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Original Article

Effect of opisids on the immunologic function of gastrointestinal cancer patients undergoing laparoscopy

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Abstract: It is well-known that the perioperative period is characterized by immunosuppression and may predispose already immunosuppressed cancer patients to tumor spread. Cancer patients typically show depression of both cellular and humoral immune functions. This study is aimed to evaluate the effect of traditional general anesthesia or without opioids on the immunologic function of the gastrointestinal cancer patients undergoing laparoscopic surgery. A total of 90 gastrointestinal cancer patients were randomly divided to 3 groups to accepted three anesthetic methods. RE and SE of Entropy, mean arterial pressure and heart rate during the entire anesthesia process were detected at nine times and changes in the proportion of $CD3^+$, $CD4^+$, $CD8^+$ and $CD16^+/56^+$ were measured by flow cytometry and concentration of IFN-γ and IL-10 were measured by enzyme-linked immunoassay (ELISA) at four phases. No significant differences were observed in RE and SE of Entropy, mean arterial pressure and heart rate at the nine points in the three groups ($P>0.05$). As the same time, the time of ambulation after operation, postoperative anal exhaust time and discharge time also showed no significant differences in the three groups. However, Changes in the proportion of $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+/56^+$, IFN-γ and IL-10 displayed significant differences in three groups. This results indicates that patients in laparoscopic surgery who received the general anesthesia combined with spinal anesthesia without opioids has better protective effect on postoperative immunologic function than those received general anesthesia combined with spinal anesthesia with opioids and general anesthesia alone.

Keywords: Opioid, gastrointestinal cancer, immunologic function, postoperative recovery

Introduction

Gastric cancer is the fourth most common cancer worldwide and the second leading cause of cancer related deaths in the world [1]. Surgery has been the cornerstone of gastric cancer treatment. The overall survival of patients with gastrointestinal cancer remains poor despite improved diagnostic and treatment strategies. Although surgical resection is still one of the first priorities, surgery may inevitably induce profound systemic neuroendocrine, metabolic, inflammatory and immunological stress [2].

Regional anesthesia, including spinal and epidural anesthesia, reduces the stress response caused by surgery, which is believed to be a mediator of postoperative immunosuppression [3]. Regional anesthesia attenuates the surgical stress response by blocking afferent neural transmission. The addition of regional anesthesia to general anesthesia results in less overall use of opioids and volatile anesthetics [4]. Spinal anesthesia is known to prevent or attenuate an excessive stress response during or after surgery, which prevents noxious afferent input from reaching the central nervous system [5]. Both preclinical and clinical studies have suggested that the addition of spinal blockade to general anesthesia attenuates the metastasis-promoting effect of surgery in the tumor-bearing host [6].

In most studies, the immune function has been evaluated in vitro by measuring the natural killer activity or mitogen-induced proliferation of lymphocytes in the presence or absence of opioid peptides in the incubation media [7]. Opioid administration, both in perioperative and chronic stage, has been shown to suppress cell-mediated and humoral immunity [8]. This includes NK cell activity, production of immune-stimulat-

ing cytokines, phagocytic activity, and antibody production. Th cells are sub-groups of lymphocytes that play a central role in orchestrating host immune responses through their capacity to help other cells in the immune system [9]. In the scenario of cancer, Th1 cells mediate anti-tumor reactivity, by producing interferon-γ (IFN-γ), resulting in tumor regression [10]. The Th subsets are known for their altered frequencies, distribution and balance in cancer-bearing patients [11]. More importantly, recent research has revealed that the balance of Th subsets determines the direction of anti-tumor immune responses and hence patient clinical outcomes [12]. Proper peri-operative management, including selection of suitable anesthetic methods, may help recover the disturbed balances of Th subsets or even maintain the balance of anti-tumor responses. IL-10, mainly secreted by Th2 cells, has immunosuppressive effects. Foreign scholars [13] has studied that the mRNA of IL-10 was detected in basal cell carcinoma by the RT-PCR assay and IL10 was detected in its cancer strain culture supernatant by ELISA and in tissue sections by immunohistochemistry, which prompts cancer cells to secrete IL-10 inhibiting the immune response.

In this study, under the control of the depth of anesthesia, the 24 h postoperative VAS pain score, recovery time, extubation time, postoperative anal exhaust time, discharge time and the time of ambulation after operation were measured and compared between general anesthesia with opioids (group A), general anesthesia combined with spinal anesthesia with opioids (group B) and general anesthesia combined with spinal anesthesia without opioids (group C). We investigated Th cell subset changes in the peripheral blood of gastrointestinal cancer patients before anesthesia induction (T1), at the end of surgery (T2) and 24 hours (T3) and 72 hours (T4) after operation. In particular, we compared the differences in Th cell subsets changes between group A, group B and C to determine whether anesthetic methods have an impact on immune responses and postoperative recovery.

Patients and methods

Patients

The study was approved by the Ethics Committee of First Affiliated Hospital of Nanchang University and patients were given oral written informed consent.

Ninety (ASA) scores I-II patients suffering from gastrointestinal cancers who underwent curative surgery from May to September 2013 were allocated randomly by closed envelopes to receive traditional general anesthesia with opioids (group A, n=30); general anesthesia combined with spinal anesthesia with opioids (group B, n=30) and general anesthesia combined with spinal anesthesia without opioids (group C, n=30). No patient had lymph nodes and metastasis. Patients with severe diseases involved in heart, lung, brain, liver, kidney or taking drugs that affect immune function or immune system disorders before operation were refused to participate in this study. The patients who have intraoperative laparotomy surgery or bleeding occurs or hypotension or death or hemolysis case pathological results showed positive surgical margins and lymph cancer positive biopsy were excluded from the study. Informed consent about the objective and methods of our study was obtained from patients in all cases.

Anesthesia and analgesia

In the anesthetic room, before insertion of the spinal catheter, non-invasive blood pressure (NIBP), heart rate (HR), ECG, pulse oxygen saturation (SpO_2), pressure of end-tidal carbon dioxide ($PETCO_2$), reaction entropy (RE) and state entropy (SE) were recorded routinely and continuously.

In group A, anaesthesia was induced with dexmedetomidine 1 ug/kg, propofol 1.5-2.5 mg/kg, atracurium and fentanyl 2-4 ug/kg for 10 minute. Anaesthesia was maintained with propofol 4-12 mg/kg·h, atracurium 0.6-0.7 mg/kg·h, remifentanil 4-8 ug/kg·h. Postoperative analgesia was achieved with intravenous infusions of sufentanilinitially 1.5-2.5 ug/kg for 2 days. In both group B and C, an epidural catheter was inserted at T9-10 or T12-L1 or L2-3 intervertebral space by a paramedian technique, which was confirmed by 1% lidocaine with 0.5% ropivacaine 5 ml+5 ml to exclude spinal anaesthesia. Induction of epidural anaesthesia was accomplished with intravenous infusion of dexmedetomidine 1 ug/kg for 10 minute, propofol 1.5-2.5 mg/kg, atracurium 0.3-0.6 mg/kg, fentanyl 2-4 ug/kg in group B,

Table 1. Demographic data for groups A, B and C

	Group A (N=30)	Group B (N=30)	Group C (N=30)
Gender (M/F)	14/16	15/15	13/17
Age yr (SD)	57.03 (6.67)	57.77 (5.67)	58.83 (4.58)
Mean weight kg (SD)	54.33 (8.289)	55.40 (8.815)	57.60 (10.734)
Mean height cm (SD)	160.53 (6.469)	161.97 (6.206)	161.53 (6.388)
Body mass index	21.0027 (2.29093)	21.9650 (2.638)	21.9650 (3.196)
Surgical site (Gastric/Colon/Colorectal)	12/8/10	15/7/8	10/9/11

The values in parentheses represent the standard deviation.

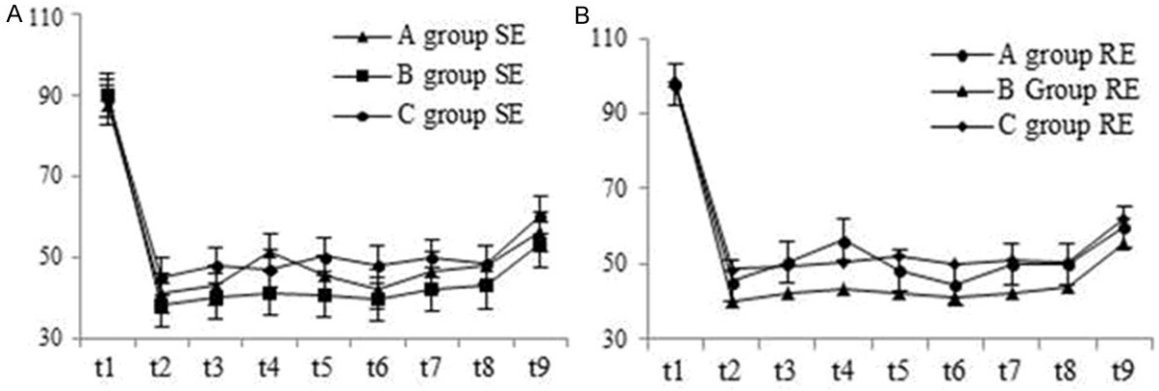

Figure 1. Change of SE (A) and RE (B) in three groups.

while no fentanyl in group C. Maintaince of Anesthesia was intravenous propofol 4-12 mg/kg·h, atracurium 0.6-0.7 mg/kg·h, remifentanil 4-8 ug/kg·h in group B but no remifentanil in group C. Postoperative Analgesia was intravenous 0.15-0.25% ropivacaine with 200 ml for two days in both group.

Flow cytometry analysis

5 mL blood samples were collected in the 100 μl of EDTA tube, treated immediately with 10 mg/mL of Brefeld in A (BFA; Sigma Chemical, St. Louis, Missouri), kept at ambient temperature, and prepared within 4 hours. Brefeld in A, a relatively nontoxic but potent inhibitor of extracellular transport, was used to block cytokine secretion, keeping the products within cells. Cell surfaces were stained with anti-CD3, anti-CD4, anti-CD8, anti-CD16 and anti-CD56 Ab. The red cells were lysed with 13 FACS Lysing Solution (Becton Dickinson) for 10 minutes at room temperature. After washing with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) and NaN3, cells were permeabilized with 0.5 mL 1 3 FACS permeabilizing Solution (Becton Dickinson) for 10 minutes at room temperature. After two washes, cells were incubated with optimal concentrations of anti-IFN-γ and anti-IL-10 mAb. Stained cells were analyzed on an EPICS/XL flow cytometer (Coulter Electronics, Inc., Hialeah, Florida).

Enzyme-linked immunosorbent assay analysis

The plasma levels of IFN-γ, IL-10 were measured by enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions (R and D Systems, Minneapolis, MN, United States). Intra-assay and inter-assay coefficients of variation for all ELISAs were <5% and <10%, respectively. All samples were measured using three independent experiments, in duplicate.

Statistical analysis

All results are presented as mean ± SD from 3 independent experiments. Statistical differences between groups were determined by the one-way analysis of variance and Student's t-test. Values of $P<0.05$ were considered statistically significant differences.

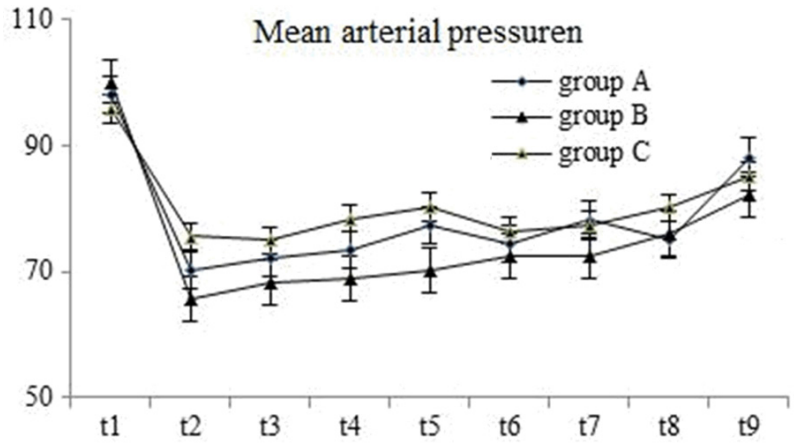

Figure 2. Chance of mean arterial pressuren in three groups.

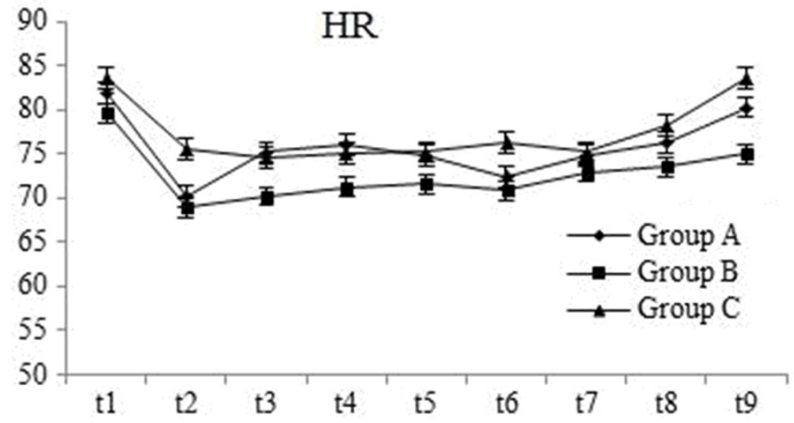

Figure 3. Chance of the HR of three groups.

minute after intubation (t4), three minutes after intubation (t5), before incision (t6), skin incision (t7), exploratory surgery (t8), at ending of administration (t9). Interestingly, the external stimuli entropy change in the both group B and C with epidural anesthesia was smaller than group A, but there was no significant difference (P>0.05) in three groups (**Figure 1**). Mean arterial pressure after induction of anesthesia in three groups decreased transiently, but decreased more greatly in group B and C with epidural anesthesia than in group A, then followed in smoothly low state. There was no significant difference (P>0.05) among the three groups at nine time points (**Figure 2**). The heart rate of the patient in three groups was not obviously, and there was no significant difference between the three groups at nine time points (P>0.05) (**Figure 3**).

Results

Patient characteristics

There were no significant differences in gender, age, weight, height, body mass index between three groups (**Table 1**).

Depth of anaesthesia

The entropy was declined rapidly to about 40 after induction of anesthesia, and then slowly raised to about 50. RE and SE raised moderately during intubation time, skin incision time and other external stimuli time, while slowed down after stimulation and gradually increased after anesthesia. There was no significant difference (P>0.05) in RE and SE in three groups before induction of anesthesia (t1), before intubation (t2), at the time of intubation (t3), one

Changes in the proportion of $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ and $CD16^+/56^+$

The results were showed in **Figure 4**. The proportion of $CD3^+$ was no statistically significantly different between three groups (P>0.05) before anesthesia induction (T1) and at the end of surgery (T2). The proportion of $CD3^+$ in Group C was increased more significantly than group A and B after operative at 24 h (T3) and 72 h (T4), and there was statistically significant difference (P<0.01) in three groups, while the differences between Group A and B was not statistically significant (P>0.05). There was no statistically significant difference in the proportion of $CD4^+$ between three groups (P>0.05) before anesthesia induction (T1). The proportion of $CD4^+$ was more significantly increased in group B and Group C than Group C and the differences between three groups were statistically sig-

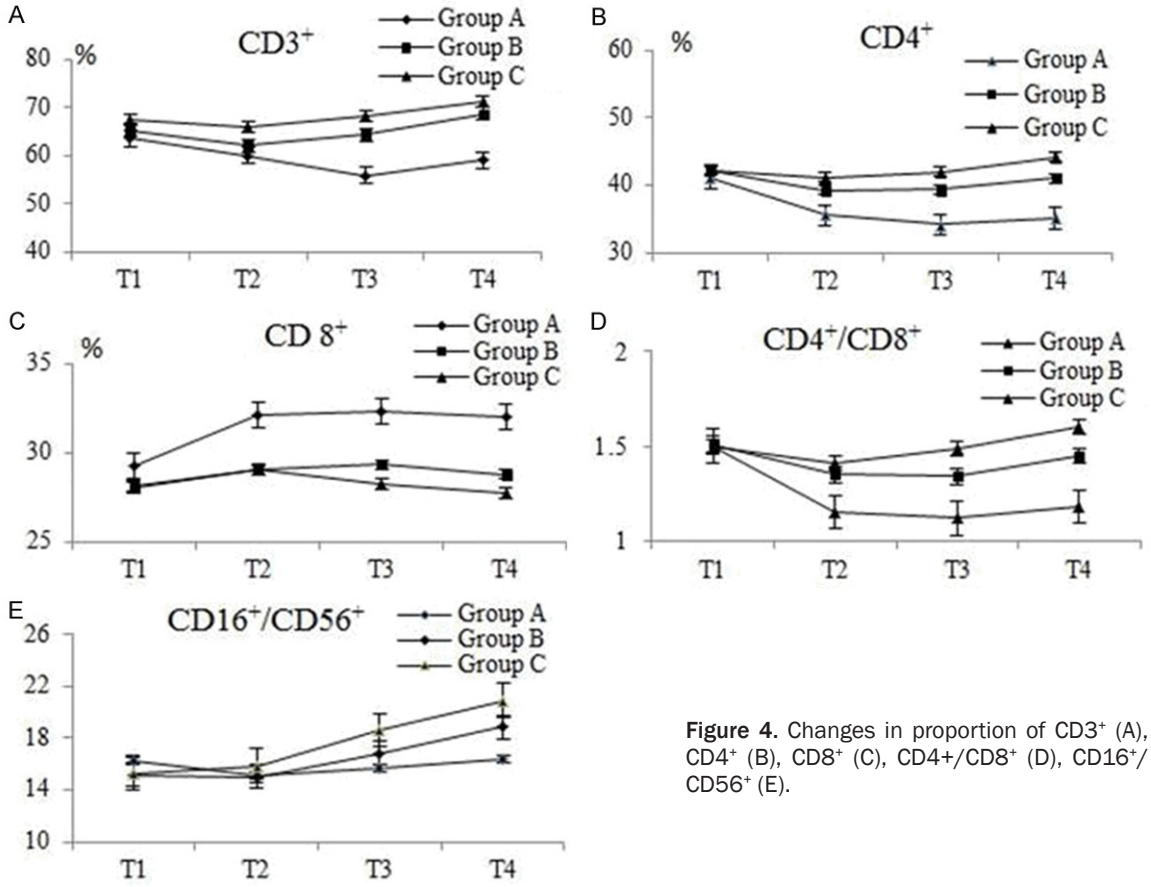

Figure 4. Changes in proportion of $CD3^+$ (A), $CD4^+$ (B), $CD8^+$ (C), CD4+/$CD8^+$ (D), $CD16^+$/$CD56^+$ (E).

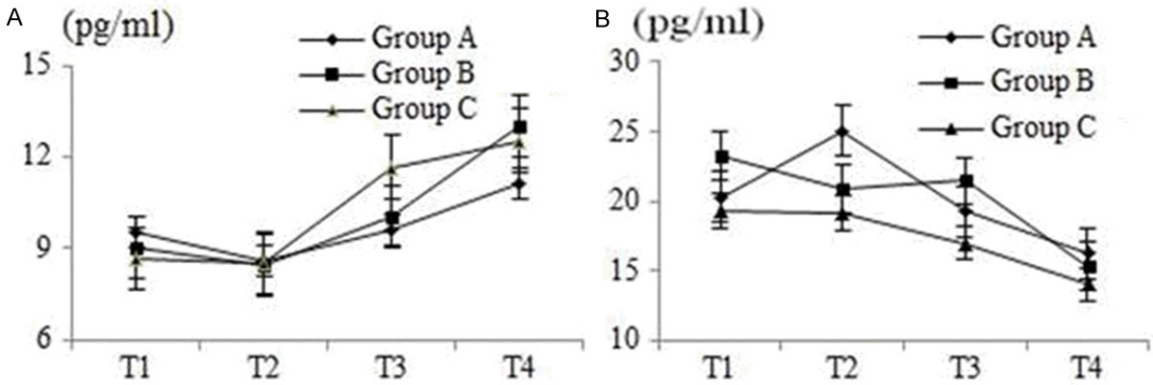

Figure 5. Changes of the concentration of IFN-γ (A) and IL-10 (B) in three groups.

nificant (P<0.05) in T2, T3, T4 phase; The proportion of $CD4^+$ in group C was increased more significantly than group B and the difference between this two groups was statistically significant (P<0.05). The proportion of $CD8^+$ between three groups had no statistically difference (P>0.05) in T1 phase, while the proportion of $CD8^+$ was significantly more decreased in group B and group C than Group A in T2, T3, T4 phase, which was statistically significant (P<0.05) between three groups. But there was no statistically difference in group B and Group C. There was no statistically significant difference (P>0.05) in the ratio of $CD4^+$/$CD8^+$ between three groups in T1 Phase, but the ratio of $CD4^+$/$CD8^+$ in group C was increased more

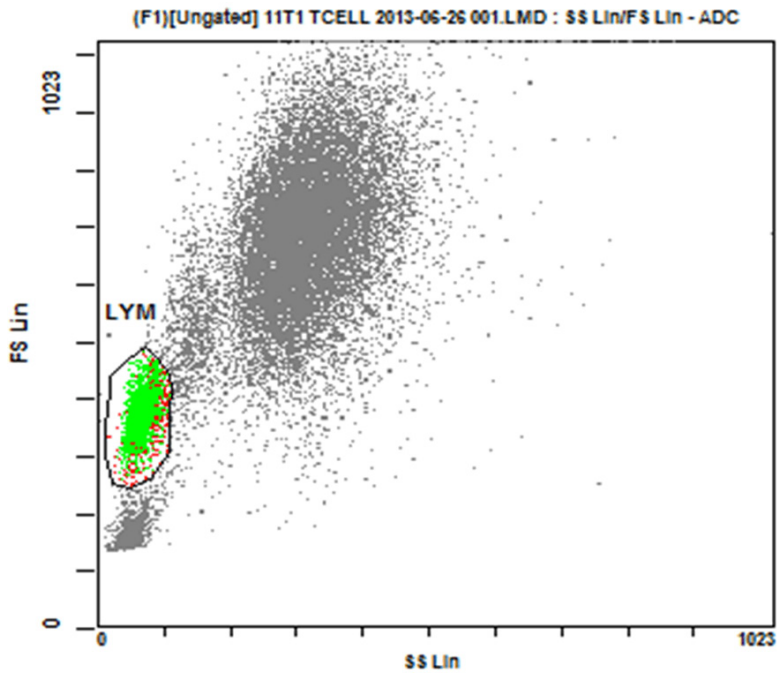

Figure 6. Dimensional flow cytometry (From lower left to upper right is red blood cell debris, lymphocytes, monocytes, neutrophils, where the aim region in lymphocytes was T cells, NK cells).

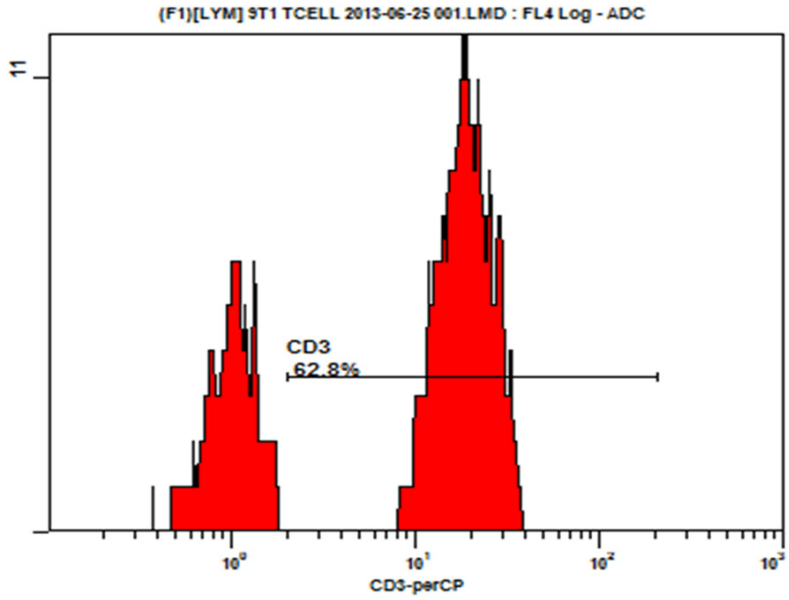

Figure 7. $CD3^+$ cells in the histogram (the proportion of $CD3^+$ cells was 62.8%).

significantly than in group A and B in T2, T3, T4 phase, when the difference was statistically significant between three groups (P<0.01), while difference between group A and B was not statistically significant. There was no statistically significant difference (P>0.05) in the ratio of $CD16^+/CD56^+$ between the three groups in T1 and T2 Phase. The ratio of $CD16^+/CD56^+$ was increased more significantly in group A and B than group C and the difference between three groups was statistically significant (P<0.05) in T3 and T4 phase, when there was statistically significant difference between three groups, but the ratio of $CD16^+/CD56^+$ in group C was increased more significantly than group B and the difference in the two groups was statistically significant (P< 0.05) (**Figures 7-9**).

Plasma concentrations of IFN-γ and IL-10

The Plasma concentrations of IFN-γ and IL-10 were measured with witch ELISA kits (Quantikine, R&D Systems and Minneapolis, MN, USA). The result was showed in **Figures 5-9**. There was no statistically significant difference in the concentration of IFN-γ in three groups in T1, T2 and T3 phase. The concentration of IFN-γ in group B and C was increased more significantly than Group A in T4 phase, when there was significant difference between three groups. There was no was significant difference in the concentration of IL-10 in three groups in T1, T3 and T4 phase. The concentration of IL-10 in group B and C was decreased more obviously than group A in T2 phase, which was characterized with statistically significant difference (P<0.005), while there was no statistically significant difference between Group B and C (P>0.05).

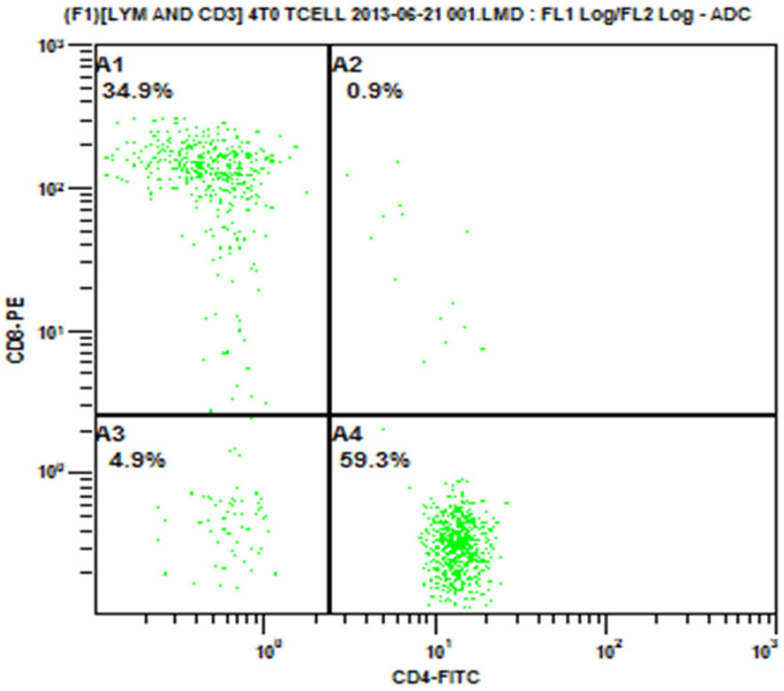

Figure 8. $CD4^+$ cells and $CD8^+$ cells parameter (The proportion of $CD8^+$ cells was 34.9% and $CD4^+$ cells were 59.3%).

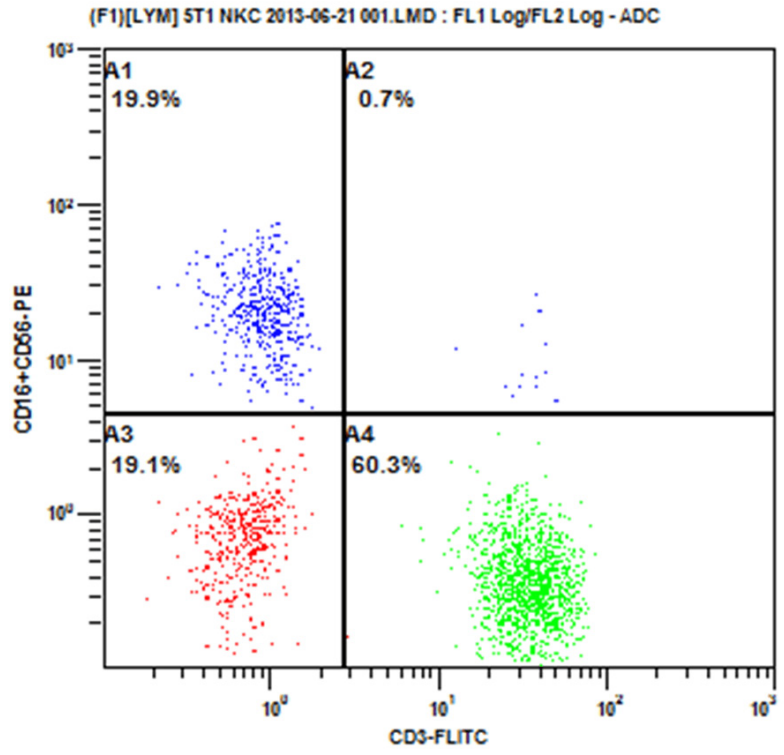

Figure 9. $CD16^+$ cells and $CD56^+$ cells parameter (The ratio of $CD16^+/CD56^+$ was 19.9%).

Postoperative recovery

Recovery time is recorded from the end of anesthesia to regaining consciousness; extubation time is measured from the start of anesthesia to full regain consciousness. The result of recovery time and extubation time was showed in **Table 2**. Compared with group A, the recovery time of group B and C was significantly reduced, while the difference in two groups was statistically significant ($P<0.05$); The recovery time of group C decreased more significantly than group A and B, while the difference in group B and Group C was statistically significant ($P<0.05$). The extubation time of Group C was reduced more significantly than Group A and B, but the difference in three groups was statistically significant ($P<0.05$); There was no statistically significant difference between group A and B ($P>0.05$).

Pain severity was assessed at rest using a visual analogue scale (VAS) with values from 0 (no pain) to 10 (worst pain imaginable). **Table 2** showed the medians and ranges of pain, sedation in each of three groups 24 hours after surgery. The scores of VAS of group B and C was decrease more significantly than group A, and the difference in three groups was statistically significant ($P<0.05$), while there was no statistically significant difference between group B and Group C ($P>0.05$). There was

Table 2. Statistics of recovery in three groups

Group	Scores of pain	Recovery time (min)	Extubation time (min)	Ambulation time (h)	Anal exhaust time (h)	Discharge time (d)
A	3.1±1.1	23.1±5.3	30.2±7.5	28±1	48±6	8±1.3
B	1.1±0.9	15.6±6.1	28.3±6.6	26±2	42±3	7.5±1.5
C	1.5±0.7	8.2±3.2	15.3±3.6	25±1.5	40±4	7±1.6

no significant difference in the mean time of Postoperative ambulation, postoperative anal exhaust and discharge from hospital in three groups (**Table 2**).

Discussion

In this study, we had compared three groups of patients undergoing major or super major operation in immunosuppression, prognosis and the survival rate. Gottschalk et al [15] has shown that anesthesia, surgical procedures and excessive stress response caused by systemic inflammation in patients had effect on cancer recurrence and prevented immunosuppression at perioperative stage. Perioperative immune function, postoperative infection and cancer metastasis and recurrence are closely related with each other, which indirectly affect the final outcome and prognosis of the patient. Thus, the surgical removal of cancer was followed by increasing the likelihood that cancer metastasis occurs and further weakens the immune system at the same time, which resulted to decrease perioperative immunity. Such views were consistent with the results in this study. In this study, it was showed that the concentration of IFN-γ, the proportion of $CD3^+$, $CD4^+$, the ratio of $CD4^+/CD8^+$ and $CD16^+/56^+$ in the patient decreased at the end of surgery and on the first postoperative day, while the concentration of IL-10 and the proportion of $CD8^+$ raised at the end of surgery and on the first postoperative day, which was consistent with the study of Tan Wei [15]. Perioperative immunosuppression may have significant impact on patient recovery and long-term survival rate; therefore, it is necessary to take measures to investigate how to protect perioperative immunity of the cancer patient.

In this study it had been noticed that the RE and SE of entropy, the mean arterial pressure and HR in general anesthesia combined with spinal anesthesia group were more stable than that of general anesthesia, which indicts that general anesthesia combined with spinal anesthesia improves long-term outcome after cancer surgery and overall survival rate. This result goes with the other studies [16]. The potential ability of regional anesthesia can be attributed to at least three different mechanisms [17]. First, regional anesthesia attenuates the immunosuppressive effect of surgery. Neuraxial anesthesia can inhibit the neuroendocrine stress response and paravertebral analgesia in humans having breast surgery has also been shown to inhibit this surgical stress response [18]. Secondly, patients who receive regional analgesia have lower opioid requirements. Paravertebral analgesia can reduce opioid requirements after breast surgery [19]. Opioids may themselves inhibit cell-mediated immunity and host antitumor defenses. Finally, when regional anesthesia is used in addition to general anesthesia, the amount of general anesthetic required during surgery is reduced. In this study, we also found that the concentration of IFN-γ, the proportion of $CD3^+$, $CD4^+$, the ratio of $CD4^+/CD8^+$ and $CD16^+/56^+$ in the patient increased at the end of surgery and on the first postoperative day in general anesthesia combined with spinal anesthesia group, compared to the general anesthesia group, while the proportion of $CD4^+$ was statistically significant different at the end of surgery and on the first postoperative day and the third postoperative day. The ratio of $CD16^+/56^+$ was statistically significant different on the first day after surgery and the third postoperative day and the concentration of IFN-γ was statistically significant different on the third postoperative day. Correspondingly, the concentration of IL-10 and the percentage of $CD8^+$ had decreased at the end of surgery, on the first postoperative day and the third postoperative day, while there was no statistically significant difference in the percentage of $CD8^+$ at the end of surgery, the first day after surgery and the third postoperative day, also was the percentage of IL-10 at the end of surgery. These results illustrated that the general anesthesia combined with spinal anesthesia has immune protection of advantages over general anesthesia advantage. Zhou D et al [20] conducted a study on general anesthesia and general anesthesia combined with epidural anesthesia on

perioperative liver Th cell differentiation, and the results showed that compared with general anesthesia, anesthesia combined hard film anesthesia can better protect the anti-tumor immune effect differentiation of Th cells, reducing the proportion of Treg cells after surgery, which is to be beneficial for patients with hepatocellular carcinoma. A study conducted by Chen WK [16] in the effect of anesthetic technique on survival in human cancers suggested that epidural anesthesia and analgesia may improve overall survival rate in patients with tumors, compared with general anesthesia.

For Postoperative recovery in this study, it was demonstrated that the recovery time and extubation time were shortened significantly in two general anesthesia combined with spinal anesthesia groups, compared to general anesthesia, but it was shorten more significantly in anesthesia combined with spinal anesthesia with opioids group than without opioids group. There was statistically significant difference in recovery time and extubation time, but no statistically significant difference in Scores of pain after 24 hours postoperative, postoperative ambulation time, postoperative flatus and discharge time in two groups, which probably due to the promotion of the idea of fast track surgery.

Several major differences between the two anesthetic methods may be responsible for the distinct patterns in Th1/Th2 balance, as well as Th17 and Treg frequencies between the two groups. First, regional anesthesia substantially attenuates surgery-induced stress responses, including increases in levels of corticosteroid hormone and catecholamine [21]. Second, opioids inhibit both cellular and humoral immune function in humans [22]. Animal experiments have shown that morphine suppresses the lymphocyte proliferative response to mitogens when given systemically, but not when given intrathecally [23]. Similarly, patients receiving an epidural mixture of opioids and local anesthetics exhibited better preservation of lymphocyte proliferation and cytokine production than those receiving intravenous opioids alone. Third, intravenous and inhalation anesthetics are associated with elevated serum concentrations of catecholamines and cortisol [24]. Glucocorticoids and catecholamines can heavily influence immunomodulation, including decreases in Th1/Th2 cytokine production and an increase in *FoxP3* mRNA expression [25]. Consequently, it is not surprising that general anesthesia used alone suppressed the surgical stress-induced immune response more profoundly than general anesthesia combined with spinal anesthesia.

Of course, other immune mediators such as immune cells, cytokines factually are involved in the various steps of tumor occurrence, including tumor formation, tumor progression and metastasis, when interactions between tumor cells and immune mediators are extensive and dynamic. Currently, the mechanisms of inflammation promoting tumorigenesis have not yet been fully revealed in molecular level, and mechanisms of the effect of inflammatory microenvironment on different caners are varied. In this study, we try to put forward an idea of non-opioid anesthesia to preliminarily investigate gastrointestinal cancer with the current high incidence by comparing the effect of three anesthesia methods on the immune function and rehabilitation in patients with gastrointestinal cancer.

Disclosure of conflict of interest

None.

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