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Effect of sevoflurane on CD4+CD25+FOXP3+ regulatory T cells in patients with gastric cancer undergoing radical surgery

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ABSTRACT

In this study, the proportion of CD4+CD25+FOXP3+ regulatory T (Treg) cells in CD4+ T cells in the peripheral blood of gastric cancer patients before anesthesia induction (T1), after surgery (T2) and the first day after surgery (T3) was studied to explore the effect of sevoflurane and propofol anesthesia on the prognosis of gastric cancer patients. Forty patients with advanced gastric cancer were recruited and randomly divided into the sevoflurane group (S group) and the propofol group (T group). Flow cytometry was used to detect the proportion of CD4+CD25+FOXP3+ Treg cells in CD4+ T cells in the peripheral blood of patients with T1, T2 and T3, respectively. Compared with stage IIB, the proportion of CD4+CD25+FOXP3+ Treg cells in T1, T2 and T3 of stage IIIA and stage IIIB patients was increased. Compared with the T group, the expression of CD4+CD25+FOXP3+ Treg cells in the peripheral blood of T2 and T3 in the S group was decreased. The results showed that the expression of CD4+CD25+FOXP3+ Treg cells might be related to the TNM stage of gastric cancer and sevoflurane could alleviate the inhibition of postoperative immune function more than propofol. Sevoflurane effectively reduced the expression level of CD4+CD25+FOXP3+ Treg cells in peripheral blood of T2 and T3 of patients with gastric cancer, providing the theoretical basis for the selection of surgical anesthetics for patients with gastric cancer.

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Introduction

Surgery is an important treatment for advanced gastric cancer, prolonging life and improving the quality of life of patients, but postoperative metastasis and recurrence are important factors affecting the survival of patients with gastric cancer. Immunosuppression is an important mechanism leading to tumor metastasis and recurrence. It is of great clinical significance to pay attention to the influence of perioperative factors on the immune function of tumor patients and improve the prognosis of patients.

Cellular immunity plays an important role in anti-tumor immunity. Many immune cells, such as natural killer (NK) cells, T lymphocytes, dendritic cells and macrophages, are involved in the anti-tumor immune process. Among them, NK cells have a direct killing effect on tumor cells (1). In general, the immune system can recognize and eliminate the mutant cells. However, the mutant cells have immune escape due to the change of some surface antigens of tumor cells, causing the cells to grow and metastasize continuously (2). The immune function of tumor patients is relatively fragile. In addition, anesthesia and surgical trauma may lead to different degrees of immune suppression. The combined action of many factors makes the immune function of patients more fragile. It had been pointed out that general anesthesia drugs may affect the immune function of the body to a certain extent (3).

In recent years, some studies had found that narcotic drugs might lead to postoperative immunosuppression. Besides the research on the effects of helper T cells, cytokines, immunoglobulin, complement and other aspects, the effects of inhaled anesthetic drugs on immune cells and immune response had also been widely concerned. Inhaled anesthetics affect innate immunity by affecting neutrophils, dendritic cells, NK cells and other cells. Previous studies had shown that inhaling anesthetics could reduce lymphocyte proliferation or increase apoptosis (4), and lead to lymphocyte apoptosis in a dose-dependent and time-dependent manner (5).

Regulatory T cells (Treg cells) are T cell subsets with immunosuppression, which are divided into natural regulatory T cells and adaptive regulatory T cells. CD4+CD25+ Treg cells are naturally induced T cells expressing CD4 and CD25 molecules, which are characterized by their high expression of the transcription factor FOXP3 (6). FOXP3 is an intranuclear transcription factor of Treg cells, which plays an important role in the differentiation, maintenance and immune function of regulatory T cells. FOXP3 has an effect on the proliferation, apoptosis, invasion and other biological behaviors of tumor cells, which may be due to the direct binding of the target gene promoter region, or the synergistic effect with other transcription factors. CD4+CD25+ Treg cells have the functions of inhibiting the immune function of activated immune cells, protecting immune homeostasis and controlling the inhibition of effector T cells by exogenous antigens (7-9). Studies had shown that the increased number and function of CD4+CD25+ FOXP3+ Treg cells in the peripheral blood of patients with gastric cancer could exert tumor immunosuppression and promote the growth and metasta-

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sis of gastric cancer (10). Therefore, reducing the number of CD4+CD25+ Treg cells or inhibiting their function in patients with gastric cancer may help to improve the prognosis of patients.

At present, there were few studies on the effects of narcotic drugs on CD4+CD25+ Treg cells. Studies had found that inhaled anesthetic isoflurane could promote the proliferation and differentiation of Treg cells by up-regulating hypoxia-inducible factor (HIF-1), increasing the secretion of interleukin-10 (IL-10), and inhibiting the effects of natural killer cells and cytotoxic T cells, leading to tumorigenesis and poor prognosis (11,12).

Sevoflurane is an inhaled general anesthetic commonly used in clinics. Previous studies found that sevoflurane could inhibit the proliferation of gastric cancer cells and promote cell apoptosis by up-regulating the expression of FOXP3 protein in gastric cancer cells. CD4+CD25+ FOXP3+ Treg cells in peripheral blood were positively correlated with them in gastric cancer tissues. FOXP3 overexpression in precancerous tissues reduced the metastatic ability of gastric cancer. Gastric cancer cells could promote the expression of the FOXP3 gene in lymphocytes, resulting in increased Treg cell expression and local immunosuppression.

Materials and Methods

Patients preparation

The research protocol had been approved by the clinical research ethics and ethics committee of the fourth hospital of Hebei Medical University (approval number: 2021186) and registered in the China Clinical Trial Registration Center (registration number: chictr2100045664). All enrolled patients signed informed consent by themselves or their families. Forty patients with advanced gastric cancer, ASA grade I or II, aged 18 ~ 75 years and with a BMI of 20-30 kg/m^2, underwent radical gastrectomy under elective general anesthesia.

Inclusion criteria

(1) ASA grade I or II; (2) age 18 ~ 75 years old; (3) body mass index (BMI) of 20 ~ 30 kg/m^2; (4) the imaging stages were T2~T4NxM0; (5) expected surgery time ≥ 2 h; (6) voluntarily participate in this clinical trial and sign the informed consent form.

Exclusion criteria

(1) Severe impairment of heart, lung, kidney and liver functions; (2) the weight was less than 80% or more than 120% of the ideal weight; (3) neoadjuvant chemotherapy or radiotherapy was performed; (4) patients with other malignant tumors; (5) those who participated in other clinical research at the same time; (6) other diseases that the researcher believed that they were not suitable for participating in this clinical trial.

Grouping

This trial was a prospective, randomized, controlled and exploratory study.

Blind method

The study designer, the patients participating in the trial and the inspectors did not know the specific grouping of the trial, but only the anesthesia management personnel knew the specific grouping.

Random method

The subjects were randomly assigned to the sevoflurane combined nerve block group (SeV group) or propofol-based total intravenous anesthesia combined nerve block group (TIVA group). After the patient signed the informed consent form and entered to the study, each subject was assigned a random number through Statistical Product and Service Solutions (SPSS) software. The statistician was responsible for placing the random number in a sealed envelope and distributing it to the anesthesia manager according to the enrollment order. The anesthesia manager opened the random envelope.

Grouping method

The subjects will be randomly assigned to two experimental groups in equal proportion: the sevoflurane group (S Group) and the TIVA group (T Group).

Preparation before anesthesia

After entering the room, the patient laid flat with a warm blanket to keep warm, opened the venous access of the upper limbs, injected compound sodium chloride 8 mL/(kg·h), connected with GE b650 monitor, and routinely monitored noninvasive arterial pressure (NIBP), electrocardiogram (ECG), blood oxygen saturation (SpO2), end-expiratory carbon dioxide (PetCO2), electroencephalogram bispectral index (BIS), surgical plethysmography index (SPI) and body temperature. SPI was used to monitor intraoperative noxious stimulation. Under local anesthesia, radial artery catheterization was performed and invasive arterial blood pressure (ABP) and pulse pressure variability (PPV) were monitored.

Anesthesia induction

Before induction, 8 mL/(kg·h) balance solution was infused intravenously. Anesthesia was induced by intravenous injection of midazolam 0.03 mg/kg, sufentanil 0.3 μg/kg, propofol 2 mg/kg, and cisatracurium 0.2 mg/kg. After endotracheal intubation, the anesthesia machine was connected for mechanical ventilation. The tidal volume was set at 8 mL/kg, the inhalation-exhalation ratio was 1.0: 1.5, and the respiratory rate was adjusted to maintain PETCO2 of 35 ~ 45 mmHg (1 mmHg = 0.133 kPa).

Anesthesia maintenance

In S group, 0.7 ~ 1.0 MAC sevoflurane was inhaled and remifentanil was pumped at a constant rate of 0.05 ~ 0.1 μg/(kg·min) to maintain anesthesia. T group received target-controlled intravenous infusion (TCI) of propofol (Ce 1 ~ 3 μg/mL) and remifentanil was pumped at a constant rate of 0.05 ~ 0.1 μg/(kg·min) to maintain anesthesia. The BIS and SPI values of both groups were maintained between 45 to 60 and between 20 to 50. According to the situation, 0.05 mg/kg cisatracurium was added to maintain T4/T1 at 0. The intraoperative fluid infusion was guided according to PPV. If PPV < 10%, the background dose balance solution was continuously injected at 4 mL/(kg·h). When PPV > 10%, 3 mL/kg hydroxyethyl starch was intravenously injected within 5 min. The observation was continued for 5 min. If the PPV was still > 10%, norepinephrine was injected intravenously with a pump.

5 mg dizocine and 0.25 mg palonosetron hydrochlo-

ride were injected intravenously 20 min before the end of surgery. After surgery, a bilateral rectus sheath block was performed and a postoperative patient-controlled analgesia pump (PCIA) was connected. The drug used for nerve block was 30 mL 0.375% ropivacaine and 5 mg dexamethasone. The PCIA formula was oxycodone 1 mg/kg, diluted to 100 mL with normal saline and 4 mL bolus, locked for 30 min. After surgery, the VAS score of patients remained ≤ 3 points. If VAS score > 3, 30 mg ketorolac ambutritol was injected intravenously.

Observation index

3 mL peripheral blood was collected before anesthesia induction (T1), after surgery (T2) and on the first day after surgery (T3), and the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in $CD4^+$ Treg cells was measured by flow cytometry. The age, sex, BMI, ASA grade and complications of the patients were recorded. The surgery method, surgery time and anesthesia time were recorded. The occurrence of adverse events such as intraoperative hypotension (systolic blood pressure < 90 mmHg, cumulative time at least 5 min) and hypertension (systolic blood pressure > 160 mmHg, cumulative time at least 5 min); VAS score at 24 h and 48 h after surgery, tumor tissue classification and pathological stage were also recorded.

Statistical analysis

SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, and continuous variables were expressed by means (standard deviation) ± S or median (interquartile interval) (M (Q)). The counting data was expressed by the number of cases (%). For the measurement data conforming to the normal distribution, the independent sample t-test was used for the comparison between groups. The rank sum test was used to compare the measurement data that did not conform to the normal distribution. The chi-square test or Fisher exact test was used for the counting data.

Results

Patients recruitment

Patients recruitment and grouping were shown in Figure 1.

General information about Patients

There was no significant difference between the two groups in gender, age, height, weight, surgery type, surgery time, anesthesia time and other general information ($P > 0.05$). The results were shown in Table 1.

Postoperative pathological stage and histological type of tumor

There was no significant difference in postoperative pathological stage and tumor histological type between the two groups ($P > 0.05$). The results were shown in Table 2.

Incidence of intraoperative hypertension and hypotension

There was no significant difference in the incidence of intraoperative hypertension and hypotension between the

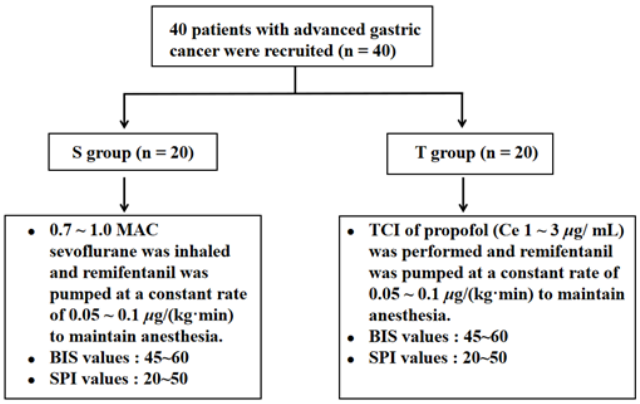

Figure 1. The flowchart of patients recruitment and grouping.

Table 1. Comparison of two groups with general information.

General information	S Group	T Group
Age ($\bar{x}$ ± s, years old)	68 ± 3	67 ± 3
Weight ($\bar{x}$ ± s, kg)	67 ± 11	65 ± 9
Height ($\bar{x}$ ± s, cm)	166.3 ± 8	163 ± 9
BMI ($\bar{x}$ ± s, kg/m^2)	23.7 ± 2.7	23.7 ± 2.2
surgery time ($\bar{x}$ ± s, min)	238 ± 47	234 ± 52
Anesthesia time ($\bar{x}$ ± s, min)	254 ± 54	254 ± 55
Gender (male/female)	12/8	13/7
Patients of radical total gastrectomy	8	10
Patients of radical proximal gastrectomy	6	7
Patients of radical distal subtotal gastrectomy	6	3

Table 2. Comparison of the two groups with TNM stage and grade of tumor cells differentiation ($n = 20$).

Group	Postoperative pathological stage			Tumor histological type	
	IIB	IIIA	IIIB	Grade II - III adenocarcinoma	Poorly differentiated adenocarcinoma
S Group	4	8	8	12	8
T Group	3	7	10	14	6

two groups ($P > 0.05$). The results were shown in Table 3.

Intraoperative bleeding volume, infusion volume and urine volume

There was no significant difference in intraoperative blood loss, infusion volume and urine volume between the two groups ($P > 0.05$), as shown in Table 4.

Postoperative VAS score

There was no significant difference in postoperative VAS scores between the two groups ($P > 0.05$). The results were shown in Table 5.

The proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in $CD4^+$ Treg cells

Compared with the S group, the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in peripheral blood increased in the T group at T2 and T3. Compared with stage IIB, the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in the peripheral blood of patients with stage IIA and IIB in both groups increased ($P < 0.05$), as shown in Table 6.

Compared with T1, the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in the peripheral blood of the S group was decreased at T2 and T3 but increased in the T group. Compared with T2, the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in the peripheral blood of patients in both groups at T3 was decreased ($P < 0.05$), as shown in Table 6.

Compared with T1, the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in peripheral blood of stage IIIA and IIIB patients in group S at T2 and T3 was decreased, and the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in peripheral blood of stage IIIA and IIIB patients in T group at T2 and T3 was increased. Compared with T2, the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in the peripheral blood of stage IIIA and IIIB patients in the S group was decreased at T3, and the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in peripheral blood of stage IIIA and IIIB patients in T group was decreased at T3 ($P < 0.05$). The results were shown in Table 6, Figures 2 and 3.

Compared with T1, the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in the peripheral blood of

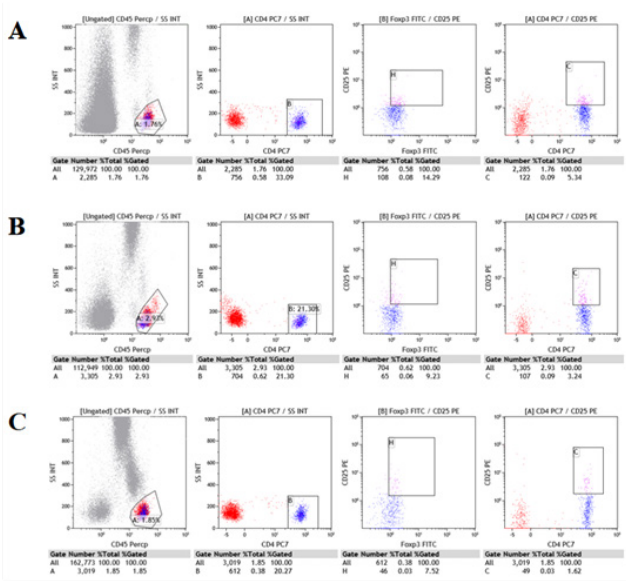

Figure 2. The proportion of Tregs in the patients of stage IIIA and IIIB in the S group. (A: blood collection before induction of anesthesia; B: blood collection after surgery; C: blood collection on the first day after the surgery).

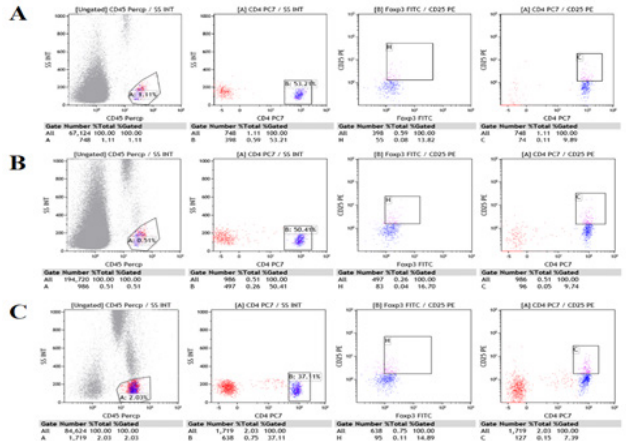

Figure 3. The proportion of Tregs in the patients of stage IIIA and IIIB in the T group. (A: blood collection before induction of anesthesia; B: blood collection after surgery; C: blood collection on the first day after the surgery).

Table 3. Comparison of occurrence of complications during surgery in the two groups (n (%)).

Group	Hypertension	Hypotension
S group	2 (10)	3 (15)
T group	3 (15)	3 (15)

Table 4. Comparison of estimated blood loss, infusion volume of urine volume during surgery in the two groups.

Group	blood loss (mL)	Infusion volume (mL)	Urine volume (mL)	Crystalloid fluid (mL)	Colloidal liquid (mL)
S Group	153 ± 47	2497 ± 508	350 ± 141	1818 ± 355	689 ± 233
T Group	118 ± 33	2245 ± 456	330 ± 145	1625 ± 321	597 ± 260

Table 5. Comparison of static VAS and dynamic VAS in two group.

Index		24 h	48 h
Static VAS score	S Group	1.17 ± 0.03	1.00 ± 0.00
	T Group	1.20 ± 0.03	1.00 ± 0.00
Dynamic VAS score	S Group	3.71 ± 0.13	2.40 ± 0.08
	T Group	3.58 ± 0.26	2.27 ± 0.07

Table 6. Comparison of the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in $CD4^+$ Treg cells in peripheral blood of the two groups (n = 20, %, $\bar{x}\pm$ s).

Experimental stage	Pathological stage	S Group	T Group
T1		12.51±0.47	12.41±3.08
	Stage IIB	7.06±2.24	6.42±0.41
	Stage IIIA	13.38±0.81[b]	13.82±0.34[b]
	Stage IIIB	14.05±0.40[b]	13.86±0.37[b]
T2		8.43±1.79	13.52±4.23a
	Stage IIB	5.98±2.03[c]	5.36±0.18[ac]
	Stage IIIA	9.17±1.67[bcd]	15.45±1.12[bcd]
	Stage IIIB	8.96±0.31[acd]	14.12±0.58[bcd]
T3		7.87±1.52	11.81±4.03[a]
	Stage IIB	5.27±1.62[c]	4.19±0.08[c]
	Stage IIIA	8.59±0.63[bcd]	14.73±0.29[bcd]
	Stage IIIB	8.39±0.30[bcd]	14.12±0.58[bcd]

Note: [a]$P < 0.05$ compared with S group, [b]$P < 0.05$ compared with stage IIB, [c]$P < 0.05$ compared with T1, [d]$P < 0.05$ compared with T2.

stage IIB patients at T2 and T3 was decreased. Compared with T2, the proportion of $CD4^+CD25^+FOXP3^+$ Treg cells in the peripheral blood of stage IIB patients at T3 was decreased ($P < 0.05$). The results were shown in Table 6, Figures 4 and 5.

Discussion

In this study, patients in both groups did not receive neoadjuvant chemotherapy or radiotherapy before surgery. There were no statistical differences between the two groups in terms of age, sex, body mass index, total dosage of opioids, surgery method, surgery time, postoperative VAS scores and other indicators, so as to ensure comparability between the two groups.

Regulatory T cells (Treg cells) are a group of cells that can regulate the immune function of the body. Treg cells play a very important role in the benign and malignant degree of tumors and immune response (13). Treg cells can regulate the proliferation of B cells and inhibit the production of antibodies (14). Treg cells can inhibit immunity by destroying cell metabolism, regulating the function of antigen-presenting cells and dissolving cells. Treg cells can also produce immune tolerance through the negative regulation of tumor immunity by the body (15). Regulatory T cells are involved in the occurrence and development of many diseases. Treg cells have the functions of inhibiting the immune function of activated immune cells, protecting immune homeostasis and controlling the inhibition of effector T cells by exogenous antigens (7-9). Treg cells are related to the occurrence and development of tumors (16).

The characteristic phenotypes of Treg cells are mainly CD4+CD25+ Treg cells and the central regulator of the forkhead family, FOXP3. CD4+CD25+ Treg cells are subpopulations of CD4+ T cells, which can regulate the immune function of the body by controlling cellular active T cells. It was found that the differentiation of CD4+CD25+ Treg cells mainly includes two types. One was that CD4+ cells were transformed into Treg cells through FOXP3 expression during thymocyte maturation. The other was that some naive T cells produced FOXP3 under the induction of transforming growth factor-β when stimulated by endogenous or exogenous antigens, forming regulatory T

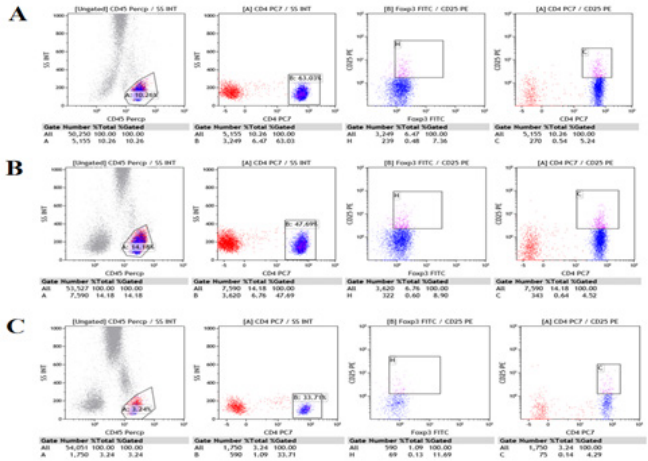

Figure 4. The proportion of Tregs in the patients of stage IIB in the S group. (A: blood collection before induction of anesthesia; B: blood collection after surgery; C: blood collection on the first day after the surgery).

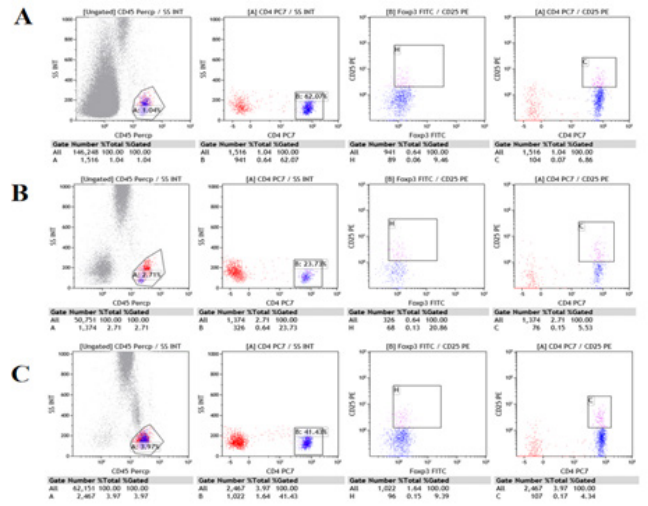

Figure 5. The proportion of Tregs in the patients of stage IIB in the T group. (A: blood collection before induction of anesthesia; B: blood collection after surgery; C: blood collection on the first day after the surgery).

cells (17,18).

FOXP3 is a highly conserved gene that is necessary for the development of CD4+CD25+ Treg cells in the thymus and peripheral generation. The transcription factor, FOXP3, is a standard for CD4+CD25+ Treg cell activation and plays a key role in regulatory T cell development and functional inhibition. CD4+CD25+FOXP3+ Treg cells belong to a group of negative regulatory cells that inhibit the function of other immune cells. FOXP3+ Treg cells can inhibit anti-tumor immune function which promotes the growth of tumors. Therefore, the proportion of CD4+CD25+FOXP3+ Treg cells was selected in this study to observe the effect of sevoflurane or propofol on the immune function of gastric cancer patients, so as to predict the prognosis of tumor patients.

Flow cytometry is a technology that uses flow cytometry to quickly measure, store and display substances (such as bacteria and cells) suspended in liquid for multiparameter rapid quantitative analysis and sorting one by one, DNA sorting and proliferation of immunophenotype, multiparameter analysis of fluorescent protein, and so on (19). Its principle is to make a single cell or other small biological particles in a fast, single straight-line flow state, and carry out multi-parameter quantitative analysis and sorting on a single cell or particle (19,20). In this study, the proportion of CD4+CD25+FOXP3+ Treg cells in peripheral blood was measured by flow cytometry.

The results of this study showed that compared with stage IIB, the proportion of CD4+CD25+FOXP3+ Treg cells in patients with stage IIA and IIB increased before induction, after surgery and on the first day after surgery, indicating that the expression of CD4+CD25+FOXP3+ Treg cells was related to the TNM stage of gastric cancer, and its expression also increased with the increase of the malignant degree of gastric cancer, which was similar to the results of Chen et al. (21).

The results of this study showed that compared with the propofol group, the expression of CD4+CD25+FOXP3+ Treg cells in the peripheral blood of patients receiving sevoflurane inhalation anesthesia was decreased after surgery and on the first day after surgery, indicating that sevoflurane could alleviate the suppression of the postoperative immune function of patients with gastric cancer more than propofol. The results showed that the expression of CD4+CD25+FOXP3+ Treg cells in the peripheral blood of patients with gastric cancer under sevoflurane anesthesia gradually decreased after surgery and on the first day after surgery, while the expression of CD4+CD25+FOXP3+ Treg cells in patients with gastric cancer under propofol anesthesia increased first and then decreased after surgery, suggesting that sevoflurane could reduce postoperative immunosuppression in patients with gastric cancer and the inhibitory effect of propofol on immune function was strengthened and then reduced.

Previous studies of our group found that sevoflurane can up-regulated FOXP3 gene expression in isolated gastric cancer cells to inhibit the biological behavior of tumor cells, while studies on patients undergoing radical gastrectomy for advanced gastric cancer found that sevoflurane could reduce the expression of CD4+CD25+FOXP3+ Treg cells in peripheral blood. The expression trend of FOXP3 in gastric cancer cells was different from that of CD4+CD25+FOXP3+ Treg cells in peripheral blood, which might be because FOXP3 in gastric cancer cells reflects local immune function, while CD4+CD25+FOXP3+ Treg cells in blood reflected systemic immune function.

This study was a small sample single-center exploratory study, and whether the expression of CD4+CD25+FOXP3+ Treg cells in peripheral blood is related to the TNM stage in patients with advanced gastric cancer needs to be observed in a large sample. This study only observed the effect of sevoflurane on the expression of CD4+CD25+FOXP3+ Treg cells in peripheral blood of patients with gastric cancer after surgery and the first day after surgery, and its long-term effect on Treg cells as well as its effect on the prognosis of patients with gastric cancer and its mechanism remain to be further discussed.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Acknowledgments

Not applicable.

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