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Expression Profiles of Immune Cells after Propofol or Sevoflurane Anesthesia for Colorectal Cancer Surgery: A Prospective Double-blind Randomized Trial

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Experimental studies alongside clinical trials yielded contradictory observations on the potential antitumor effects of intravenous- *versus* volatile anesthetics–based anesthesia regimens in the context of colorectal cancer surgery
- Various immune cells have been suggested to exert antitumor effects during the perioperative period
- The question whether propofol- *versus* sevoflurane-based anesthesia differentially affects circulating immune cells during colorectal cancer surgery is incompletely explored

What This Article Tells Us That Is New

- In this randomized controlled trial, the fraction of circulating natural killer cells and T lymphocytes was comparable between propofol- and sevoflurane-based anesthesia in patients undergoing colorectal cancer surgery
- These observations suggest that the type of general anesthetics used may minimally affect perioperative immune status

ABSTRACT

Background: The antitumor effects of natural killer cells, helper T cells, and cytotoxic T cells after cancer surgery were reported previously. This study hypothesized that propofol-based anesthesia would have fewer harmful effects on immune cells than volatile anesthetics–based anesthesia during colorectal cancer surgery.

Methods: In total, 153 patients undergoing colorectal cancer surgery were randomized and included in the analysis. The primary outcome was the fraction of circulating natural killer cells over time in the propofol and sevoflurane groups. The fractions of circulating natural killer, type 1, type 17 helper T cells, and cytotoxic T cells were investigated. The fractions of CD39 and CD73 expressions on circulating regulatory T cells were investigated, along with the proportions of circulating neutrophils, lymphocytes, and monocytes.

Results: The fraction of circulating natural killer cells was not significantly different between the propofol and sevoflurane groups until 24 h postoperatively (20.4 ± 13.4% *vs.* 20.8 ± 11.3%, 17.9 ± 12.7% *vs.* 20.7 ± 11.9%, and 18.6 ± 11.6% *vs.* 21.3 ± 10.8% before anesthesia and after 1 and 24 h after anesthesia, respectively; difference [95% CI], –0.3 [–4.3 to 3.6], –2.8 [–6.8 to 1.1], and –2.6 [–6.2 to 1.0]; $P = 0.863$, $P = 0.136$, and $P = 0.151$ before anesthesia and after 1 and 24 h, respectively). The fractions of circulating type 1 and type 17 helper T cells, cytotoxic T cells, and $CD39^+$ and $CD73^+$ circulating regulatory T cells were not significantly different between the two groups. The neutrophil to lymphocyte ratio in both groups remained within the normal range and was not different between the groups.

Conclusions: Propofol-based anesthesia was not superior to sevoflurane-based anesthesia in terms of alleviating suppression of immune cells including natural killer cells and T lymphocytes during colorectal cancer surgery.

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Most patients who have cancer will undergo at least one surgical procedure, and cancer recurrence and metastasis impose a considerable burden on these patients.[1] Recurrence after surgical resection is mainly influenced by tumor grade. In addition, perioperative factors (*e.g.*, types of anesthetics, analgesics, and other medications) influence cancer progression, recurrence, and metastasis.[2] There have been numbers of studies regarding the effects of anesthetics on cancer progression, recurrence, and metastasis.[3–7] Propofol was reported to have a greater antitumor effect than volatile anesthetics during cancer surgery. However,

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the results remain controversial, and the antitumor effects of propofol have not been confirmed.[8]

Colorectal cancer is a commonly diagnosed cancer and a leading cause of cancer-related mortality. The hazardous effects of volatile anesthetics on colorectal cancer have been investigated in several *in vitro* studies.[9,10] However, there have been few prospective randomized clinical studies regarding this issue in colorectal cancer surgery patients. In addition, *in vitro* and *in vivo* studies on colorectal cancer surgery have yielded contradictory results.[11] Recent large-scale retrospective studies yielded contradictory results regarding propofol- and volatile anesthetics–based anesthesia during colorectal cancer surgery.[12,13]

Various immune cells have antitumor effects during the perioperative period. Natural killer cells and T lymphocytes are the most important cell types due to their prominent antitumor effects after cancer surgery.[14–16] However, few clinical studies have investigated the fractions of natural killer cells and T lymphocytes in relation to the anesthetics used during colorectal cancer surgery.

In this study, we hypothesized that propofol-based anesthesia would have fewer harmful effects on circulating immune cells than equipotent doses of volatile anesthetics–based anesthesia during colorectal cancer surgery. We compared the fractions of circulating natural killer cells, circulating T lymphocytes, and related circulating immune cells between propofol- and sevoflurane-based anesthesia during colorectal cancer surgery.

Materials and Methods

Study Population

This study used a prospective, double-blind, randomized design and was approved by the Institutional Review Board of Konkuk University Medical Center, Seoul, Korea (approval number KUH1160089). The study was registered at ClinicalTrials.gov (trial registration number NCT02567942; principle investigator, S. H. Kim; date of registration, October 5, 2015) and conducted at a single tertiary medical center (Konkuk University Medical Center, Seoul, Korea).

This study was conducted in accordance with the original protocol from February 2016 to April 2018. Written informed consent was obtained from all enrolled patients. Patients were eligible for enrollment if they were aged more than 20 yr and scheduled to undergo colorectal cancer surgery. The exclusion criteria were (1) refusal to participate, (2) history of previous cancer, (3) plan for other concurrent surgery, (4) history of a hypersensitivity reaction to propofol or sevoflurane, and (5) ongoing infection. Enrolled patients were randomly assigned (at a 1:1 ratio) to an anesthetic regimen that included propofol-based anesthesia (propofol group) or sevoflurane-based anesthesia (sevoflurane group). The allocation sequence was generated by random-permuted block randomization (20 blocks with two subjects, 15 blocks with four subjects, 10 blocks with six subjects; total 45 blocks) at the clinical research coordination center of our hospital, which was not otherwise involved in the trial. The anesthesia team and care providers, including the surgical and nursing teams, were blinded to the study goals and asked to follow the protocol. A research assistant was responsible for assessing patient eligibility and enrollment; this assistant obtained informed consent from all patients. A designated data collector was responsible for data collection, including blood sampling; this individual was blinded to patient allocation. The research assistant and data collector did not participate in patient care. The research team members were blinded to the study throughout the perioperative period, and until completion of the statistical analysis.

Anesthesia Technique

Anesthesia regimens were performed by members of an anesthesia team blinded to the study. The anesthesia techniques were performed as described previously.[17] Patients in the propofol group received propofol for anesthesia induction followed by maintenance agents as target-controlled infusion. Patients in the sevoflurane group were administered thiopental sodium for anesthesia induction and then ventilated using a sevoflurane–oxygen–air mixture throughout the surgery. Propofol and sevoflurane were administered at equipotent doses to maintain a consistent bispectral index of 40 to 60 in both groups. Target-controlled intravenous infusion of remifentanil at 5 ng · ml^{-1} was performed and maintained until the end of surgery in both groups. During the anesthesia regimen, the mean systemic blood pressure was maintained within 20% of baseline or above 60 mmHg by using appropriate doses of vasopressors or inotropics. After surgery, intravenous patient-controlled analgesia (PCA) was supplied on demand to all patients. The total PCA volume was 200 ml, consisting of 2,000 μg (40 ml) fentanyl, 0.6 mg (4 ml) ramosetron, and 156 ml normal saline. The PCA device (Gemstar Pump; Hospira, USA) was programed to deliver 0.03 ml · kg · h^{-1} as the basal infusion rate and 0.05 ml · kg^{-1} on demand with a lockout time of 15 min. After tracheal extubation, all patients were transferred to the intensive care unit. Postoperative medical treatment and decision-making were performed by the attending surgeon in accordance with the standard institutional regimen, and there were no adverse events during the study.

Blood Samples

Venous blood samples were collected into ethylenediaminetetraacetic acid tubes immediately before anesthesia induction (Preop), on arrival in the postanesthesia care unit (Post 1 h), and at 24 h postoperatively (Post 24 h).

Flow Cytometric Analysis

Flow cytometric analysis was performed to evaluate immune cells in accordance with previously published methods. The

fraction and apoptosis rate of circulating natural killer cells were examined.[18–20] The fractions of circulating $CD4^+$ T cells, such as type 1 helper T cells and type 17 helper T cells, were examined,[21,22] as were the fraction and apoptosis of rate circulating $CD8^+$ T cells.[23] In addition, the fraction of $CD39^+$ and $CD73^+$ circulating regulatory T cells were analyzed.[24] Peripheral blood mononuclear cells were isolated from blood samples using density-gradient centrifugation over a Ficoll-Hypaque gradient (GE Healthcare, USA). The peripheral blood mononuclear cells were washed with phosphate-buffered saline (137 mM NaCl, 2.7 M KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) and resuspended in Roswell Park Memorial Institute 1640 medium supplemented with 1% penicillin and 10% fetal bovine serum. All data were collected using the flow cytometers BD FACS Aria (Becton Dickinson, USA) and BD FACS Calibur (Becton Dickinson, USA) and then analyzed with software (FlowJo; Tree Star, USA).

Analysis of the Fraction and Apoptosis Rate of Circulating Natural Killer Cells. To isolate natural killer cells from among peripheral blood mononuclear cells, cells were stained with phycoerythrin-cyanine 7-conjugated anti-human CD16 (catalog number 25-0168-42; eBioscience, USA) and allophycocyanin-conjugated anti-human CD56 (catalog number 557711; BD Biosciences, USA) for 30 min, after which $CD56^+CD16^+$ cells (natural killer cells) were purified from peripheral blood mononuclear cells using a flow cytometer in accordance with the manufacturer's protocol. To determine the apoptosis rate of natural killer cells, phycoerythrin-cy7–conjugated anti-human CD16 (catalog number 25-0168-42; eBioscience) and allophycocyanin-conjugated anti-human CD56 (catalog number 557711; BD Biosciences) were used. After 30 min, the cells were washed with a cell staining buffer (catalog number 420201; BioLegend, USA). Next, 5y3 cells were mixed with 300 μl annexin V–binding buffer (catalog number 422201; BioLegend) and fluorescein isothiocyanate–conjugated annexin V (catalog number 640906; BioLegend) antibody at room temperature for 15 min.

Analysis of Fractions of Circulating Type 1 and Type 17 Helper T Cells. To determine the fractions of $CD4^+$ interferon-γ^+ type 1 helper T cells and $CD4^+$ interleukin-$17A^+$ type 17 helper T cells among peripheral blood mononuclear cells, peripheral blood mononuclear cells were washed with flow cytometric analysis buffer (0.1% bovine serum albumin in phosphate-buffered saline). The cells were then stained with peridinin chlorophyll–conjugated anti-human CD4 (catalog number 347324; BD Biosciences) at room temperature for 30 min, washed with a fluorescence-activated cell sorting buffer (0.1% bovine serum albumin in phosphate-buffered saline), and then stimulated with 50 ng $\cdot$ ml^{-1} phorbol myristate acetate (Sigma–Aldrich, USA) and 1 μg $\cdot$ ml^{-1} ionomycin (Sigma–Aldrich) in the presence of Golgi stop (catalog number 554724; BD Biosciences) for 4 h at 37°C. Stimulated cells were washed with a flow cytometric analysis buffer and fixed for 10 min with 4% paraformaldehyde (catalog number 554655; BD Biosciences). After fixation, the cells were permeabilized with solutions (FACS Perm 2; catalog number 340973; BD Biosciences) in accordance with the manufacturer's instructions and then stained with fluorescein isothiocyanate–conjugated anti-human interferon-γ (catalog number 554700; BD Biosciences) and phycoerythrin-conjugated anti-human interleukin-17A (catalog number 12-7179-42; eBioscience) antibody for 30 min.

Analysis of Fraction and Apoptosis Rate of Circulating Cytotoxic T Cells. To isolate $CD8^+$, T cells in peripheral blood mononuclear cells were stained for 30 min with phycoerythrin-conjugated anti-human CD8 (catalog number 555367; BD Biosciences). Subsequently, $CD8^+$ cells were purified from peripheral blood mononuclear cells using the flow cytometric instrument in accordance with the manufacturer's protocol. To determine the apoptosis rate of $CD8^+$ T cells among peripheral blood mononuclear cells, phycoerythrin-conjugated anti-human CD8 (catalog number 555367; BD Biosciences) was used. After staining for 30 min, the cells were washed with a cell staining buffer (catalog number 420201; BioLegend). Next, 5y3 cells were mixed with 300 μl of annexin V–binding buffer (catalog number 422201; BioLegend) and fluorescein isothiocyanate–conjugated annexin V (catalog number 640906; BioLegend) antibody at room temperature for 15 min.

Analysis of Fractions of $CD39^+$ and $CD73^+$ Circulating Regulatory T Cells. To investigate the fractions of $CD39^+$ and $CD73^+$ regulatory T cells among peripheral blood mononuclear cells, the cells were stained with fluorescein isothiocyanate–conjugated anti-human CD39 (catalog number 561444; BD Biosciences), phycoerythrin-cy7–conjugated anti-human CD73 (catalog number 561258; BD Biosciences), peridinin chlorophyll–conjugated anti-human CD4 (catalog number 347324; BD Biosciences), and allophycocyanin-conjugated anti-human CD25 (BD catalog number 555434; BD Biosciences) antibody for 30 min. Subsequently, the cells were washed with fluorescence-activated cell sorting buffer (0.1% bovine serum albumin in phosphate-buffered saline) and fixed for 20 min with fix/perm buffer (catalog number 421401; BioLegend). After fixation, the cells were permeabilized with solutions (FACS Perm 2; catalog number 421403; BD Biosciences) in accordance with the manufacturer's instructions and stained with phycoerythrin-conjugated anti-human forkhead box P3 antibody (catalog number 320208; BioLegend) for 30 min.

Determination of Differential Numbers of Leukocytes

Data regarding differential numbers of leukocytes were collected at Preop, Post 1 h, and Post 24 h. The absolute neutrophil count was divided by the absolute lymphocyte count to obtain the neutrophil to lymphocyte ratio.

Clinical Measurements

The site of colorectal cancer, rates of preoperative chemotherapy and radiation therapy, pathologic stage, pathologic type of colorectal cancer, degree of cancer cell differentiation, and maximum size of the mass were recorded for all patients. Furthermore, the expression levels of proteins such as epidermal growth factor receptor, and *p53* were determined in pathologic examinations, as were the rates of mutations in genes such as *KRAS*, *NRAS*, and *BRAF*. Finally, the rates of microsatellite instability and loss of heterozygosity were recorded.

The rates of phenylephrine or ephedrine use, the total amount of fluid administered during operation, and the total doses of opioids administered during the perioperative period were recorded. Postoperative pain was assessed using a visual analog scale that ranged from 0 mm (no pain) to 100 mm (worst pain imaginable) up to 24 h postoperatively.

Statistical Analysis

The primary outcome measure was the fraction of circulating natural killer cells over time in the propofol and sevoflurane groups, while the secondary outcome measure was the fraction of circulating helper T cells over time in each group. The sample size was calculated using software (G*Power; version 3.1.9.2; Universität Kiel, Kiel, Germany) based on a pilot study with 10 patients/group. The standardized effect size (*i.e.*, the magnitude of differences between groups) was 0.229 for changes in the fraction of circulating natural killer cells and 0.302 for changes in the fraction of circulating helper T cells. These values indicated that sample sizes of 76 and 44 patients/group were needed for analyzing the fractions of circulating natural killer and helper T cells, respectively. The sample size achieved 80% power to detect differences in the mean fraction of circulating natural killer cells between the two groups at the 5% significance level (α). Finally, we assigned 76 patients to each group and enrolled 217 patients in the study, assuming a 30% dropout rate.

The fractions of circulating immune cells were compared between randomized groups by means of separate analysis of covariance models at Post 1 h and Post 24 h while adjusting for Preop scores for each outcome. Independent two-tailed *t* tests were used to compare means for continuous, normally distributed variables between the propofol and sevoflurane groups. When the data were not normally distributed, the Mann–Whitney *U* test was used. The chi-squared test was used to compare the means of categorical variables between the two groups. Normally distributed continuous data are presented as the means ± SD, while nonnormally distributed data are presented as the medians (25 to 75%). For categorical variables, the numbers of patients (n) and proportions (%) were calculated. All calculations were performed using software (IBM SPSS; version 20.0; SPSS Inc., USA). In all analysis, $P < 0.05$ was taken to indicate statistical significance.

Results

A total of 217 patients were eligible for the study from February 2016 to April 2018. A total of 64 patients were excluded for the following reasons: 32 due to refusal to participate, 15 due to a history of previous cancer, 10 because other types of concurrent surgery were planned, 2 due to a history of a hypersensitivity reaction to propofol, and 5 due to ongoing infection. Therefore, 153 patients (76 in the propofol group and 77 in the sevoflurane group) were included in the final analysis, and the trial ceased when the recruitment target was met (fig. 1).

The patient demographics are presented in table 1 and Supplemental Digital Content 1 (http://links.lww.com/ALN/C772). The types of operation, duration of anesthesia, and perioperative opioid and nonsteroidal anti-inflammatory drug use rates were similar between the two groups. There were also no differences in underlying disease, perioperative medications, and postoperative pain scores between the two groups.

The colorectal cancer data for both groups are presented in table 2 and Supplemental Digital Content 2 (http://links.lww.com/ALN/C773). All patients in both groups were pathologically diagnosed with adenocarcinoma. The site of colorectal cancer, rates of preoperative chemotherapy and radiation therapy, pathologic stage, and degree of cancer cell differentiation were similar between the propofol and sevoflurane groups. The maximum size of the mass; expression levels of epidermal growth factor receptor and *p53* in pathologic specimens; rates of mutations in *KRAS*, *NRAS*, and *BRAF* genes; and rates of microsatellite instability and loss of heterozygosity were similar between the two groups.

The fraction of circulating natural killer cells was not significantly different between the propofol and sevoflurane groups up to 24 h postoperatively (20.4 ± 13.4% *vs.* 20.8 ± 11.3%, 17.9 ± 12.7% *vs.* 20.7 ± 11.9%, and 18.6 ± 11.6% *vs.* 21.3 ± 10.8% at Preop, Post 1 h, and Post 24 h, respectively; difference [95% CI], –0.3 [–4.3 to 3.6], –2.8 [–6.8 to 1.1], and –2.6 [–6.2 to 1.0]; $P = 0.863$, $P = 0.136$, and $P = 0.151$, at Preop, Post 1 h, and Post 24 h, respectively; table 3). The apoptosis rate of circulating natural killer cells was also not significantly different between the two groups (5.7% [3.0 to 16.3%] *vs.* 7.3% [3.3 to 11.9%], 6.2% [2.9 to 14.0%] *vs.* 7.1% [2.3 to 9.6%], and 6.8% [3.5 to 15.1%] *vs.* 7.0% [2.8 to 10.6%] at Preop, Post 1 h, and Post 24 h, respectively; difference [95% CI], –0.2 [–2.0 to 2.1], –0.9 [–3.0 to 1.1], and –1.0 [–3.0 to 0.8]; $P = 0.855$, $P = 0.084$, and $P = 0.142$, respectively; table 3).

The fractions of circulating type 1 and type 17 helper T cells were not significantly different between the two groups (type 1 helper T cells: $P = 0.830$, and $P = 0.580$ at Post 1 h, and Post 24 h, respectively; type 17 helper T cells: $P = 0.590$, and $P = 0.133$ at Post 1 h, and Post 24 h,

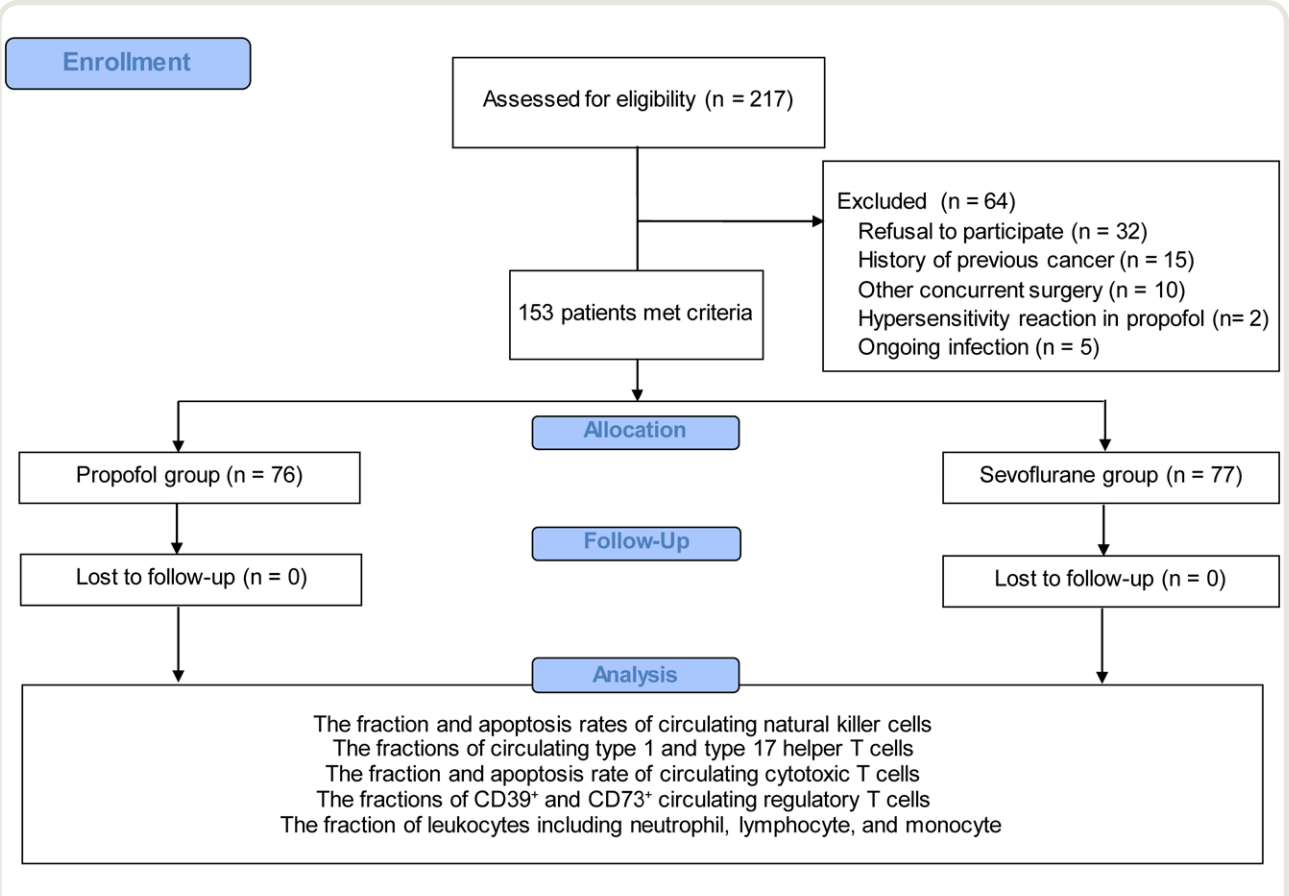

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

respectively). Furthermore, the fraction of circulating cytotoxic T cells were not significantly different between the groups ($P = 0.439$ and $P = 0.676$ at Post 1 h and Post 24 h, respectively). However, the apoptosis rates of circulating cytotoxic T cells were significantly different between the groups ($P = 0.877$ and $P = 0.019$ at Post 1 h and Post 24 h, respectively; table 3). The fractions of $CD39^+$ and $CD73^+$ circulating regulatory T cells were not significantly different between the groups ($CD39^+$, $P = 0.471$ and $P = 0.452$ at Post 1 h and Post 24 h, respectively; $CD73^+$, $P = 0.661$ and $P = 0.820$ at Post 1 h and Post 24 h, respectively; table 3).

Laboratory values related to inflammation are presented in table 3. The proportions of circulating neutrophils, lymphocytes, and monocytes were similar between the propofol and sevoflurane groups up to postoperative day 1 (table 3). The neutrophil–lymphocyte ratio remained in the normal range in both groups and was not significantly different between the groups (table 3).

Discussion

This study showed that the fraction of circulating natural killer cells in patients with propofol-based anesthesia did not differ from those with sevoflurane-based anesthesia during colorectal cancer surgery. The fractions of circulating type 1 and type 17 helper T cells and cytotoxic T cells were also not significantly different between the two groups. There were no differences in the fractions of $CD39^+$ and $CD73^+$ circulating regulatory T cells between the two groups during colorectal cancer surgery.

Natural killer cells and T lymphocytes exert antitumor effects mediated by several mechanisms during the perioperative period. Natural killer cells play roles in antibody-dependent cytotoxicity and enhance innate and adaptive immunity through the secretion of an array of cytokines, growth factors, and chemokines.[14] Especially, natural killer cells are known to attenuate colorectal cancer progression during the perioperative period.[16] Previous studies also revealed the antitumor effects of helper T cells and regulatory T cells.[25] In particular, type 1 and type 17 helper T cells were shown to support immune responses in the cancer microenvironment. Type 1 helper T cells promote interleukin-2 secretion and activate cytotoxic T cells and natural killer cells in colorectal cancer,[26] while type 17 helper T cells amplify the inflammatory response by inducing interleukin-17A and elicit the initiation of colorectal cancer.[27]

Table 1. Patient Demographic Data

Characteristic	Propofol Group (n = 76)	Sevoflurane Group (n = 77)
Sex		
Male	39 (51.3%)	40 (51.9%)
Female	37 (48.7%)	37 (48.1%)
Age, yr	62.2 ± 9.8	64.4 ± 11.3
Height, cm	162.0 (154.5–168.0)	163.0 (156.0–170.0)
Weight, kg	59.4 ± 9.2	61.9 ± 11.0
ASA physical status class		
I	33	29
II	35	37
III	8	11
Operation		
Hemicolectomy	16 (21.1%)	14 (18.2%)
Transverse colectomy	4 (5.3%)	1 (1.3%)
Low anterior resection	35 (46.1%)	41 (53.2%)
Anterior resection	12 (15.8%)	14 (18.2%)
Abdominoperineal resection	9 (11.8%)	7 (9.1%)
Duration of anesthesia, min	197 (165–223)	190 (161–240)
Duration of operation, min	155 (130–180)	150 (130–198)
Anesthetics		
Propofol, mg	1,513 (1,108–2,130)	0 (0–0)
Remifentanil, μg	2,118 (1,773–2,715)	2,059 (1,845–2,637)
Thiopental sodium, mg	0 (0–0)	300 (250–350)
Minimal end-expiratory concentration of sevoflurane, vol%	0.0 (0.0–0.0)	1.0 (1.0–2.0)
Maximal end-expiratory concentration of sevoflurane, vol%	0.0 (0.0–0.0)	2.0 (2.0–2.0)
Rocuronium, mg	78 (70–98)	70 (60–90)
Preoperative NSAID medication	5 (6.6%)	2 (3.9%)
Perioperative fentanyl, μg	962 (757–1,242)	993 (783–1,213)

The data are expressed as numbers (%) or means ± SD or medians (25 to 75%).
ASA, American Society of Anesthesiologists; NSAID, nonsteroidal anti-inflammatory drug.

On the other hand, regulatory T cells had tumor-promoting effects caused by surface expression of enzymes such as CD39 and CD73. The high rates of CD39 and CD73 expression in regulatory T cells promote cancer progression by suppressing type 1 and type 17 helper T cells and impairing the tumor cell-killing effects of natural killer cells and cytotoxic T cells.[28,29]

Table 2. Profiles of Colorectal Cancer

Characteristic	Propofol Group (n = 76)	Sevoflurane Group (n = 77)	*P* Value
Site of colorectal cancer			0.434
Ascending	12 (15.8%)	13 (16.9%)	
Transverse	4 (5.3%)	1 (1.3%)	
Descending	2 (2.6%)	1 (1.3%)	
Rectal	34 (44.7%)	43 (55.8%)	
Sigmoid	24 (31.6%)	19 (24.7%)	
Preoperative chemotherapy	7 (9.2%)	8 (10.4%)	1.000
Preoperative radiation therapy	8 (10.5%)	7 (9.1%)	0.979
Pathologic type of cancer			1.000
Adenocarcinoma	76 (100.0%)	77 (100.0%)	
Stage of cancer			0.071
0	0 (0.0%)	4 (5.2%)	
1	24 (31.6%)	21 (27.3%)	
2	17 (22.4%)	27 (35.1%)	
3	28 (36.8%)	18 (23.4%)	
4	7 (9.2%)	7 (9.1%)	
Degree of cancer cell differentiation			0.774
Well	7 (9.2%)	8 (10.4%)	
Moderate	64 (84.2%)	65 (84.4%)	
Poor	5 (6.6%)	4 (5.2%)	

The data are expressed as numbers (%).

Table 3. Laboratory Results during Colorectal Cancer Surgery

	Time	Propofol Group (n = 76)	Sevoflurane Group (n = 77)	Difference (95% CI)	*P* Value
Fraction of circulating immune cells					
Natural killer cells, %	Preop	20.4 ± 13.4	20.8 ± 11.3	–0.3 (–4.3 to 3.6)	0.863
	Post 1 h	17.9 ± 12.7	20.7 ± 11.9	–2.8 (–6.8 to 1.1)	0.136
	Post 24 h	18.6 ± 11.6	21.3 ± 10.8	–2.6 (–6.2 to 1.0)	0.151
Type 1 helper T cells, %	Preop	12.9 ± 9.8	10.8 ± 8.2	2.0 (–0.8 to 4.9)	0.163
	Post 1 h	12.7 ± 8.1	11.2 ± 8.4	1.5 (–1.1 to 4.1)	0.830
	Post 24 h	11.8 ± 7.9	10.3 ± 8.6	1.4 (–1.2 to 4.0)	0.580
Type 17 helper T cells, %	Preop	2.6 ± 2.5	2.4 ± 2.1	0.2 (–0.5 to 1.0)	0.565
	Post 1 h	2.5 ± 2.2	2.2 ± 2.6	0.3 (–0.5 to 1.1)	0.590
	Post 24 h	2.0 ± 2.0	2.5 ± 2.2	–0.5 (–1.2 to 0.2)	0.133
Cytotoxic T cells, %	Preop	24.9 ± 10.3	27.7 ± 8.9	–2.8 (–5.9 to 0.3)	0.073
	Post 1 h	25.5 ± 10.0	28.5 ± 10.3	–3.0 (–6.2 to 0.3)	0.439
	Post 24 h	25.8 ± 10.3	27.1 ± 10.1	–1.3 (–4.6 to 2.0)	0.676
Apoptosis rate of circulating immune cells					
Natural killer cells, %	Preop	5.7 (3.0 to 16.3)	7.3 (3.3 to 11.9)	–0.2 (–2.1 to 2.0)	0.855
	Post 1 h	6.2 (2.9 to 14.0)	7.1 (2.3 to 9.6)	–0.9 (–3.0 to 1.1)	0.084
	Post 24 h	6.8 (3.5 to 15.1)	7.0 (2.8 to 10.6)	–1.0 (–3.0 to 0.8)	0.142
Cytotoxic T cells, %	Preop	7.6 (3.2 to 12.3)	8.3 (3.8 to 11.3)	0.0 (–1.9 to 2.0)	0.964
	Post 1 h	7.2 (2.6 to 12.6)	7.2 (4.5 to 11.1)	0.1 (–1.8 to 1.9)	0.877
	Post 24 h	6.3 (2.3 to 10.4)	8.1 (5.2 to 12.8)	–1.7 (–3.6 to 0.1)	0.019
Fraction of CD39 and CD73 on circulating regulatory T cells					
CD39, %	Preop	14.6 ± 7.0	13.7 ± 6.8	0.9 (–1.3 to 3.1)	0.410
	Post 1 h	15.2 ± 7.6	13.8 ± 7.7	1.3 (–1.1 to 3.8)	0.471
	Post 24 h	16.2 ± 10.6	14.7 ± 7.4	1.5 (–1.4 to 4.4)	0.452
CD73, %	Preop	15.9 ± 5.7	17.2 ± 5.9	–1.3 (–3.1 to 0.6)	0.173
	Post 1 h	16.2 ± 6.1	17.2 ± 5.0	–1.0 (–2.8 to 0.8)	0.661
	Post 24 h	17.2 ± 9.2	18.1 ± 6.8	–0.9 (–3.4 to 1.7)	0.820
Differential count of white blood cells					
Neutrophil, %	Preop	61.7 ± 10.3	60.4 ± 9.9	1.3 (–1.9 to 4.6)	0.420
	Post 1 h	75.1 ± 9.2	74.2 ± 12.0	1.0 (–2.5 to 4.4)	0.762
	Post 24 h	79.9 ± 7.2	78.4 ± 8.5	1.5 (–1.0 to 4.0)	0.344
Lymphocyte, %	Preop	28.7 ± 9.2	29.0 ± 8.9	–0.2 (–3.1 to 2.7)	0.872
	Post 1 h	18.0 ± 8.2	18.2 ± 8.6	–0.3 (–3.0 to 2.4)	0.898
	Post 24 h	13.0 ± 5.8	13.3 ± 5.7	–0.2 (–2.1 to 1.6)	0.841
Monocyte, %	Preop	7.1 ± 1.8	7.6 ± 2.1	–0.5 (–1.1 to 0.1)	0.128
	Post 1 h	5.5 ± 1.5	5.4 ± 1.7	0.2 (–0.4 to 0.7)	0.218
	Post 24 h	6.2 ± 1.8	6.8 ± 2.1	–0.6 (–1.2 to 0.5)	0.178
Neutrophil lymphocyte ratio, %	Preop	2.5 ± 1.2	2.4 ± 1.2	0.1 (–0.3 to 0.4)	0.765
	Post 1 h	5.3 ± 2.7	5.3 ± 3.5	0.0 (–1.0 to 1.0)	0.883
	Post 24 h	7.7 ± 4.1	7.6 ± 4.6	0.1 (–1.3 to 1.5)	0.953

The data are expressed as means ± SD or median (25 to 75%). The intergroup differences at 1 and 24 h after surgery were adjusted for preoperative values.
Post 1 h, on arrival in the postanesthesia care unit; Post 24 h, at 24 h postoperatively; Preop, immediately before anesthesia induction.

Accordingly, these immune cells have been investigated in the context of cancer immunotherapy because they play crucial roles in the cancer microenvironment.[30,31]

Propofol is the most commonly used intravenous anesthetic agent. Previous *in vitro* studies revealed a potential antitumor mechanism for propofol involving enhancement of natural killer cell activity.[4,32] In contrast to propofol, volatile anesthetics have been implicated in immunosuppression and direct stimulation of both cancer cell survival and proliferation.[33,34] However, numerous other *in vitro* studies reported conflicting results.[35–39] Müller-Edenborn *et al.*[38] reported that volatile anesthetics reduce colon cancer cell invasion through a preconditioning effect, and Kvolik *et al.*[39] showed that volatile anesthetics induce apoptosis in colorectal cancer cells. Furthermore, most recent clinical studies did not identify differences in cancer progression, recurrence, or metastasis between propofol- and volatile anesthetic–based anesthesia.[8,12,40–42] The main limitation of most *in vitro* studies is that they did not faithfully replicate the *in vivo* conditions of the cancer microenvironment, which may be responsible for the discrepant results of numerous previous studies regarding the potential antitumor effects of propofol. We did not find any differences in the fractions of immune cells between propofol- and sevoflurane-based anesthesia in the current study. Our results indicate that propofol-based anesthesia at clinically relevant concentrations has no advantages over sevoflurane-based anesthesia with regard to circulating immune cells during colorectal cancer surgery.

There are several reasons for the lack of advantage of propofol with regard to the fractions of immune cells compared to volatile anesthetics during cancer surgery. Indeed, the progression of cancer and clinical course of cancer treatment are complex, highly orchestrated processes. Surgical stimulation elicits natural killer cell dysfunction by increasing inflammation[15] and contributes to the spread of cancer cells after cancer surgery.[43] However, multiple groups have suggested that the impact of anesthesia after minor surgery may be smaller than after major surgery. In particular, the impact of anesthesia on immunosuppression could differ according to the intensity of surgery.[41,44] Therefore, anesthetic-related immunomodulation could differ according to the types of cancer and surgery. The age at initial cancer occurrence could also influence the subsequent disease course. Gottschalk *et al.*[45] reported that a blunted sympathetic response after epidural analgesia was associated with colorectal cancer recurrence only in older patients, and the association was absent in younger patients. In addition, numerous perioperative factors including physiologic stress, hyperglycemia, and hypothermia can also cause a substantial imbalance in the antitumor immune response. Cancer heterogeneity, genetic influences, and physiologic disturbances during the perioperative period can influence the fate of cancer cells remaining in the microenvironment after cancer surgery. These factors have profound effects on the initiation and progression of colorectal cancer, response to standard antitumor therapy, and final clinical outcome.[16] Moreover, recent reviews have attempted to draw overall conclusions regarding whether anesthetics affect cancer progression, recurrence, and metastasis. However, the conclusions were unclear because of heterogeneity and conflicting findings among the studies.[2,46,47] In a recent meta-analysis, Yap *et al.*[44] demonstrated a benefit of propofol during cancer surgery on patient survival. However, their meta-analysis included only one small, randomized trial of 80 patients undergoing surgery for breast cancer, and the cancer types exhibited considerable heterogeneity. Notably, several large, retrospective studies reported similar effects of propofol and volatile anesthetics on cancer recurrence and patient survival.[12,42] In addition, a large-scale prospective study showed that the effects of anesthetics on cancer were similar between propofol- and sevoflurane-based anesthesia.[41] Our findings indicated that clinically relevant doses of these anesthetics do not lead to the fractions of immune cells during colorectal cancer surgery.

This clinical trial had several limitations. First, we did not investigate the prognosis of colon cancer according to anesthesia type after colorectal cancer surgery. In addition, we investigated immune cells within 24 h postoperatively. Therefore, we could not assess the overall perioperative immune status, which may change for 1 to 4 weeks postoperatively.[48,49] However, we assumed that the prognosis would be similar because the neutrophil–lymphocyte ratio, a good marker of cancer prognosis,[50,51] was similar between the groups. Second, we did not investigate the relation of circulating tumor cells with circulating immune cells during cancer surgery. However, this could provide important information in patients with metastasis or higher cancer stages due to their circulating characteristics. As the current study included few patients with high cancer stages, we assumed that the effects of anesthetics on circulating tumor cells may not have been evident. Finally, our study group investigated the effects of anesthetics on immune cells during the perioperative period in similar studies.[52–54] Therefore, the novelty of the current study may be limited. We suggest that researchers should focus on the numerous factors that may influence cancer progression, recurrence, and metastasis, other than immune cells. Finally, the apoptosis rate of cytotoxic T cells at Post 24 h was significantly higher in the sevoflurane groups in the current study. However, we considered this significant difference to be clinically meaningless because the fraction of cytotoxic cells during whole study periods and apoptosis rate of cytotoxic T cells at Post 1 h was not differ between the two groups. Therefore, it is highly possible that the significant difference is merely a statistical observation.

In conclusion, the fractions of circulating natural killer cells, helper T cells, and cytotoxic T cells did not differ between propofol-based anesthesia– and sevoflurane-based anesthesia during colorectal cancer surgery. There were also no differences in the expression levels of CD39 and CD73 in circulating regulatory T cells between the groups. Numerous perioperative factors could affect perioperative immune status during colorectal cancer surgery, and our findings indicate that the effects of anesthetics may be minimal.

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Competing Interests

The authors declare no competing interests.

Reproducible Science

Full protocol available at: yshkim75@daum.net. Raw data available at: yshkim75@daum.net.

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A Legacy with *Long*-evity: Commemorating Crawford W. Long, M.D.

"In commemoration of the 70th anniversary of the discovery of ether anesthesia…the noble achievement of a great son of this grand old school," the University of Pennsylvania (*crest, upper left*) posthumously awarded this medallion (*lower right*) not to William T. G. Morton, but to a quiet, country physician from Georgia, Crawford W. Long, M.D. The glowing orations from that day are preserved in the copy of Penn's *University Bulletin* held in the Wood Library-Museum Collection. Inscribed to Long, "First to Use Ether as an Anaesthetic in Surgery, March 30, 1842," the medallion accepted by his daughter Frances Long Taylor in 1912 is now displayed in the John Morgan Building as part of the University of Pennsylvania Art Collection in Philadelphia. A fierce advocate of his legacy, Long's daughter would pen his biography *Crawford W. Long and the Discovery of Ether Anesthesia* in 1928, eventually inspiring the United States' annual celebration of Doctor's Day each March 30. This year will be the 180th anniversary of Long's anesthetic! Many thanks to this humble man not interested in fame, fortune, or priority, and whose legacy is fondly and prominently celebrated each spring. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology. www.woodlibrarymuseum.org)

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