

Effect of High-Dose Remifentanyl Combined With Propofol on Perioperative Immune-Related Biomarkers in Patients Undergoing Radical Lung Cancer Surgery: A Randomized Trial

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Background: Lung cancer surgery is associated with perioperative immune suppression and inflammatory responses, which may influence postoperative recovery and oncological outcomes. The immunomodulatory effects of different doses of remifentanyl remain unclear. This study aimed to investigate the effects of high-dose remifentanyl combined with propofol on perioperative immune-related biomarkers in patients undergoing radical lung cancer surgery.

Methods: A total of 120 patients were assessed for eligibility; 12 were excluded before randomization; 108 were randomly assigned to three remifentanyl groups (target plasma concentrations: 4, 8, and 16 ng/mL) and included in the analysis. Baseline characteristics, intraoperative variables, postoperative pain, recovery outcomes, and immune-inflammatory parameters were systematically compared among the three groups. Immune indices were dynamically assessed at multiple perioperative time points, and generalized estimating equations were used for repeated-measures analyses with appropriate corrections for multiple comparisons.

Results: The three groups were comparable in baseline characteristics, surgical duration, anesthetic duration, and most intraoperative parameters ($p > 0.05$). Compared with the low-dose group, the high-dose group showed significantly increased natural killer (NK) cell counts at 24 and 72 h postoperatively ($p < 0.05$). In addition, the high-dose group exhibited significantly reduced levels of several inflammatory cytokines, including interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor- α (TNF- α), and interferon- γ (IFN- γ), at selected postoperative time points ($p < 0.05$); however, these differences were time-dependent and not consistently observed across all measurements. Postoperative pain scores at 4 and 48 h were significantly lower in the high-dose group compared with the low-dose group ($p < 0.05$). No significant differences were observed among groups in recovery quality or the incidence of postoperative nausea and vomiting (PONV) ($p > 0.05$). No serious adverse events occurred.

Conclusion: High-dose remifentanyl (16 ng/mL) combined with propofol was associated with changes in selected immune and inflammatory biomarkers at some time points. VAS scores were lower at 4 and 48 h in the high-dose group. No serious adverse events were observed; however, the high-dose group had a greater vasopressor requirement. These findings are exploratory and hypothesis-generating and require further validation.

Clinical Trial Registry: Chinese Clinical Trial Registry (ChiCTR2300077233).

Keywords: remifentanyl; immune-related biomarkers; radical lung cancer surgery; propofol; natural killer cells

Introduction

Lung cancer is the most common malignancy of the respiratory system and remains the leading cause of cancer-related mortality worldwide, with its incidence steadily increasing globally [1]. Currently, radical surgical resection is the primary treatment option for early-stage lung cancer, involving extensive removal of tumor tissue and surrounding structures [2,3]. Postoperative prognosis, including the prevention of recurrence and distant metastasis, is closely associated with the patient's perioperative immune status [4].

Perioperative immune function in lung cancer patients is frequently compromised due to surgical trauma, stress responses, pain stimulation, and anesthetic interventions, particularly opioid use [5]. Surgical stress induces the release of pro-inflammatory cytokines such as interleukin 6 (IL6), tumor necrosis factor- α (TNF- α), and interleukin-1 β (IL1 β), which may impair immune surveillance by suppressing T lymphocyte and natural killer (NK) cell function [6,7,8]. Moreover, various anesthetic agents exert distinct immunomodulatory effects. For example, fentanyl and ketamine have been shown to suppress NK cell activity in a dose-dependent manner [9], whereas propofol may increase NK cell counts and enhance cellular immune responses [10]. These findings highlight the critical importance of modulating perioperative inflammation to preserve immune-related biomarkers.

Opioids exert immunomodulatory effects primarily through μ -opioid receptors expressed on immune cells, thereby inhibiting T cell proliferation, NK cell cytotoxicity, and cytokine release [11,12]. Remifentanyl is a potent, ultra-short-acting μ -opioid receptor agonist with unique pharmacokinetic properties. It undergoes rapid metabolism by nonspecific esterases, allowing for precise dose titration and rapid recovery [13,14]. These characteristics make remifentanyl particularly suitable for oncologic surgery requiring stable hemodynamic control and fast emergence.

Notably, remifentanyl may influence immune function through direct interactions with immune receptors [15]. Other perioperative factors, such as surgical pain, anesthetic techniques, and adjunctive agents, may further modulate immune responses [16]. A previous clinical study has reported that anesthesia with remifentanyl and propofol, combined with dexmedetomidine, can attenuate perioperative inflammation in lung cancer surgery [17]. High-dose remifentanyl has also been shown to reduce stress responses during abdominal laparoscopic surgery and influence cardiac conduction in pediatric anesthesia [18,19].

Given these findings and the delicate immune balance in oncologic patients, it is clinically relevant to investigate whether different doses of remifentanyl exert distinct immunological effects. Therefore, this study aimed to assess the impact of varying remifentanyl concentrations (4, 8, and 16 ng/mL) in combination with propofol, on perioperative

immune-related biomarkers in patients undergoing radical lung cancer resection.

Methods

Study Design

This prospective, randomized, double-blind study protocol was reviewed and approved by the Zhejiang Provincial Hospital of Chinese Medicine Ethics Committee (2023-KLS-141-02). Written informed consent was obtained from all participants. The study was initially submitted for registration in January 2023; however, due to multiple rounds of required protocol revisions and amendments, along with unavoidable administrative delays at the clinical trial registry, formal registration was not completed before the initiation of patient recruitment. Consequently, participant enrollment commenced in October 2023 following ethical approval. The study was subsequently registered in the Chinese Clinical Trial Registry (<https://www.chictr.org.cn/showproj.html?proj=190362>) (ChiCTR2300077233), on November 02, 2023. All procedures were performed in accordance with institutional ethical standards and the Declaration of Helsinki.

Study Population

From October 08, 2023, to July 31, 2024, a total of 120 patients scheduled to undergo radical lung cancer resection surgery at Zhejiang Provincial Hospital of Traditional Chinese Medicine were prospectively enrolled.

Inclusion criteria: (1) aged 18–75 years; (2) undergoing radical lung cancer resection; (3) no known allergies to the study medications; and (4) normal cognitive function and voluntary participation.

Exclusion criteria: (1) severe dysfunction of the heart, liver, kidneys, or other major organs; (2) immune deficiency or endocrine/metabolic disorders; (3) treatment with radiotherapy, chemotherapy, or immunosuppressants within one month prior to surgery; and (4) pregnancy or lactation.

Dropout criteria: (1) voluntary withdrawal; and (2) loss to follow-up.

Randomization and Blinding

A computerized random number table was used to generate the allocation sequence. The randomization sequence was generated by an independent statistician who was not involved in patient recruitment, anesthesia management, or outcome assessment. Allocation concealment was achieved using the sequentially numbered, opaque, sealed envelope (SNOSE) method. Each envelope contained the assigned group allocation and was opened only after patient enrollment and immediately before anesthesia induction. Participants were enrolled by clinical research coordinators. Group allocation was performed by an anesthesiologist who was not otherwise involved in the trial, according

to the pre-generated randomization sequence. Patients were randomly assigned in a 1:1:1 ratio to three groups: low-dose (L; target plasma concentration of 4 ng/mL), medium-dose (M; 8 ng/mL), or high-dose (H; 16 ng/mL) remifentanyl. All 120 patients were assessed for eligibility. Before randomization, 12 patients withdrew (4 declined to participate and 8 no longer met the inclusion criteria). Therefore, 108 patients were randomized (L: 37; M: 36; H: 35) and all completed the study. Blinding was implemented for participants, healthcare providers, and outcome assessors. Remifentanyl was administered using target-controlled infusion (TCI) pumps programmed by an independent anesthesiologist according to the assigned group. The TCI pump screens were concealed, and identical infusion protocols and syringes were used across all groups to maintain blinding of the attending anesthesiologists, surgical staff, and patients. Outcome assessors and laboratory personnel responsible for immune and inflammatory marker measurements remained blinded to group allocation throughout data collection and analysis.

Interventions

Anesthesia Induction and Maintenance

All patients received general anesthesia with endotracheal intubation. Standard intraoperative monitoring included electrocardiography, pulse oximetry, noninvasive blood pressure, body temperature, and bispectral index (BIS). Anesthesia was induced using 0.5 µg/kg sufentanil (Yichang Humanwell Pharmaceutical Co., Ltd., batch number 91A12281, Yichang, China), 0.2 mg/kg cisatracurium besylate (Jiangsu Hengrui Medicine Co., Ltd., batch number 240125, Lianyungang, China), and propofol via target-controlled infusion (TCI; Mindray BeneFusion eSP, Shenzhen, China) at 4.5 µg/mL. Following intubation, the propofol TCI was adjusted to 3 µg/mL. Continuous infusion of remifentanyl (Yichang Humanwell Pharmaceutical Co., Ltd., batch number 10A12341) was started at group-specific target concentrations (4, 8, or 16 ng/mL; prepared at 40 µg/mL). Muscle relaxant (2.5 mg cisatracurium) was administered every 30 minutes. BIS was maintained between 40 and 60 by adjusting the propofol infusion. End-tidal CO₂ was maintained at 35–45 mmHg, and FiO₂ was set at 50%. Propofol was stopped 5 minutes before the end of surgery, and remifentanyl was discontinued at the time of skin closure. All patients underwent video-assisted thoracoscopic lobectomy with one-lung ventilation using a double-lumen endotracheal tube.

Multimodal Analgesia

Fifteen minutes before the end of surgery, 100 mg flurbiprofen axetil was administered intravenously. Local infiltration with 10 mL of 0.75% ropivacaine was performed at the incision site. Postoperatively, patient-controlled analgesia (PCA) was provided using a pump (Zhejiang Smith Medical Instrument Co., Ltd., model ZB-100, Hangzhou,

China) containing sufentanil (1.5 µg/mL, 100 mL total), set for a 2 mL bolus dose with a 10-minute lockout and no background infusion. The total sufentanil consumption was calculated based on the actual volume delivered from the PCA pump (recorded to one decimal place), which may result in values that are not exact multiples of the single bolus dose.

Prevention of Postoperative Nausea and Vomiting (PONV)

All patients received 0.075 mg palonosetron (Hangzhou Jiuyuan Gene Engineering Co., Ltd., batch number 240312, Hangzhou, China) preoperatively. If PONV occurred, 0.625 mg droperidol (Jiangsu Nhwa Pharmaceutical Co., Ltd., batch number 240228, Xuzhou, China) was administered as rescue therapy. PONV was defined as any episode of nausea, retching, or vomiting, or the need for rescue antiemetic medication, within 48 hours after surgery. Severity was graded as mild (nausea only), moderate (1–2 vomiting episodes), or severe (≥3 episodes or requiring repeated rescue therapy).

Management of Adverse Events

In cases of intraoperative hemodynamic instability, vasoactive agents were administered after ruling out other potential causes. Norepinephrine was administered when mean arterial pressure (MAP) <65 mmHg, with an initial bolus of 4–8 µg, followed by repeated boluses or continuous infusion titrated to maintain MAP ≥65 mmHg. The same protocol was applied uniformly across all three groups. Transient hypotension requiring norepinephrine was prespecified as an expected hemodynamic effect of the anesthesia protocol rather than a serious adverse event, because it occurred within the context of the protocol and resolved with protocol-directed treatment. Respiratory depression was defined as respiratory rate <8 breaths/min or SpO₂ <90% requiring intervention. Delayed emergence was defined as failure to open eyes or respond to verbal commands within 30 minutes after the end of surgery.

Observational Indicators

The primary outcome was the relative NK cell count at 72 hours postoperatively (T3). Secondary outcomes included: (1) other immune cell subsets (CD3⁺, CD4⁺, CD8⁺, CD4⁺/CD8⁺ ratio, and CD3⁺ count); (2) inflammatory cytokines (IL2, IL4, IL6, IL10, TNF-α, IFN-γ); (3) perioperative hemodynamics (MAP and HR); (4) intraoperative parameters (duration of surgery and anesthesia, propofol dosage, norepinephrine dosage, time to eye opening, extubation time, and orientation recovery); (5) postoperative pain (VAS scores and sufentanil consumption); and (6) incidence of PONV.

Immune-Related Biomarker Measurements

The main results are as follows: Peripheral venous blood was collected at four time points: before surgery (T0), end of surgery (T1), 24 hours post-surgery (T2), and

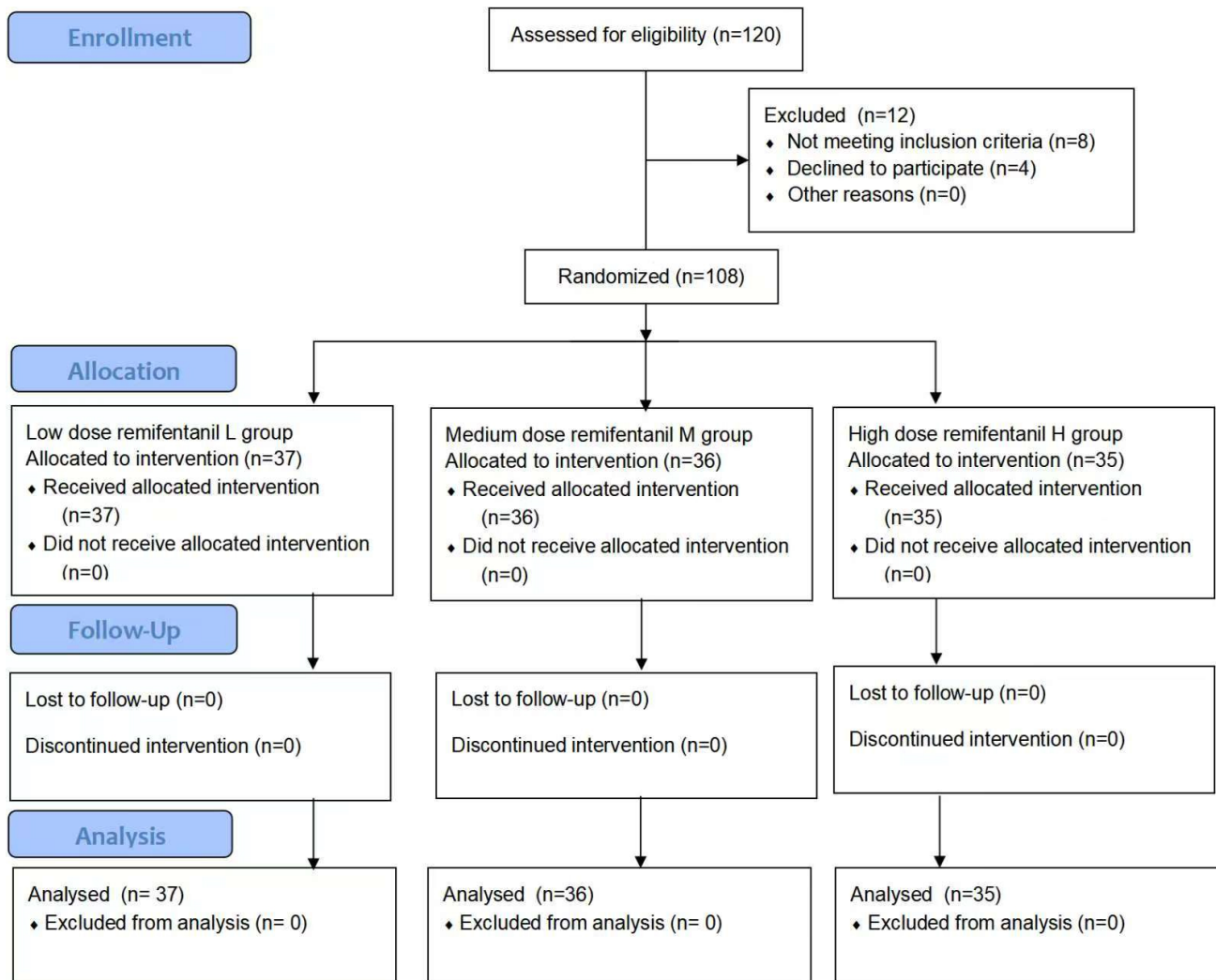


Fig. 1. Study flow chart.

72 hours post-surgery (T3). Serum levels of cytokines, including IL2 (H003-1-2), IL4 (H005-1-2), IL6 (H007-1-2), IL10 (H009-1-2), TNF- α (H052-1-2), and IFN- γ (H025-1-2), were measured using Enzyme-Linked Immunosorbent Assay (ELISA) kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), with absorbance detected using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Flow cytometry (CytoFLEX, Beckman Coulter, Suzhou Industrial Park, Suzhou, China) was used to analyze peripheral immune cell subsets. Briefly, peripheral blood samples were processed to obtain peripheral blood mononuclear cells (PBMCs) using density gradient centrifugation with Ficoll-Paque (GE Healthcare, Marlborough, MA, USA). Cells were washed and resuspended in PBS containing 2% fetal bovine serum. For immunophenotyping, cells were incubated with fluorochrome-conjugated monoclonal antibodies for 30 min at 4 °C in the dark. The following antibodies were used: CD3 (clone OKT3, FITC, 317306), CD4 (clone RPA-T4, PE, 300508), CD8 (clone RPA-T8, PerCP-Cy5.5, 344709), CD16 (clone 3G8,

APC, 302012), CD56 (clone HCD56, PE-Cy7, 318317) and CD45 (clone HI30, BV421, 304032). All antibodies were purchased from BioLegend (San Diego, CA, USA). After staining, cells were washed and immediately analyzed by flow cytometry. Data were acquired on a CytoFLEX flow cytometer and analyzed using CytExpert software (version 2.7, Beckman Coulter, CA, USA). Lymphocytes were first gated based on forward scatter (FSC) and side scatter (SSC) characteristics, followed by doublet exclusion and gating on CD45⁺ leukocyte populations. CD3⁺ T cells were further subdivided into CD4⁺ and CD8⁺ subsets. NK cells were defined as CD3⁻CD16⁺CD56⁺ populations after exclusion of CD3⁺ T cells. Absolute counts of NK cells and CD3⁺ T cells were determined using a single-platform flow cytometry assay with counting beads (CountBright™ Absolute Counting Beads, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. A known number of counting beads was added to each sample before acquisition. Absolute cell counts were calculated as follows: absolute cell count (cells/ μ L) = (number of ac-

quired target cells $\times$ total number of beads) / (number of acquired beads $\times$ sample volume). The absolute counts were then converted to $10^6/L$ for analysis. Quality control included daily instrument calibration and compensation using standardized beads, regular validation of the antibody panel with reference samples, and processing of all samples within 6 h of collection.

Secondary results are as follows:

Perioperative Hemodynamics

Mean arterial pressure (MAP) and heart rate (HR) were monitored at different time points during the operation.

Intraoperative Parameters

Intraoperative parameters included duration of surgery and anesthesia, total doses of remifentanyl, propofol, and norepinephrine, as well as time to eye opening, extubation time, and orientation recovery.

Postoperative Nausea and Vomiting (PONV)

The incidence of PONV was recorded at 24 and 48 hours after the operation.

Postoperative Pain Assessment

Postoperative pain was assessed using the Visual Analog Scale (VAS) at 4, 8, 24, and 48 hours after surgery. Sufentanil consumption via PCA was recorded at 24 and 48 hours. The VAS score ranged from 0 (no pain) to 10 (worst possible pain), with higher scores indicating greater pain intensity [20].

Statistical Analysis

Statistical analysis was performed using SPSS version 25.0 (IBM Corp., NY, USA). Normality of continuous variables was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. Data with normal distribution were presented as mean $\pm$ standard deviation (SD) and compared using one-way ANOVA or Welch ANOVA when variances were unequal. Non-normally distributed data were expressed as median (P_{25} , P_{75}) and analyzed using the Kruskal-Wallis H test. Repeated measures were evaluated using generalized estimating equations (GEE), with an exchangeable working correlation structure and robust sandwich standard errors. The identity link function and normal distribution were specified for continuous outcomes. Model parameters were estimated using generalized estimating equations. To assess the robustness of the repeated-measures results, three GEE models were constructed. Model 1 (primary analysis) was unadjusted. Model 2 (exploratory analysis) was adjusted for remifentanyl dosage, norepinephrine dosage, and total sufentanil consumption within 24 h postoperatively. Model 3 (exploratory analysis) was adjusted for duration of surgery, duration of anesthesia, and total sufentanil

consumption within 48 h postoperatively. Because these variables may lie on the causal pathway between randomized treatment and outcomes, results from Models 2 and 3 were interpreted as exploratory and hypothesis-generating only. No missing data were present for the primary and secondary immune outcomes, as all 108 enrolled patients completed the perioperative blood sampling protocol. Pair-wise comparisons among groups and time points were adjusted for multiple testing using the Bonferroni correction where appropriate for predefined comparisons. Categorical variables were expressed as counts (percentages) and compared using the χ^2 test or Fisher's exact test, as appropriate. Multiple comparisons were corrected using the Bonferroni or Tamhane T2 method. A two-sided p -value < 0.05 was considered statistically significant.

Sample Size Calculation

Based on a previous study [21], the relative NK cell count at 72 hours postoperatively was selected as the primary outcome for sample size estimation. In that study, the NK cell counts at 72 hours postoperatively were 13.07 ± 1.12 , 15.28 ± 1.33 , and 15.08 ± 1.32 in the three groups, respectively. Using PASS software (version 21.0.3; NCSS, LLC, Kaysville, UT, USA), with $\alpha = 0.05$, power = 0.80, and $k = 3$ groups, the required sample sizes were calculated as 18, 21, and 21 patients for the three groups, respectively. To ensure adequate statistical power across all groups and maintain a balanced design for implementation and analysis, the largest calculated sample size among the three groups (21 patients) was adopted as the baseline sample size for each group. Considering an anticipated dropout rate of 20%, the final sample size was increased to 27 patients per group.

Results

Baseline Characteristics and Intraoperative Parameters

Patient recruitment is illustrated in Fig. 1. A total of 120 patients were eligible for this study, of whom 4 declined to participate and 8 did not meet the inclusion criteria. Ultimately, 108 patients were included in the analysis. Aside from tumor type data (L: 1 [2.70%]; M: 5 [13.89%]; H: 6 [17.14%]), all other baseline characteristics and the available tumor type data were comparable among the three groups, suggesting overall baseline comparability ($p > 0.05$, Table 1). In terms of intraoperative parameters, the total intraoperative remifentanyl dose increased in a stepwise manner from the L to the H group ($p < 0.001$, Table 1). The norepinephrine dose was also significantly higher in the H group than in the L and M groups ($p = 0.003$, Table 1), and was therefore accounted for in subsequent adjusted analyses.

Table 1. Comparison of baseline characteristics.

Characteristics	L group (n = 37)	M group (n = 36)	H group (n = 35)	χ^2/F	<i>p</i>
Gender [n (%)]				0.751	0.687
Male	13 (35.14)	16 (44.44)	15 (42.86)		
Female	24 (64.86)	20 (55.56)	20 (57.14)		
Age (year)	60.14 ± 11.55	56.33 ± 11.24	59.46 ± 11.35	1.149	0.321
BMI (kg/m ²)	22.29 ± 2.88	22.02 ± 2.48	22.79 ± 2.46	0.778	0.462
Type of tumor [n (%)]					0.144*
Adenocarcinoma	33 (89.19)	30 (83.33)	25 (71.43)		
Squamous carcinoma	3 (8.11)	1 (2.78)	4 (11.43)		
Unregistered	1 (2.70)	5 (13.89)	6 (17.14)		
Hyperlipemia [n (%)]					0.239*
No	35 (94.59)	36 (100.00)	32 (91.43)		
Yes	2 (5.41)	0 (0.00)	3 (8.57)		
Hypertension [n (%)]				0.026	0.987
No	24 (64.86)	23 (63.89)	23 (65.71)		
Yes	13 (35.14)	13 (36.11)	12 (34.29)		
Diabetes [n (%)]					0.599*
No	32 (86.49)	33 (91.67)	33 (94.29)		
Yes	5 (13.51)	3 (8.33)	2 (5.71)		
Coronary heart disease [n (%)]					0.465*
No	35 (94.59)	36 (100.00)	33 (94.29)		
Yes	2 (5.41)	0 (0.00)	2 (5.71)		
Hepatopathy [n (%)]				1.374	0.503
No	26 (70.27)	29 (80.56)	28 (80.00)		
Yes	11 (29.73)	7 (19.44)	7 (20.00)		
Duration of surgery (min)	120.00 (85.00, 160.00)	117.50 (93.50, 150.00)	100.00 (82.50, 136.00)	2.004	0.367
Duration of anesthesia (min)	150.00 (120.00, 200.00)	150.00 (122.25, 186.25)	150.00 (120.00, 170.00)	0.581	0.748
Remifentanyl dosage (μg)	1346.38 ± 712.96	2243.78 ± 1149.64 ^a	4370.06 ± 1342.27 ^{ab}	70.076	<0.001
Propofol dosage (mg)	890.00 (640.00, 1220.00)	900.00 (684.75, 1262.50)	1000.00 (800.00, 1110.00)	1.153	0.562
Norepinephrine dosage (μg)	0.00 (0.00, 80.00)	0.00 (0.00, 90.00)	240.00 (0.00, 410.00) ^{ab}	11.511	0.003

Notes: ^a*p* < 0.05 vs. Low dose remifentanyl group; ^b*p* < 0.05 vs. Medium dose remifentanyl group. *Fisher's exact probability method.

Abbreviation: BMI, body mass index; L, Low dose remifentanyl; M, Medium dose remifentanyl; H, High dose remifentanyl.

Group and Time Effects on Postoperative Immune Responses

As shown in Table 2 and **Supplementary Fig. 1**, immune cell profiles were assessed at four time points (T0: before surgery; T1: end of surgery; T2: 24 h post-surgery; T3: 72h post-surgery). At T3, CD4⁺ (%) was significantly lower in the H groups than in the M group (*p* < 0.05, Table 2 and **Supplementary Fig. 1B**). Repeated-measures analysis indicated significant effects of time on CD4⁺ (%), CD8⁺ (%) and CD4⁺/CD8⁺ (*p* < 0.001) (Table 2). No significant group effects were observed for CD3⁺ (%), CD4⁺ (%), CD8⁺ (%), or CD4⁺/CD8⁺ (*p* > 0.05, Table 2). Significant group × time interactions were observed for CD4⁺ (%) (*p* = 0.015), CD8⁺ (%) (*p* = 0.006), indicating differential immune recovery among the three groups over time (Table 2). To further assess robustness, three repeated-measures

models were constructed (Table 3). No significant group effects were observed for CD3⁺, CD4⁺, CD8⁺ or CD4⁺/CD8⁺ in any model (*p* > 0.05, Table 3). Significant time effects were observed for CD4⁺, CD8⁺ and CD4⁺/CD8⁺ ratio (*p* < 0.001), indicating postoperative changes over time (Table 3). Furthermore, significant group × time interactions were found for CD4⁺ and CD8⁺ in all models (*p* < 0.05), suggesting differential recovery patterns of immune parameters among the three groups across time points (Table 3).

Group and Time Effects on Postoperative Inflammatory Indicators

As displayed in Table 4 and **Supplementary Fig. 2**, IL2, IL4, IL6, IL10, TNF-α, IFN-γ, CD3⁺ counts and NK cell counts were assessed at different time points (T0: before surgery; T1: end of surgery; T2: 24h post-surgery;

Table 2. Comparison of postoperative immune parameters among the three groups.

Item	N	Time	CD3 ⁺ (%)	CD4 ⁺ (%)	CD8 ⁺ (%)	CD4 ⁺ /CD8 ⁺
L	37	T0	71.40 (61.67, 81.10)	42.97 (35.17, 47.07)	22.34 (16.11, 28.60)	1.76 (1.54, 2.07)
		T1	69.26 (59.10, 76.59)	39.97 (34.48, 47.73)	19.17 (16.13, 26.23)	2.07 (1.37, 2.65)
		T2	69.60 (62.33, 76.21)	40.75 (35.63, 47.03)	20.27 (16.07, 26.11)	1.88 (1.66, 2.64)
		T3	72.12 (60.34, 83.00)	42.53 (39.63, 50.76) ^{*+^}	18.45 (14.00, 28.31)	2.12 (1.75, 2.98) ^{*^}
M	36	T0	74.13 (64.81, 82.27)	41.64 (35.18, 51.56)	24.66 (20.20, 30.00)	1.53 (1.32, 2.78)
		T1	69.52 (60.50, 80.48)	44.76 (36.77, 51.18)	20.80 (17.05, 25.67) [*]	2.07 (1.77, 2.65) [*]
		T2	70.47 (60.78, 79.35)	44.15 (35.77, 51.96)	21.69 (17.11, 24.90) [*]	2.09 (1.55, 2.74) [*]
		T3	69.26 (62.08, 76.48)	48.88 (40.10, 58.00) ^{*+^}	24.04 (18.89, 28.12) ^{*+^}	2.33 (1.62, 2.79) [*]
H	35	T0	71.37 (59.73, 77.18)	39.63 (36.72, 45.82)	21.66 (17.01, 29.64)	1.95 (1.30, 2.42)
		T1	69.31 (58.40, 80.09)	43.20 (35.51, 50.74)	18.53 (15.69, 24.92) [*]	2.30 (1.55, 2.92) [*]
		T2	71.27 (59.08, 77.46)	43.17 (35.54, 52.95)	20.12 (16.53, 26.10) [*]	2.00 (1.67, 2.64) [*]
		T3	68.79 (58.49, 79.58)	40.34 (35.66, 48.44) ^b	19.89 (16.41, 26.52) [*]	1.83 (1.48, 2.73) [*]
Wald χ^2_{group}			0.410	2.033	0.586	0.313
p_{group}			0.815	0.362	0.746	0.855
Wald χ^2_{time}			5.183	18.615	50.828	49.091
p_{time}			0.159	<0.001	<0.001	<0.001
Wald $\chi^2_{\text{group*time}}$			8.703	15.596	18.258	10.342
$p_{\text{group*time}}$			0.191	0.015	0.006	0.111

Notes: ^b $p < 0.05$ vs. Medium dose remifentanyl group at the same time point; * $p < 0.05$ vs. T0 within the same group; ⁺ $p < 0.05$ vs. T1 within the same group; [^] $p < 0.05$ vs. T2 within the same group.

Abbreviation: T0, before surgery; T1, at the end of surgery; T2, 24 hours post-surgery; T3, 72 hours post-surgery; L, low dose remifentanyl; M, medium dose remifentanyl; H, high dose remifentanyl; CD, cluster of differentiation.

T3: 72h post-surgery). At T2, the H group exhibited significantly reduced levels of IL4, IL10, TNF- α , and IFN- γ compared with the L and M groups ($p < 0.05$, **Supplementary Fig. 2B,D–F** and Table 4). At T3, the level of IL2, IL6, IL10 and IFN- γ was significantly lower in the H group than in the L and M groups ($p < 0.05$, **Supplementary Fig. 2A,C,D,F** and Table 2). At T1, the level of IFN- γ in the H group was significantly lower than that in the L and M groups ($p < 0.001$, **Supplementary Fig. 2F** and Table 4). At T3, NK counts were significantly higher in the M and H groups than in the L group ($p < 0.05$, **Supplementary Fig. 2H** and Table 4). For the primary outcome, the relative NK cell count at 72 h is explicitly stated as L group vs. H group (mean difference, -3.152 ; 95% CI, -5.870 to -0.434). The corresponding comparison between the L group and M group was also significant (mean difference, -4.521 ; 95% CI, -8.095 to -0.948). Between-group comparisons at T0, T1, T2, and T3 are presented in **Supplementary Table 1**. Similarly, at T2, NK cell counts in the H group were significantly higher than in the L group ($p < 0.01$, **Supplementary Fig. 2H** and Table 4). Significant group effects were observed for IL2, IL4, IL6, IL10, TNF- α , IFN- γ and NK cell counts ($p < 0.05$), whereas CD3⁺ count showed no significant group effect ($p = 0.941$) (Table 4). Significant time effects were observed for IL4, IL6, IL10, IFN- γ , CD3⁺ count, and NK cell counts ($p < 0.05$), whereas IL2 and TNF- α did not show significant time-dependent changes ($p > 0.05$) (Table 4). Furthermore, significant group $\times$ time interactions were found for IL4, IL6, IL10, IFN- γ , CD3⁺ counts

and NK cell counts ($p < 0.05$, Table 4), indicating differential inflammatory response patterns among the three groups over time. The main effect analysis revealed significant between-group differences in IL2, IL4, IL6, IL10, TNF- α , and IFN- γ across the three models ($p < 0.05$, Table 5). In addition, NK cell counts showed a significant group effect in Models 1 and 2 ($p < 0.05$), whereas CD3⁺ counts did not differ significantly among groups in any model ($p > 0.05$). For the time effect, IL4, IL6, IL10, and IFN- γ showed significant changes over time ($p < 0.05$), whereas IL2 and TNF- α did not exhibit significant time-dependent variation ($p > 0.05$, Table 5). Moreover, CD3⁺ counts and NK cell counts demonstrated significant time effects across all models ($p < 0.001$), indicating dynamic postoperative changes (Table 5). Regarding the interaction effect, IL4, IL6, IL10, IFN- γ , CD3⁺ counts and NK cell counts demonstrated significant group $\times$ time interactions ($p < 0.05$, Table 5), indicating that their postoperative temporal trends differed among the three remifentanyl dose groups. In contrast, IL2 and TNF- α showed no significant interaction effects ($p > 0.05$, Table 5).

Perioperative Hemodynamics

MAP and HR were measured before anesthesia induction, before skin incision, after skin incision, 60 minutes after the start of surgery, at the end of surgery, and at extubation. 60 minutes after the start of surgery, MAP was higher in the L group than in the M group ($p < 0.05$, Table 6 and

Table 3. Main effects of immune parameters among the three groups.

Parameter	Group effect		Time effect		Group $\times$ time interaction	
	Wald	<i>p</i>	Wald	<i>p</i>	Wald	<i>p</i>
	χ^2		χ^2		χ^2	
Model 1						
CD3 ⁺	0.410	0.815	5.183	0.159	8.703	0.191
CD4 ⁺	2.033	0.362	18.615	<0.001	15.596	0.016
CD8 ⁺	0.586	0.746	50.828	<0.001	18.258	0.006
CD4 ⁺ /CD8 ⁺	0.313	0.855	49.091	<0.001	10.342	0.111
Model 2						
CD3 ⁺	2.489	0.288	5.183	0.159	8.703	0.191
CD4 ⁺	2.708	0.258	18.615	<0.001	15.596	0.016
CD8 ⁺	1.316	0.518	50.828	<0.001	18.258	0.006
CD4 ⁺ /CD8 ⁺	0.160	0.923	49.091	<0.001	10.342	0.111
Model 3						
CD3 ⁺	0.416	0.812	5.183	0.159	8.703	0.191
CD4 ⁺	2.004	0.367	18.615	<0.001	15.596	0.016
CD8 ⁺	0.452	0.798	50.828	<0.001	18.258	0.006
CD4 ⁺ /CD8 ⁺	0.051	0.975	49.091	<0.001	10.342	0.111

Notes: Model 1 was unadjusted for confounding factors; Model 2 was adjusted for remifentanyl dosage, norepinephrine dosage, and total sufentanil consumption within 24 h postoperatively; Model 3 was adjusted for duration of surgery, duration of anesthesia, and total sufentanil consumption within 48 h postoperatively.

Abbreviation: CD, cluster of differentiation.

Supplementary Fig. 3A). At extubation, MAP was higher in the H group than in the M group ($p < 0.05$, Table 6 and **Supplementary Fig. 3A**). At the end of surgery, HR in the M group was lower than that in the L group ($p < 0.01$, Table 6 and **Supplementary Fig. 3B**). At the remaining time points, no statistically significant differences in MAP or HR were found among the three groups ($p > 0.05$, Table 6 and **Supplementary Fig. 3**). Both MAP and HR showed significant changes over time ($p < 0.001$, Table 6) and significant group-by-time interactions ($p < 0.001$, Table 6). The norepinephrine usage rate was higher in the H group than in the L and M groups ($p < 0.05$; **Supplementary Table 2**).

Incidence of PONV and Recovery Times

The incidences of PONV at 24 and 48 hours postoperatively were 5.41% and 5.41% in the L group, 8.33% and 5.56% in the M group, and 5.71% and 8.57% in the H group, respectively (Table 7). No significant differences in PONV incidence were observed among the three groups at either time point ($p > 0.05$, Table 7). There were no significant differences among the three groups in terms of time to eye opening, extubation time, or time to orientation recovery ($p > 0.05$, Table 8).

Postoperative Pain

Postoperative pain was evaluated using VAS scores at 4, 8, 24, and 48 hours after surgery (**Supplementary Fig. 4A**). The H group showed significantly lower VAS scores at 4 and 48 hours postoperatively compared to the L group ($p < 0.05$, Table 9 and **Supplementary Fig. 4A**). No significant differences were observed at 8 and 24 hours (Table 9 and **Supplementary Fig. 4A**). Sufentanil consumption at 24 hours postoperatively was significantly lower in the M group compared to the L group ($p < 0.05$), whereas no significant differences were noted among the groups at 48 hours (Table 9 and **Supplementary Fig. 4B**). Repeated-measures analysis indicated significant effects of time on both VAS and SF, and a significant group effect on VAS, but no significant group $\times$ time interactions (Table 9). These findings suggest that high-dose remifentanyl may be associated with a modest and time-limited analgesic effect, but the clinical relevance of this observation remains uncertain.

Adverse Event

No serious adverse events, including respiratory depression, delayed emergence, or malignant arrhythmias, were observed in any group throughout the study period. Transient intraoperative hypotension requiring norepinephrine administration was managed according to the standardized protocol and was not classified as an adverse event.

Discussion

This study evaluated the impact of different doses of remifentanyl combined with propofol anesthesia on perioperative immune-related biomarkers, inflammation, postoperative recovery, and analgesia in patients undergoing radical lung cancer resection. Overall, only selected immune and inflammatory parameters exhibited significant between-group differences, while most clinical recovery outcomes remained comparable. The findings suggest that high-dose remifentanyl (16 ng/mL), when combined with propofol, was associated with changes in specific immune and inflammatory markers and modest improvement in early postoperative analgesia; however, these effects were not consistently observed across all parameters and time points. Importantly, no significant differences were observed in broader clinical recovery outcomes or safety profiles, including the incidence of postoperative nausea and vomiting, which is consistent with our previous findings [22].

Natural killer (NK) cells are pivotal components of the innate immune system and play essential roles in tumor surveillance and early defense against malignancy [7,23]. Surgical stress is known to suppress NK cell activity postoperatively. In our study, NK cell counts decreased after surgery in all groups, consistent with previous reports. Notably, NK cell counts—one of the few immune param-

Table 4. Comparison of postoperative inflammatory indicators among the three groups.

Group	N	Time	IL2 (pg/ mL)	IL4 (pg/ mL)	IL6 (pg/mL)	IL10 (pg/ mL)	TNF- α (pg/mL)	IFN- γ (pg/ mL)	CD3 ⁺ count (10 ⁶ /L)	NK count (10 ⁶ /L)	NK (%)					
L	37	T0	2.65 (1.90, 2.97)	1.05 (0.45, 1.45)	3.91 (3.25, 5.50)	2.40 (1.81, 2.80)	4.19 (3.24, 5.00)	7.96 (4.25, 10.77)	1217.00 (884.00, 1510.00)	253.00 (156.00, 338.00)	14.27 (9.33, 22.08)					
			T1	2.24 (1.62, 2.46)	0.89 (0.67, 1.21)	38.45 (24.20, 59.22)*	3.30 (2.55, 5.20)	4.34 (3.10, 4.89)	8.10 (4.35, 10.77)	463.00 (284.00, 669.00)*	91.00 (43.00, 254.00)*	13.47 (10.00, 20.49)				
				T2	2.25 (1.67, 2.57)	1.19 (0.72, 1.53)	29.49 (22.46, 51.81)*	4.72 (3.32, 6.50)*	4.33 (3.67, 4.83)	8.75 (3.68, 10.55)	607.00 (376.00, 856.00)*	113.00 (79.00, 143.00)*	10.25 (6.24, 16.60)**			
		T3			2.45 (2.01, 3.12)	1.16 (0.69, 1.56)	28.92 (24.23, 53.20)*	3.50 (2.72, 4.32)*	4.29 (3.49, 4.73)	9.08 (3.28, 10.20)	725.00 (506.00, 1034.00)**	125.00 (76.00, 165.00)*	10.35 (6.94, 14.48)**			
			M		36	T0	2.49 (1.67, 3.50)	0.71 (0.34, 1.45)	5.47 (4.19, 6.00)	2.73 (1.89, 3.61)	3.85 (3.33, 5.24)	9.69 (5.47, 12.31)	1112.00 (797.50, 1422.50)	238.50 (161.75, 328.50)	14.68 (10.47, 21.68)	
				T1			2.30 (1.82, 3.05)	1.06 (0.95, 1.32)	30.73 (23.70, 40.52)*	3.62 (2.77, 6.09)	4.10 (3.49, 4.75)	9.53 (3.29, 11.54)	464.50 (351.25, 620.50)*	78.50 (47.25, 120.00)*	12.42 (9.20, 15.88)	
		T2					2.65 (2.00, 3.13)	1.35 (0.58, 2.02)**	26.67 (23.40, 37.77)*	3.80 (2.98, 4.61)*	4.06 (3.59, 4.99)	11.19 (5.86, 13.52) ⁺	672.50 (570.75, 858.25)**	117.50 (72.75, 176.50)**	9.34 (7.27, 13.49)*	
						T3	2.45 (1.74, 3.14)	1.15 (0.42, 1.64) [^]	28.74 (20.82, 41.45)*	2.90 (2.42, 3.54)**	4.35 (3.61, 5.05)	10.43 (6.47, 12.01) [^]	845.50 (725.00, 992.25)**	164.50 (101.75, 226.00)**	12.97 (9.83, 20.35) ^a	
				H			35	T0	2.18 (1.82, 2.65)	0.40 (0.30, 0.60)	4.28 (3.25, 5.80)	1.98 (1.37, 2.45)	3.91 (3.15, 4.72)	5.69 (4.53, 8.16)	1275.00 (1033.50, 1479.00)	316.00 (221.00, 439.50)
		T1							1.99 (1.65, 2.21) ^b	0.53 (0.35, 1.21) ^b	28.36 (20.09, 36.41) ^{a*}	3.21 (2.40, 4.96)*	3.61 (3.19, 4.14)	2.78 (2.31, 5.90) ^{ab*}	485.00 (350.50, 749.50)*	103.00 (66.00, 156.00)*
						T2			2.06 (1.66, 2.38) ^b	0.54 (0.38, 1.06) ^{ab}	25.67 (14.86, 40.11)*	3.19 (1.80, 4.21) ^{ab*+}	3.79 (3.08, 4.12) ^{ab}	2.85 (2.39, 5.72) ^{ab*}	663.00 (590.50, 844.00)*	168.00 (118.50, 296.50) ^{a*}
								T3	1.88 (1.69, 2.25) ^{ab}	0.41 (0.32, 1.31) ^a	11.26 (4.08, 15.88) ^{ab*+^}	1.80 (0.85, 3.30) ^{ab+^}	3.69 (3.29, 4.21) ^a	2.88 (2.47, 6.11) ^{ab*^}	793.00 (580.00, 908.00)* ⁺	197.00 (100.50, 264.50) ^{a*}
Wald χ^2_{group}									17.162	11.344	13.435	15.080	9.566	19.891	0.122	7.644
p_{group}						<0.001			0.003	0.001	<0.001	0.008	<0.001	0.941	0.022	0.459
Wald χ^2_{time}						5.465		11.033	389.667	84.888	0.501	74.382	233.092	118.546	30.295	
p_{time}						0.141		0.012	<0.001	<0.001	0.919	<0.001	<0.001	<0.001	<0.001	
Wald			9.970		16.833	68.198		30.145	8.346	32.190	27.762	25.715	40.011			
$\chi^2_{\text{group*time}}$																
$p_{\text{group*time}}$			0.126		0.010	<0.001		<0.001	0.214	<0.001	<0.001	<0.001	<0.001			

Notes: ^a $p < 0.05$ vs. Low dose remifentanyl group at the same time point; ^b $p < 0.05$ vs. Medium dose remifentanyl group at the same time point; ^{*} $p < 0.05$ vs. T0 within the same group; ⁺ $p < 0.05$ vs. T1 within the same group; [^] $p < 0.05$ vs. T2 within the same group.

Abbreviation: T0, before surgery; T1, at the end of surgery; T2, 24 hours post-surgery; T3, 72 hours post-surgery; L, low dose remifentanyl; M, medium dose remifentanyl; H, high dose remifentanyl; IL, interleukin; TNF- α , tumor necrosis factor- α ; IFN- γ , interferon- γ ; NK, natural killer.

Table 5. Main effects of inflammatory indicators among the three groups.

Parameter	Group effect		Time effect		Group × time interaction	
	Wald χ^2	<i>p</i>	Wald χ^2	<i>p</i>	Wald χ^2	<i>p</i>
Model 1						
IL2	17.162	<0.001	5.465	0.141	9.970	0.126
IL4	11.344	0.003	11.033	0.012	16.833	0.010
IL6	13.435	0.001	389.667	<0.001	68.198	<0.001
IL10	15.080	<0.001	84.888	<0.001	30.145	<0.001
TNF- α	9.566	0.008	0.501	0.919	8.346	0.214
IFN- γ	19.891	<0.001	74.382	<0.001	32.190	<0.001
CD3 ⁺ count	0.122	0.941	233.092	<0.001	27.762	<0.001
NK count	7.644	0.022	118.546	<0.001	25.715	<0.001
NK	1.559	0.459	30.295	<0.001	40.011	<0.001
Model 2						
IL2	16.415	<0.001	5.465	0.141	9.970	0.126
IL4	18.281	<0.001	11.033	0.012	16.833	0.010
IL6	21.001	<0.001	389.667	<0.001	68.198	<0.001
IL10	15.241	<0.001	84.888	<0.001	30.145	<0.001
TNF- α	13.594	0.001	0.501	0.919	8.346	0.214
IFN- γ	21.575	<0.001	74.382	<0.001	32.190	<0.001
CD3 ⁺ count	0.149	0.928	233.092	<0.001	27.762	<0.001
NK count	9.702	0.008	118.546	<0.001	25.715	<0.001
NK	2.637	0.268	30.295	<0.001	40.011	<0.001
Model 3						
IL2	13.560	0.001	5.465	0.141	9.970	0.126
IL4	8.070	0.018	11.033	0.012	16.833	0.010
IL6	9.138	0.010	389.667	<0.001	68.198	<0.001
IL10	11.038	0.004	84.888	<0.001	30.145	<0.001
TNF- α	9.670	0.008	0.501	0.919	8.346	0.214
IFN- γ	23.008	<0.001	74.382	<0.001	32.190	<0.001
CD3 ⁺ count	0.097	0.953	233.092	<0.001	27.762	<0.001
NK count	5.491	0.064	118.546	<0.001	25.715	<0.001
NK	1.116	0.572	30.295	<0.001	40.011	<0.001

Notes: Model 1 was unadjusted for confounding factors; Model 2 was adjusted for remifentanyl dosage, norepinephrine dosage, and total sufentanil consumption within 24 h postoperatively; Model 3 was adjusted for duration of surgery, duration of anesthesia, and total sufentanil consumption within 48 h postoperatively.

Abbreviation: IL, interleukin; TNF- α , tumor necrosis factor- α ; IFN- γ , interferon- γ ; CD, cluster of differentiation; NK, natural killer.

ters showing a significant group effect—were higher in the high- and medium-dose remifentanyl groups at 24 and 72 hours postoperatively than in the low-dose group. This observation may reflect modulation of the perioperative stress response; however, the clinical significance of this difference remains uncertain. The potential mechanism may involve attenuation of neuroendocrine stress responses and reduction of pro-inflammatory cytokines [24], although further mechanistic validation is required.

Regarding T lymphocyte subsets, most parameters, including CD3⁺ (%), CD8⁺ (%), CD4⁺/CD8⁺ ratio, and CD3⁺ counts, did not show consistent between-group differences, although time-dependent postoperative changes were observed. The observed changes over time likely reflect the transient impact of surgical stress on T cell populations rather than the effects of remifentanyl dose. These findings suggest that varying remifentanyl doses did not exert a clear or consistent influence on T cell-mediated immunity during the perioperative period. Given the importance of T lymphocytes in tumor immune surveillance, the lack of significant between-group differences in these parameters further limits the interpretation of any clinically meaningful immunomodulatory effect.

The study also revealed complex but clinically relevant changes in inflammatory cytokines. IL10, a key anti-inflammatory cytokine, and IFN- γ , a pro-inflammatory and immune-activating cytokine, were both significantly reduced in the high-dose group at 24 and 72 hours postoperatively. While these findings may suggest modulation of the inflammatory balance, the simultaneous reduction in both pro- and anti-inflammatory mediators makes the overall immunological interpretation complex and uncertain. Additionally, levels of IL6 and TNF- α —major mediators of acute surgical stress—were markedly reduced in the high-dose group, consistent with the anti-inflammatory properties of remifentanyl reported in prior studies [25,26]. However, it should be emphasized that these biomarkers represent surrogate endpoints, and their relationship with clinically meaningful outcomes remains unclear.

One of the concerns with high-dose opioid use is opioid-induced hyperalgesia and delayed recovery. In our study, patients in the high-dose group reported lower VAS scores at 4 and 48 hours postoperatively, and no signs of opioid-induced hyperalgesia were observed. These findings may be attributed to the multimodal analgesia protocol, which included flurbiprofen and incision site infiltration with ropivacaine. This aligns with literature indicating that combining non-opioid analgesics with regional techniques can mitigate opioid-related adverse effects [27]. Although these findings suggest a possible modest and time-limited analgesic effect, the clinical relevance of this observation remains uncertain.

Furthermore, no differences were observed among the groups in awakening time, extubation time, or recovery of orientation, and no patients experienced respiratory de-

Table 6. Perioperative hemodynamics in the three groups of patients.

Group	N	Time	MAP (mmHg)	HR (bpm)
L	37	Pre-anesthesia induction	104.00 (94.00, 111.00)	73.00 (67.00, 80.00)
		Pre-incision	78.00 (72.00, 85.00) [#]	59.00 (52.00, 65.00) [#]
		Post-incision	85.00 (76.00, 92.00) [#]	60.00 (53.00, 66.00) [#]
		60 minutes into the surgery	82.00 (77.00, 85.00) [#]	60.00 (55.00, 67.00) [#]
		End of surgery	85.00 (77.00, 88.00) [#]	62.00 (58.00, 72.00) [#]
		At extubation	97.00 (90.00, 105.00) [#]	71.00 (63.00, 80.00)
M	36	Pre-anesthesia induction	104.00 (98.00, 108.00)	76.00 (70.00, 83.25)
		Pre-incision	79.00 (70.75, 83.00) [#]	56.00 (51.75, 61.25) [#]
		Post-incision	82.00 (72.75, 86.25) [#]	56.50 (52.00, 63.25) [#]
		60 minutes into the surgery	80.00 (75.00, 85.00) ^{a#}	59.50 (55.00, 65.25) [#]
		End of surgery	88.50 (82.00, 95.25) [#]	60.00 (54.00, 62.25) ^{a#}
		At extubation	94.50 (87.75, 99.00) [#]	70.00 (64.25, 78.00) [#]
H	35	Pre-anesthesia induction	105.00 (99.50, 111.00)	72.00 (66.00, 77.50)
		Pre-incision	76.00 (69.50, 83.00) [#]	58.00 (53.50, 64.50) [#]
		Post-incision	85.00 (74.00, 91.50) [#]	60.00 (55.00, 64.00) [#]
		60 minutes into the surgery	85.00 (76.00, 88.00) [#]	61.00 (58.00, 66.00) [#]
		End of surgery	87.00 (80.00, 90.00) [#]	63.00 (56.50, 65.50) [#]
		At extubation	98.00 (90.00, 103.50) ^b	70.00 (65.00, 78.00)
Wald χ^2_{group}			0.680	0.952
p_{group}			0.712	0.621
Wald χ^2_{time}			905.866	610.306
p_{time}			<0.001	<0.001
Wald $\chi^2_{\text{group*time}}$			52.313	35.435
$p_{\text{group*time}}$			<0.001	<0.001

Notes: ^a $p < 0.05$ vs. Low dose remifentanyl group; ^b $p < 0.05$ vs. Medium dose remifentanyl group.

[#] $p < 0.05$ vs. the pre-anesthesia.

Abbreviation: L, low dose remifentanyl; M, medium dose remifentanyl; H, high dose remifentanyl; MAP, mean arterial pressure; HR, heart rate.

Table 7. Comparison of the incidence of PONV in groups H, M and L at 24 hours and 48 hours post-operation [n (%)].

Item	N	Postoperative 24 h	Postoperative 48 h
L	37	2 (5.41)	2 (5.41)
M	36	3 (8.33)	2 (5.56)
H	35	2 (5.71)	3 (8.57)
p		0.896*	0.794*

Notes: *Fisher's exact probability method.

Abbreviation: L, Low dose remifentanyl; M, Medium dose remifentanyl; H, High dose remifentanyl.

pression or other serious adverse events. The high-dose group required more norepinephrine, indicating a dose-related hemodynamic effect. Therefore, within the dosing range used in this study, high-dose remifentanyl should not be simply regarded as safe; its hemodynamic effects require further evaluation under controlled anesthetic conditions. The absence of differences in these clinically relevant recovery endpoints further suggests that the overall impact of remifentanyl dose on perioperative recovery may be limited. This finding is consistent with the known pharmacoki-

netic profile of remifentanyl, which is characterized by rapid clearance and minimal residual sedation [28].

Overall, our findings support the possibility that high-dose remifentanyl combined with propofol may confer perioperative benefits in some immune, inflammatory, and analgesic outcomes; however, high-dose remifentanyl was also associated with greater norepinephrine requirement, and no clear dose-response relationship was established. Importantly, this study was designed as a randomized, double-blind trial to characterize perioperative immune-inflammatory changes associated with different remifentanyl doses. Rather than establishing causal mechanisms, the findings provide descriptive clinical evidence of immune modulation in the context of opioid-based anesthesia. While the observed changes in NK cell counts and cytokine profiles appear transient and time-dependent, they may reflect complex neuro-immune interactions during surgical stress and anesthetic exposure. Therefore, the current results should be interpreted as hypothesis-generating evidence that may inform future mechanistic studies rather than immediate modification to clinical practice.

Table 8. Comparison of the recovery indicators among the three groups (min).

Item	N	Time to eye-opening	Extubation time	Time to orientation recovery
L	37	9.00 (5.00, 13.00)	10.00 (10.00, 15.00)	15.00 (10.00, 18.00)
M	36	8.00 (5.75, 12.00)	10.00 (7.00, 15.00)	12.00 (10.00, 16.25)
H	35	10.00 (7.00, 15.00)	13.00 (9.50, 20.00)	15.00 (10.00, 20.00)
<i>H</i>		2.340	3.789	2.436
<i>p</i>		0.310	0.150	0.296

Abbreviation: L, Low dose remifentanyl; M, Medium dose remifentanyl; H, High dose remifentanyl; *H*, *H*-statistic of the Kruskal-Wallis *H* test.

Table 9. Comparison of postoperative pain among the three groups.

Item	N	VAS (score)				SF (μg)	
		Postoperative 4 h	Postoperative 8 h	Postoperative 24 h	Postoperative 48 h	Postoperative 24 h	Postoperative 48 h
L	37	1.00 (1.00, 2.00)	1.00 (0.00, 2.00)	1.00 (1.00, 1.00)	1.00 (0.00, 1.00)	6.00 (3.00, 18.00)	15.00 (9.00, 30.00)
M	36	1.00 (0.75, 1.25)	1.00 (0.75, 2.00)	1.00 (1.00, 2.00)	1.00 (0.00, 1.00)	6.00 (3.00, 9.75) ^a	15.50 (11.25, 24.00)
H	35	1.00 (0.00, 1.00) ^a	0.00 (0.00, 1.00)	1.00 (0.00, 1.00)	0.00 (0.00, 0.50) ^a	9.00 (6.00, 15.00)	18.00 (10.50, 24.00)
Wald χ^2		6.436	4.449	3.296	7.926	9.974	2.642
<i>p</i>		0.040	0.108	0.192	0.019	0.007	0.267

Notes: ^a*p* < 0.05 vs. Low dose remifentanyl group.

Abbreviation: L, Low dose remifentanyl; M, Medium dose remifentanyl; H, High dose remifentanyl; VAS, visual analogue scale; SF, sufentanil.

VAS: Wald $\chi^2_{\text{group}} = 8.282$, *p* = 0.016, Wald $\chi^2_{\text{time}} = 43.966$, *p* < 0.001, Wald $\chi^2_{\text{group*time}} = 4.321$, *p* = 0.633;

SF: Wald $\chi^2_{\text{group}} = 5.100$, *p* = 0.078, Wald $\chi^2_{\text{time}} = 86.513$, *p* < 0.001, Wald $\chi^2_{\text{group*time}} = 1.068$, *p* = 0.586.

This study has several limitations. First, the sample size was relatively small and derived from a single center, which may limit the generalizability of the findings. In addition, the allocation into three groups further reduced the statistical power for detecting between-group differences. Second, the follow-up period was limited to 72 hours postoperatively; thus, long-term immunological outcomes and cancer recurrence were not assessed. Therefore, the observed changes in immune cell counts and cytokines represent surrogate endpoints and may not directly reflect functional immune competence or clinically meaningful outcomes. Lastly, the effect of potential confounding factors, such as psychological stress or genetic variations, was not evaluated. Although statistical adjustments were applied for predefined comparisons, the residual risk of type I error due to multiple testing across biomarkers and time points cannot be fully excluded. Therefore, the observed significant findings should be interpreted cautiously and considered hypothesis-generating rather than confirmatory. The study was also not specifically powered to assess clinical endpoints, and no clear primary clinical outcome was defined, which limits the strength of causal inference.

Conclusion

In conclusion, high-dose remifentanyl (16 ng/mL) combined with propofol anesthesia regimen was associated with changes in selected perioperative immune and inflam-

matory markers and modest improvements in early postoperative pain control in patients undergoing radical lung cancer surgery. These findings suggest that this anesthetic strategy may offer potential immunological and analgesic benefits in the perioperative setting of oncologic surgery; however, careful hemodynamic monitoring is warranted.

Availability of Data and Materials

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

Author Contributions

WQZ, MLZ, and CWS designed the research study; WQZ, MLZ, XYC, XYY, CWS, FSL, and LY performed the research; XYC, XYY, and FSL collected and analyzed the data. WQZ, MLZ, XYC, and LY have been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

This prospective, randomized, double-blind study protocol was reviewed and approved by the Zhejiang Provincial Hospital of Chinese Medicine Ethics Committee (2023-KLS-141-02). Written informed consent was obtained from all participants. All procedures were performed in accordance with the ethical standards of the institution and the Declaration of Helsinki.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.24976/Discover.Med.202638213.260>.

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