Effects of different methods of anesthesia and analgesia on immune function and serum tumor marker levels in critically ill patients

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Abstract. This study investigated the effects of different anesthesia and analgesia methods on immune function and serum tumor marker levels of critically ill patients undergoing tumor resection surgery. Seventy-six critically ill patients with indications for tumor resection surgery were selected in The Second Affiliated Hospital of Zhengzhou University from September 2015 to August 2016. The patients were randomly divided into a control and an observation group (38 patients each). The patients in the control group were treated with general anesthesia and postoperative intravenous analgesia, while the patients in the observation group were treated with general anesthesia and epidural anesthesia and postoperative epidural analgesia. Venous blood samples were collected at 30 min before anesthesia (T1), 2 h after the beginning of the surgery (T2), immediately after surgery (T3), 24 h after surgery (T4) and 72 h after surgery (T5). The viable cell percentage of T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$) and natural killer (NK) cells were measured by flow cytometry. The levels of carcinoembryonic antigen, sugar chain antigen 199, sugar chain antigen 125, neuron specific enolase and cytokeratin 19 were detected by electrochemiluminescence at 24 h before and after operation. Our results showed the levels of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ in the control group at T3-T5 were significantly lower than those at T1 ($p<0.05$). The $CD3^+$ level in observation group at T3 was also significantly lower than the level at T1 ($p<0.05$), but it increased at T4 and T5 and showed no significant difference compared with the initial level ($p>0.05$). The levels of $CD4^+$ and $CD4^+/CD8^+$ in the observation group were significantly higher than those in the control group at T2-T5 ($p<0.05$). And, the levels of $CD3^+$ and $CD4^+$ were significantly higher than those in the control group at T4 ($p<0.05$). The level of $CD4^+/CD8^+$ was significantly higher than that in the control group at T5 ($p<0.05$). No significant differences were found in the levels of $CD8^+$ and NK cells between the 2 groups at any of the time-points ($p>0.05$). No significant differences were found either in any of the tested tumor markers in either group after 24 h. Even without differences on the tumor marker levels, these results suggest that general anesthesia combined with epidural anesthesia and analgesia produces milder deleterious effects on the immune function of perioperative critically ill patients than general anesthesia combined with intravenous analgesia.

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Introduction

Tumors pose a serious threat to human health, and malignant tumors are one of the main causes of human death all over the world (1). Surgical resection is the main method for the treatment of malignant tumors, however numerous studies have shown that various factors during the surgery can alter cellular immunity. Especially for critically ill patients, cell immunosuppression due to surgery increases the risks for postoperative infections and tumor metastasis and recurrence, thereby shortening the survival time of patients (2). Besides the surgical operation per se, different methods of anesthesia and analgesia also have been claimed to impact the patient's immune function (3). Therefore, maintaining an appropriate immune balance by careful management of anesthesia and analgesia can improve the prognosis for patients (4). In this study, we investigated the effects of different anesthesia and analgesia techniques on the immune function and the levels of peripheral blood tumor markers on patients, in order to provide reliable experimental information that can guide the choice of suitable techniques that should promote optimal outcomes.

Patients and methods

Clinical data

General information. Seventy-six critically ill patients with indications for malignant tumor resection were selected in The Second Affiliated Hospital of Zhengzhou University from 2015 to 2016. The inclusion criteria included the presence

of malignant tumors confirmed by computed tomography/magnetic resonance imaging (CT/MRI) and pathology with indications for surgical resection, an expected survival time longer than 3 months, and an agreement to participate in the study in the form of a written signed informed consent. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Zhenzhou University. Patients with abnormal coagulation results or allergy to experimental drugs were excluded from the study. The patients were randomly divided into a control and observation group, with 38 patients in each. There were no significant differences in terms of general information between the patients in the two groups (p>0.05) (Table I).

Table I. Comparison of baseline data between two groups.

Items	Control group (n=38)	Observation group (n=38)	t/χ^2	P-value
Sex (Male/female)	21/17	23/15	0.054	0.816
Age (years)	45-76	45-75		
Average age (years)	57.36±4.49	58.13±4.52	0.745	0.458
Weight (kg)	63.76±6.24	62.23±6.36	1.059	0.293
Tumor type (n, %)			0.492	0.483
Liver cancer	24 (63.15)	22 (57.89)		
Colorectal cancer	14 (36.85)	16 (42.11)		
Duration of anesthesia (min)	254.78±31.26	251.43±33.39	0.451	0.653
Surgical duration (min)	203.68±34.37	204.75±35.58	0.113	0.894

Methods

Anesthesia method. Patients in the control group were treated with tracheal intubation and general anesthesia. The anesthesia was induced using a target-controlled micropump infusion of propofol (approval no. H20084531; Zhejiang Jiuxu Pharmaceutical Co., Ltd., Zhejiang, China) at 3-4 μg/ml, a single injection of fentanyl (approval no. H42022076; Yichang Renfu Pharmaceutical Co., Ltd., Yichang, China) at 2-4 μg/kg and a single injection of cisatracurium (approval no. H20060869; Jiangsu Hengrui Medicine Co., Ltd., Jiangsu, China) at 0.02 mg/kg. The tracheal tube was placed under bispectral index (BIS) value of 55 or less. Tracheal intubation was used to maintain anesthesia, remifentanil was injected (approval no. H20143314; Jiangsu Nhwa Pharmaceutical Co., Ltd., Jiangsu, China) at 0.1-0.2 μg/kg/min, and cisatracurium was intermittently injected. The intravenous injection of fentanyl (0.1 mg) was administered 5 min before cutting the skin.

The patients in the observation group received general anesthesia combined with epidural anesthesia. Before induction of anesthesia, the patient's T8-10 intervertebral space was punctured to place the epidural tube. Lidocaine (1%) (approval no. H14023559; Jincheng Hayes Pharmaceutical Co., Ltd., Jincheng, China) was injected first using 3 ml. The blocking plane was detected by the temperature discrimination method, and it was deemed successful whenever it spanned the T4-T12 levels. General anesthesia was performed in the same way as for the control group. Epidural administration of 0.375% ropivacaine (approval no. H20050325; Guangdong Shunfeng Pharmaceutical Co., Ltd., Guangdong, China) and 1% lidocaine (5-10 ml) was performed using a micropump sustained infusion time 5-8 ml/h.

Postoperative analgesia. The patients in the control group were treated with intravenous analgesia, and intravenous infusion of 1 μg/ml sufentanil (approval no. H20054172; Yichang Renfu Pharmaceutical Co., Ltd., Yichang, China) was performed at 5 ml/h.

The patients in the observation group were given epidural analgesia with sufentanil (0.2 μg/ml) and ropivacaine (0.12%) at 5 ml/h. The epidural catheter was removed when the density of platelets was higher than 100,000/mm^3, the INR value was <1.5, and the clotting time returned to normal.

Indicator detection. Venous blood samples (2-3 ml) were collected at 30 min before anesthesia (T1), 2 h after the beginning of the surgery (T2), immediately after surgery (T3), 24 h after surgery (T4) and 72 h after surgery (T5). The blood samples were centrifuged to collect serum. Mouse monoclonal anti-human CD3 (dilution, 1:50; cat. no. 300308), mouse monoclonal CD4 (dilution, 1:50; cat. no. 100405) and mouse monoclonal CD8 antibodies (dilution, 1:50; cat. no. 300912) and mouse monoclonal anti-human natural killer (NK) cell (CD3-CD16-CD56) antibody (dilution, 1:50; cat. no. 308745) were all purchased from BioLegend (San Diego, CA, USA) and were added and incubated for 30 min at 4˚C. The viable cell percentage of T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$) and NK cells were measured by flow cytometry.

Additionally, venous blood samples (3-5 ml) were collected at 24 h before and after surgery. The levels of carcinoembryonic antigen (CEA), sugar chain antigen 199 (CA199), CA125, neuron specific enolase (NSE) and cytokeratin 19 (CYFRA21-1) were detected by electrochemiluminescence using a kit from Roche Diagnostics (Basel, Switzerland).

Statistical analysis. Data were processed using SPSS 19.0 statistical software (SPSS, Inc., Chicago, IL, USA). The measurement data were expressed as mean ± standard deviation (mean ± SD), and the t-test was used for analysis. Data were expressed as rate and χ^2 test was used for analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

The levels of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ in both groups started to decrease at T2. All the indicator levels in the control group at T3-T5 were significantly lower than the indicator levels at T1 (p<0.05). The $CD3^+$ level in observation group was significantly lower at T3 than it had been at T1 (p<0.05), but the difference became insignificant again at subsequent time-points (p>0.05).

Interestingly, the levels of $CD4^+$ and $CD4^+/CD8^+$ in the observation group were significantly higher than those in control group at T2-T5 (p<0.05). Also, the levels of $CD3^+$ and $CD4^+$ were significantly higher than those of the control group at T4 (p<0.05). The level of $CD4^+/CD8^+$ was significantly higher than that of the control group at T5 (p<0.05). No

Table II. Comparison of T lymphocyte subset levels between two groups at different times.

Items	Groups	T1	T2	T3	T4	T5
$CD3^+$ (%)	Control	54.83±9.54	50.36±7.42	43.56±6.45[a]	44.74±3.66[a]	48.74±3.63[a]
	Observation	55.36±9.35	51.45±7.57	45.86±6.37[a]	51.47±4.73[b]	51.47±4.78
$CD4^+$ (%)	Control	32.56±6.45	28.76±3.34	22.36±4.38[a]	21.48±3.87[a]	24.48±3.25[a]
	Observation	33.21±6.32	28.36±3.26[a]	24.42±3.45[a]	25.25±3.65[a,b]	27.25±3.45[a]
$CD8^+$ (%)	Control	21.74±3.06	20.32±2.27	20.46±2.35	22.56±2.14	23.53±2.26
	Observation	21.92±3.12	23.86±2.33	20.83±2.47	24.24±2.18	23.47±2.38
$CD4^+/CD8^+$	Control	1.54±0.36	1.36±0.33	1.13±0.47[a]	1.01±0.34[a]	1.06±0.32[a]
	Observation	1.52±0.32	1.22±0.47[a]	1.16±0.45[a]	1.07±0.28[a]	1.24±0.48[a,b]

[a]Comparison with T1 within a group, $p<0.05$; [b]comparison between control and observation groups, $p<0.05$.

Table III. Comparison of NK cells percentage between two groups at each time-point (%).

Groups	T1	T2	T3	T4	T5
Observation	16.73±3.54	18.86±3.31	19.53±3.46	17.76±3.63	16.73±3.56
Control	17.32±3.35	19.35±3.54	19.84±3.47	18.27±3.75	16.49±4.83
t-test	0.746	0.623	0.390	0.602	0.247
P-value	0.457	0.535	0.698	0.549	0.805

NK, natural killer.

Table IV. Comparison of tumor markers between two groups before and 24 h after surgery.

Indicators	Groups	Before surgery	24 h after surgery	t-test	P-value
CEA (ng/ml)	Control	3.16±1.62	2.98±1.03	0.640	0.524
	Observation	3.19±1.58	2.96±1.04	0.750	0.456
t-test		0.082	0.084		
P-value		0.935	0.933		
CA199 (U/ml)	Control	14.06±3.43	13.96±3.54	0.125	0.901
	Observation	14.67±3.21	13.93±3.53	0.956	0.342
t-test		0.800	0.037		
P-value		0.426	0.971		
CA125 (U/ml)	Control	13.58±3.36	13.18±3.15	0.535	0.594
	Observation	13.87±3.87	13.36±3.17	0.628	0.532
t-test		0.349	0.248		
P-value		0.728	0.805		
NSE (ng/ml)	Control	17.26±3.47	16.73±3.84	0.631	0.530
	Observation	17.78±3.64	16.53±3.82	1.460	0.148
t-test		0.637	0.228		
P-value		0.526	0.820		
CYFRA21-1 (ng/ml)	Control	2.75±1.18	2.71±1.13	0.151	0.880
	Observation	2.77±1.03	2.73±1.15	0.45	0.28
t-test		0.160	0.076		
P-value		0.873	0.940		

CEA, carcinoembryonic antigen; CA199, sugar chain antigen 199; CA125, sugar chain antigen 125; NSE, neuron specific enolase; CYFRA21-1, cytokeratin 19.

significant differences were found in levels of $CD8^+$ between the two groups at any of the time-points ($p>0.05$) (Table II).

Comparison of the level of NK cells between two groups. No significant differences were found in the level of NK cells between the two groups at any of the time-points ($p>0.05$) (Table III).

Comparison of tumor markers between two groups before and 24 h after surgery. No significant differences in CEA, CA199, CA125, NSE and CYFRA21-1 levels were found before or after surgery, and no significant differences were found between groups either ($p>0.05$) (Table IV).

Discussion

Tumorigenesis is closely related to the function of the immune system. The immune system can identify, monitor and kill tumor cells in many cases, while the tumor cells can inhibit the immune system monitoring in others (5). The immune response to tumors is mainly mediated by T cells, and T cell subsets have become the main indicators in the diagnosis and prognosis of certain tumors (6). $CD3^+$, which is expressed by all mature T cells on their surface, can assist other T cells to recognize antigen receptors. Mature T cells can be divided into $CD4^+$ T and $CD8^+$ T subsets based on their different surface molecules. $CD4^+$ exerts an immunoregulatory function and can assist T cells. $CD8^+$ T cells are mainly cytotoxic and inhibitory T cells and can cause immune function disorders (7,8). NK cells are the most important innate immune cells that can non-specifically recognize and kill tumor cells (9).

An impaired and low cellular immune function is common in the critically ill patients with malignant tumors. The use of large amounts of opioids during the perioperative period to relieve pain combined with a response induced by the anesthesia and surgery and other stresses can further alter the immune function, increasing the risks for an inflammatory response, postoperative infection or adverse effects such as tumor recurrence or metastasis (10,11). The results of this study show that $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ cells in both groups started to decrease from 2 h after the beginning of the surgery, probably due to stress responses to trauma and anesthesia, resulting in increased secretion of adrenaline and catecholamines, that led to T lymphocyte immune function inhibition. General anesthesia can inhibit the limbic system in the cerebral cortex, however the stimulation through nociceptive pathways is not effectively blocked, resulting in a stress response of the body that can affect the cellular immune function (12,13). The patients in the observation group were treated with epidural anesthesia, which blocks the nerve impulses caused by surgical traumatic stimulation at the spinal level and, therefore does not affect immune function. The secretion of catecholamines, plasma cortisol and other stress-induced hormones, and the activity of sympathetic nerves can also be inhibited, which in turn decrease the effects on hypothalamus-pituitary-adrenal cortical axis and alleviate cell immunosuppression (14). Alternatively, general anesthesia combined with regional block anesthesia can reduce the required dose of general anesthetics, which reduces the immunosuppression effects (15).

Many studies have shown that epidural analgesia reduces the postoperative stress response (16). The results of our study showed that $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ levels in the control group at T3-T5 were significantly lower than those at T1 ($p<0.05$). On the other hand, the $CD3^+$ level in observation group at T3 was significantly lower than that at T1, but the level increased again soon thereafter and showed no significant difference at T4 or T5. The levels of $CD4^+$ and $CD4^+/CD8^+$ in the observation group at T2-T5 were significantly lower than those at T1. Also, the levels of $CD3^+$ and $CD4^+$ were significantly higher in the observation group than those in the control group at T4 ($p<0.05$). The level of $CD4^+/CD8^+$ was significantly higher than those of the control group at T5 ($p<0.05$). A possible explanation is that the use of postoperative epidural analgesia can reduce the postoperative stress response more significantly than intravenous analgesia. Epidural analgesia can inhibit the secretion of the adrenal glands and reduce protein degradation and insulin resistance. The drug used in epidural analgesia can directly prevent the transmission of the injury stimulus to central nervous system to achieve analgesia. More importantly, the use of epidural analgesia can be supplemented with systemic application of opioids, so as to ensure that the immune protection mechanism will remain so after the surgery (17). No significant differences were found in the levels of CD8 between the two groups at any of the time-points. This may be due to the fact that the groups of patients were treated with the same surgical procedures and the degrees of injury of the two groups were not significantly different from each other. Therefore, the direct cell killing effect of $CD8^+$ T cells on target cells was not obviously affected.

Our study found no significant differences in the levels of NK cells between the two groups at any of the time-points ($p>0.05$). A possible explanation is that the total number of lymphocytes affected the relative counting performed by flow cytometry. So, even though the total number of lymphocytes may have been reduced, the relative number of NK cells could have stayed the same or be even increased in some situations.

NSE, CYFRA21-1, CA199, CA125, CEA and other tumor markers have been used clinically in the diagnosis of tumors and evaluation of treatment efficacy (18). CEA is an antigen in cancer tissues. As the most widely used tumor marker, CEA is highly expressed in a variety of cancer tissues (colon, stomach, liver and lung cancer) (19). NSE, a neuron and neuroendocrine cell-specific enzyme, is highly specific for liver cancer tissue. The positive percentage of NSE in liver cancer tissues can be up to 60-80%. NSE is also useful in the evaluation of the treatment efficacy. CYFRA21-1, which exists in the cytoplasm of tumor cells, is released into the serum after tumor cell necrosis. CA199 and CA125 are generally used in the diagnosis of colorectal, liver and gallbladder cancers. Increased tumor marker levels can be used to diagnose tumors (20). Our study found no significant differences in any of the levels of tumor markers tested between T1 and T4. This surprising result may be due to the fact that minimally invasive surgery was performed in both groups of patients, compared with the traditional open surgery minimally invasive surgery is less traumatic with less intraoperative blood loss, needs a shorter operation time, and requires a smaller anesthetic dosage, making any differences in the tumor marker levels undetectable.

Taken together, the results in our study suggest that the effects of general anesthesia combined with epidural anesthesia and analgesia on the immune function of perioperative patients were significantly lower than the effects of general anesthesia combined with intravenous analgesia. Even though the levels of tumoral markers were not different between the groups of patients studied, the general anesthesia combined with epidural anesthesia and analgesia seems better at protecting the patient's postoperative cellular immune function.

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