33.49±6.92	32.61±5.52	0.560
	24h after operation	25.39±8.55$^{\#\#\#}$	21.20±7.81$^{\#\#\#}$	0.037
	48h after operation	32.70±6.39	28.91±6.11$^{\#}$	0.014
$CD8^+$T cells（%）	Before surgery	23.45±4.37	24.34±5.09	0.437
	24h after operation	20.83±7.02$^{\#}$	20.28±6.54$^{\#\#}$	0.734
	48h after operation	23.47±4.10	24.57±6.21	0.391
$CD4^+/CD8^+$	Before surgery	1.49±0.36	1.39±0.30	0.226
	24h after operation	1.32±0.51	1.15±0.52$^{\#}$	0.163
	48h after operation	1.41±0.27	1.21±0.29	0.003

CD=Clusters of Differentiation. $^{\#}P$＜0.05 versus “before surgery” for each group. $^{\#\#}P$＜0.01 versus “before surgery” for each group, $^{\#\#\#}P$＜0.001 versus “before surgery” for each group.

TABLE 4. Rescue Analgesic Dose, Postoperative Nausea and Vomiting scores，Respiratory Depression and Hospital Stay

	Naloxone（n=35）	Non-naloxone （n=34）	*P* Value
Rescue analgesic dose(mg)	0.00（0.00-0.00）	25.0（0.00-62.50）	0.034
Nausea score 24h after operation	0.00（0.00-0.00）	1.00（0.00-1.00）	0.002
vomiting score 24h after operation	0.00（0.00-0.00）	0.00（0.00-1.00）	0.132
Nausea score 48h after operation	0.00（0.00-0.00）	0.00（0.00-0.00）	0.179
vomiting score 48h after operation	0.00（0.00-0.00）	0.00（0.00-0.00）	0.424
Respiratory depression 1h, n（%）	0.00（0.00）	0.00（0.00）	，0.99
Respiratory depression 6h, n（%）	0.00（0.00）	0.00（0.00）	，0.99
Respiratory depression 12h, n（%）	0.00（0.00）	0.00（0.00）	，0.99
Respiratory depression 24h, n（%）	0.00（0.00）	0.00（0.00）	，0.99
Respiratory depression 48h, n（%）	0.00（0.00）	0.00（0.00）	，0.99
Hospital stay（day）	4.66±1.39	5.35±1.77	0.074

TABLE 5. Changes in White Blood Cell Count and Neutrophil Percentage After Surgery

		Naloxone（n=35）	Non-naloxone （n=34）	*P* Value
WBC count/uL	Before surgery	7.81±1.23	7.78±1.25	0.907
	24h after operation	11.96±3.13$^{###}$	13.21±3.41$^{###}$	0.115
	48h after operation	9.93±2.86$^{##}$	10.79±2.87$^{###}$	0.214
Neutrophil Percentage（%）	Before surgery	71.74±7.06	72.49±6.71	0.656
	24h after operation	83.57±5.71$^{###}$	85.48±4.39$^{###}$	0.124
	48h after operation	76.47±8.55$^{##}$	79.41±6.67$^{###}$	0.117

$^{##}$*P*，0.01 versus “before surgery” for each group. $^{###}$*P*，0.001 versus“ before surgery” for each group.

Figures

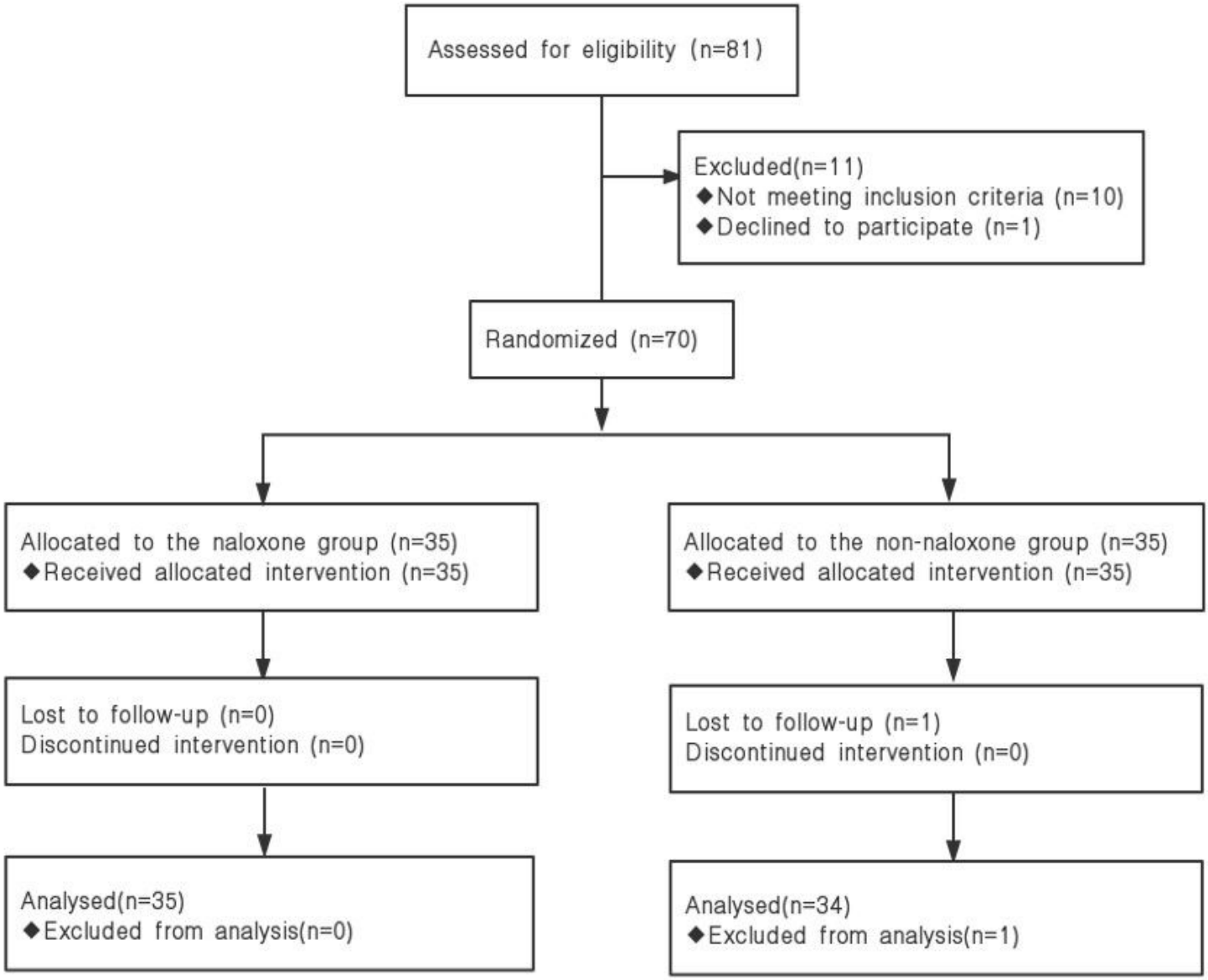

Figure 1

Diagram Showing Flow of Study Participants.

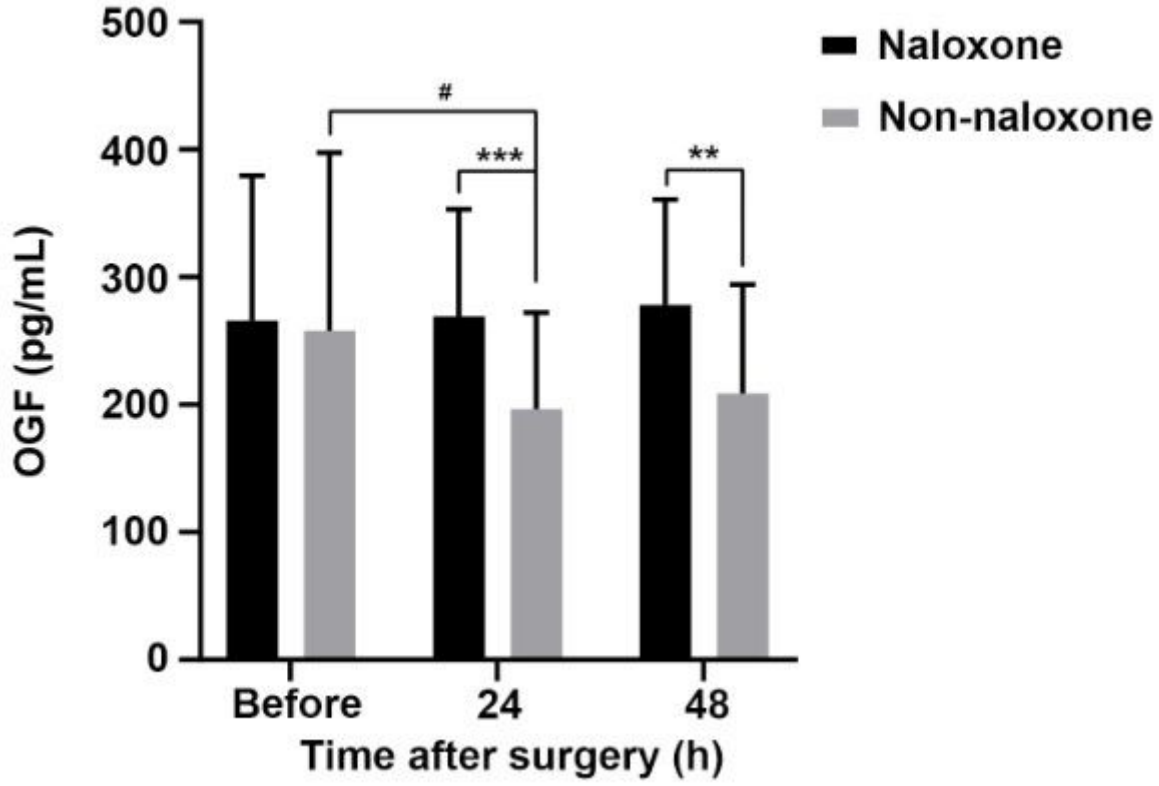

Figure 2

Changes in OGF levels after surgery. OGF levels are presented as means ± SD. Significantly different from the non-naloxone group at **P□0.01□***P□0.001. #P□0.05 versus “before surgery” for each group.

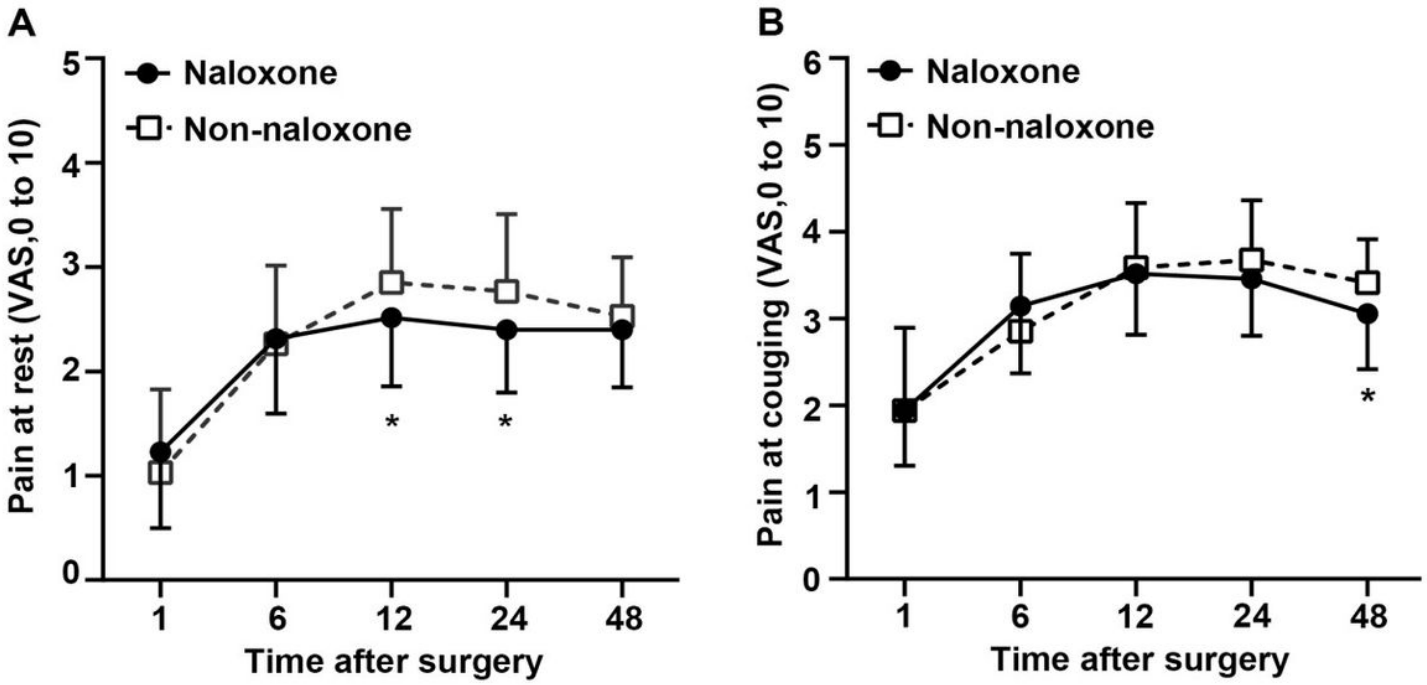

Figure 3

Visual analog scale for pain (A) at rest and (B) while coughing 1, 6, 12, 24, and 48 hours after surgery. Data are expressed as means ± SD. Significantly different from the non-naloxone group at *P□0.05.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- YunLinCONSORT2010Checklist.doc