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*Original paper*

# ***The effects of two different anesthesia and analgesia techniques on NK cell cytotoxicity in patients undergoing surgery for colorectal cancer***

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## **Abstract**

Perioperative anesthesia and analgesia may contribute in emphasizing immunosuppression in cancer patients. The natural killer (NK) cells play an important role in anti-tumor immunity. The aim of this study was to evaluate the effects of two different techniques of anesthesia and analgesia on the immune function by NK cell cytotoxicity (NKCC %) in surgical patients for elective colorectal cancer surgery. Thirty-three patients operated for colorectal cancer with resection were selected to receive general anesthesia with postoperative opioids and non-steroid anti-inflammatory drugs (NSAID), the GA group, or general anesthesia combined with epidural anesthesia and analgesia, the GAE Group. NKCC (%) for immune modulation was determined before and 24 h after surgery. Preoperative values NKCC (%) were found to be similar between the two groups (16.43 in the GA group vs. 16.65 in the GAE group). A decrease of NKCC % was recorded at 24 h after surgery in both groups: from 16.43 $\pm$ 1.8 to 11.94 $\pm$ 1.3 ( $p < 0.05$ ) in the GA group and from 16.66  $\pm$ 1.4 to 14.52 $\pm$ 1.2 in the GAE group. Analysis of the individual decrease expression revealed a decline by 24 % the GA group compared with 12 % in the GAE group ( $p = 0.007$ ). Combined general anesthesia with epidural analgesia is superior to general anesthesia alone in regards of preserving the NK cell cytotoxicity, thus delivering a lower impact on immune function for patients with elective colorectal surgery.

## **Keywords**

Anesthesia, analgesia, NK cell, cytotoxicity, colorectal cancer.

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## Introduction

For many malignant tumors, surgical resection is the mainstay treatment, but surgery itself can promote the development of metastasis by releasing tumor cells into circulation, augmenting angiogenesis or suppressing the cellular immune system (ZAHARESCU et al, 2017 [1]).

Natural killer (NK) cells are a critical component of the anti-tumor immune response, they play a major role in innate and adaptive immunity (ISVORANU, 2017 [2]). Their main function is recognition and killing of virus-infected cells or tumor cells. There are very-well documented studies that report a significant alteration of NK cell functions in cancer (SHAKHAR and BLUMENFELD, 2003 [3], ISVORANU, 2015 [4], ISVORANU, SURCEL et al, 2019 [5]). The immune system is suppressed by surgery, which promotes inflammation (VOGELAAR, LIPS et al, 2016 [6]). Also, antitumor immune potential can have a significant impact on postoperative outcomes in cancer patients. Animal models and retrospective clinical data suggest an association between anesthetic and analgesic techniques and cancer recurrence or metastasis. Postoperative suppression of the immune response may affect the tumor metastases disseminated after surgery (SALO, 1992 [7]). Stress response after surgery leads to depression of natural killer (NK) cell functions which is associated with increased tumor growth and metastasis in animal models (BEN-ELIYAHU, PAGE et al, 1999 [8]).

Opioids are a common first-choice analgesic for postoperative pain management, but there is an emerging interest in the possible association between perioperative use of opioids and recurrence or metastasis after cancer surgery. Immunomodulation induced by opioids occurs through direct action on immune cells (AL-HASHIMI, SCOTT et al, 2013 [9]). There is evidence that indicates Fentanyl of suppressing postoperative NK cell function both in patients and animal studies, which may increase the risk of tumor metastasis (SHAVIT, BEN-ELIYAHU et al, 2004 [10], FORGET, COLLET et al, 2010 [11]). An increased risk of cancer recurrence after radical prostatectomy was associated with postoperative opioid analgesia when is compared with epidural analgesia (BIKI, MASCHA et al, 2008 [12]). To avoid the immunosuppressive effects of opioids in cancer patients undergoing surgery an alternative choice is the use of non-opioid analgesic techniques (KIM, KIM et al, 2016 [13]).

The goal of this study was to compare the impact of two different anesthesia techniques – General Anesthesia (GA) and combined General Anesthesia with Epidural Anesthesia (GAE) – on the status of the immune function, assessed by the NK cell cytotoxicity (NKCC %), in patients for elective colorectal cancer surgery.

## Material and Methods

Our study is a prospective clinical study conducted in the Intensive Care Unit and General Surgery Ward of Witting Clinical Hospital Bucharest, Romania, from January 2015 to August 2018. The design of the study and the patient enrollment protocol was approved by the internal Ethics Committee of Witting Clinical Hospital, Bucharest, Romania, also a written informed consent was obtained.

Forty-five patients were enrolled, undergoing colorectal surgery with tumor resection; 33 completed the study. Patients with American Society of Anesthesiology (ASA) physical status I-III were randomized into two groups to receive either general anesthesia (GA, n=16) or general anesthesia combined with epidural anesthesia/analgesia (GAE, n=17). GA Group received general anesthesia (opioids – Sevoflurane) with postoperative opioids – NSAIDs analgesia and GAE Group received a combined anesthesia – general anesthesia and epidural anesthesia/postoperative analgesia.

The postoperative pain scores were assessed using a visual analogue scale (VAS) and the use of perioperative opioids were also evaluated.

Anesthesia was maintained in the GA group with Sevoflurane 1.5-2 % to maintain BIS values of 40-60 and Fentanyl 1 µg/kg was added intraoperatively as required; meanwhile in the GAE group we used Sevoflurane < 1.5% with the addition of epidural anesthesia which was maintained with the use of 1% Ropivacaine during surgery. Postoperative analgesia consisted in a multimodal approach (opioids + NSAIDs) for the GA group while for the GAE group we used the epidural analgesia (infusion of Ropivacaine 0.2-0.3% and 5 µg/ml Fentanyl).

Blood samples were obtained to determine NKCC (%) at the following moments: T1, before the induction of anesthesia and T2 at 24 hours after surgery.

### Isolation and cryopreservation of peripheral blood mononuclear cells (PBMC)

PBMC were isolated by ficoll gradient centrifugation from heparinized blood. After whole blood was mixed with the same volume of Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Invitrogen, CO), it was laid on a Biocoll separating solution density 1.077 g/ml (Biochrom, GmbH) and centrifuged at 2000 rpm for 20 minutes. After the centrifugation, the layer of PBMC was harvested and washed twice with RPMI 1640 and resuspended in culture media that contained 20% dimethyl sulfoxide (DMSO). For cryopreservation, PBMC were aliquoted at a concentration of  $1 \times 10^7$  cells per cryogenic vial. The cells were placed in a -80°C freezer for 24 hours and then transferred into a liquid nitrogen freezer until

use. The PBMC were used within 2 months of the date they were frozen. Frozen PBMC were thawed in a 37°C water bath until a small amount of frozen material was left, then washed in complete media. After that the cells were incubated overnight at 37°C in 5% CO<sub>2</sub> atmosphere.

### NK cell cytotoxicity assay

NK cell cytotoxicity was assessed with the CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega Co., WI) using K562 cells as a target cell line. By this colorimetric assay lactate dehydrogenase (LDH), a cytosolic enzyme that is released upon cell lysis, is quantitatively measures. Peripheral blood mononuclear cells ( $2 \times 10^5$  cells/well; effector cell, E) were mixed with K562 cells ( $2 \times 10^4$  cells/well; targeted cell, T) were mixed in a 96-well and incubated at 37°C with 5% CO<sub>2</sub> for 4 hours. The NK cell cytotoxicity of effector cells was measured at 490 nm (Absorbance Microplate Reader Sunrise-Basic Tecan, Grödig, Austria) and was calculated using the following equation:

$$\text{NKCC\%} = (\text{Experimental} - \text{Effector Spontaneous} - \text{Target Spontaneous}) / (\text{Target Maximum} - \text{Target Spontaneous}) \times 100$$

where “Experimental” is the experimental LDH release of co-cultured effector and target cells, “Effector Spontaneous” and “Target Spontaneous” express the spontaneous released LDH of the effector and target

cells alone, respectively, and “Target Maximum” is the maximum LDH release of target cells.

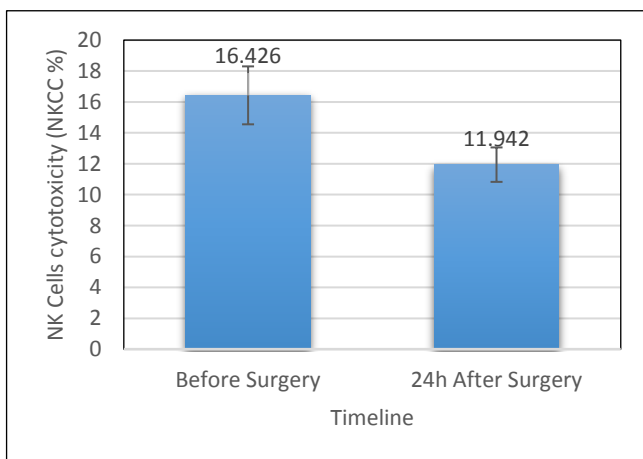
Collected data was introduced in a local database using the Microsoft Excel Office software. The statistical analysis was performed using the SPSS v16.0 software (SPSS Inc. Chicago, IL, USA) and was presented as mean  $\pm$  standard error (SEM). For statistical analysis, the Student t-test (two-tailed, assuming equal variance) was used for statistical evaluation of the differences. The difference between the individual decreases of the NKCC% between the two groups was analyzed for statistical relevance. The results were considered significant when *p* values were  $< 0.05$ .

## Results

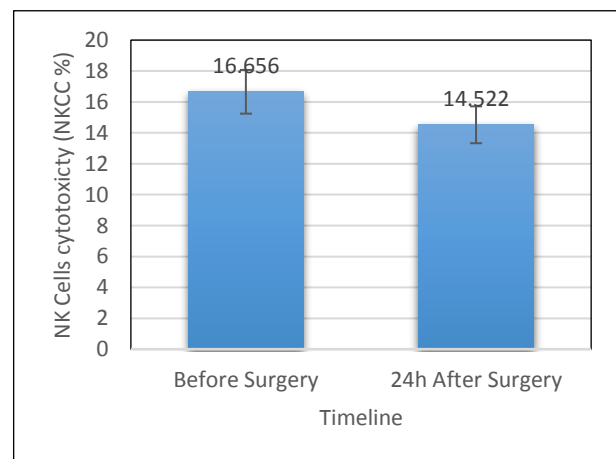
There were no significant differences in terms of patient characteristics and surgical factors between the two groups.

Before surgery (T1), the quantitative values of the Natural Killer (NK) cells cytotoxicity (NKCC %) are similar in both groups: 16.43% in the GA group vs. 16.65% in the GAE group.

At T2 (24 h after surgery), we found a decrease of NKCC (%) in both groups: in GA group NKCC (%) decreased from 16.43  $\pm$  1.87 to 11.94  $\pm$  1.11 (*p*=0.048) and in GAE group from 16.65  $\pm$  1.41 to 14.52  $\pm$  1.19, with a statistical significance in GA group (Figure 1 and 2).



**Figure 1.** NKCC (%) evolution at 24 hours postoperatively at GA group, a decrease with 24% was observed (*p*=0.048).



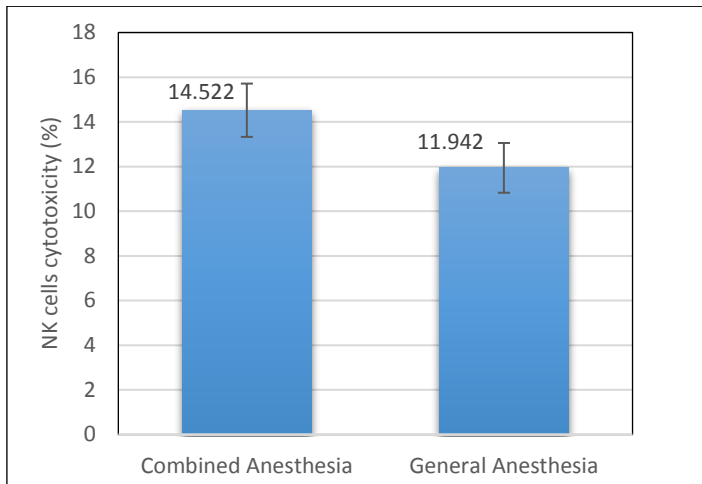
**Figure 2.** NKCC (%) evolution at 24 hours at GAE group, a decrease of 12%.

Analysis of the NKCC% level recorded a decline by 24% in the GA group compared with 12% in the GAE group (Figure 3).

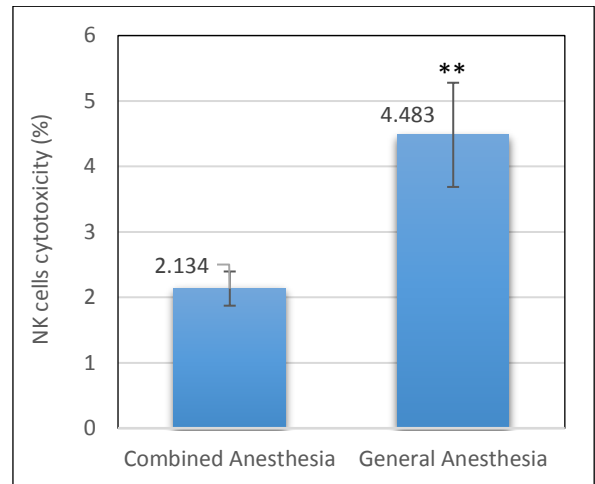
Regarding the individual evolution of NKCC(%), between the two moments T2 and T1, the results show a more pronounced decrease in GA group compared with GAE group, with 4.48  $\pm$  0.8 vs. 2.13  $\pm$  0.26 (*p* =0.007).

This comparative analysis of individual decreases revealed a statistical relevant value (*p* = 0.007) (Figure 4).

The evolution of NKCC depending on the operative times and type of anesthesia revealed that a lower decrease of NKCC in patients who were given combined anesthesia than those who were given only general anesthesia.



**Figure 3.** Comparison of the levels of the NKCC% at 24 h after surgery between the two groups.



**Figure 4.** Comparison of the differences of NKCC% (preoperatively versus postoperative) between the two groups ( $p = 0.007$ ).

## Discussions

The immune system of patients during the perioperative period can be affected directly or indirectly by anesthesia and other related drugs and thus can affect treatment and prognosis of colorectal cancer patients. The effects of anesthesia on immunity have been studied on multiple factors, including NK cells, B-lymphocytes, T-lymphocytes, macrophages, leukocytes and some cytokines (TNF- $\alpha$ , IL-1, IL-6, IL-8, IL-10, IFN, IFN-7, soluble Interleukin-2 receptor (sIL-2R) (ZAHARESCU, MOLDOVAN et al, 2017 [1]).

In our study, the cytotoxic capacity of natural killer cells (NKCC %) registered a decrease at 24 h after surgery in both groups with statistically significant correlation in GA group. Between the two moments T2-T1, NKCC% shows a drop rate more pronounced in GA group with 24% compared with GAE group, with 12% ( $p = 0.007$ ).

Therefore, we note that patients who received only general anesthesia and postoperative analgesia with opioids + NSAIDs systemically developed a significant reduction in the cytotoxic capacity of NK cells compared to those who received combined general anesthesia with postoperative epidural analgesia, proving the favorable effects of combined anesthesia.

There is quite a challenge to study in a comparative manner the effects of different anesthesia methods on perioperative immunity in patients with cancers, the results of available studies showing that the degree of impairment of immunosuppression varies depending on the type of anesthesia.

These findings can be partially explained by the stress induced by surgery, which inhibits the activity of NK cells responsible for lysis of tumor cells and promotes the stimulation of pro-angiogenic factors. Perioperative immunosuppression can be attributed to activation of the sympathetic nervous system and the hypothalamic-

pituitary axis. Catecholamine released from sympathetic nervous system activation bind to NK cell adrenoceptors, T lymphocytes, and macrophages leading to inhibition of NK cell activity and stimulation of LTh2 production by increasing balance in their favor (KUROSAWA, 2012 [14], DIVATIA and AMBULKAR, 2014 [15]).

Animal experiments show that tumor growth is associated with impaired cytotoxic capacity of NK cells, counteracted by beta-blockers and NSAIDs (PANTZIARKA, BOUCHE et al, 2016 [16]).

On human subjects, Kwon et al. (KWON, KWON et al, 2015 [17]) demonstrated a reduction in the cytotoxic capacity of NK cells for 3 months postoperatively in patients with hepatocellular cancer and Crane et al. demonstrated that the resumption of the cytotoxic function of NK cells in patients with glioblastoma was associated with increased expression of NKG2D receptors and reduced circulating MHC Class I Polypeptide-Related Sequence A (MICA) values (CRANE, HAN et al 2010 [18]).

Cata J.P. found that NK cell count in the perioperative period seem to have a biphasic response: increased number of circulating NK cells intraoperatively and minutes after surgery, effect mediated by epinephrine acting on  $\beta_2$ -adrenergic receptors, followed by a sustained drop for several days up to a month in stage II or III of colorectal cancer surgery. However the study does not mention anything about the NKCC(%) (CATA, CONRAD et al, 2015 [19]).

A link between the number and function of NK cells, on one hand and prognosis the other hand was found in patients with solid tumors (ANGKA, KHAN et al, 2017 [20]). Some studies in solid tumors have shown that NK-CD8+ cell association is related to a better prognosis compared with NK-CD3+-CD4+ cell association, suggesting an intra-tumor interdependence between NK cells and CD8+ lymphocytes (SCONOCCHIA, EPPENBERGER et al, 2014 [21], Donadon, HUDSPETH et al, 2017 [22]). In colorectal cancers, the presence of intra-tumor NK cells

has been associated with a better prognosis. These cells can exert a protection against cancer-initiating cells, cells with greater resistance to anti-tumor treatment but are more sensitive to NK cells. Therefore increasing the NKCC might contribute to reducing the risk of recurrence (PERNOT, TERME et al, 2014 [23]).

Regarding the comparison of NKCC in two types of anesthesia (general anesthesia versus combined general anesthesia with regional anesthesia) our results are in accordance with other studies conducted so far. Wada et al demonstrated in animal models that epidural or spinal blockade results in a reduction of the immune suppression after surgery and protection against postoperative metastasis (WADA, SEKI et al, 2007 [24]).

Exadaktylos et al studied 129 cases of mastectomy for primary breast cancer and found lower cancer recurrence and metastasis 2.5 years after surgery in patients that received paravertebral anesthesia with general anesthesia compared to those that had received general anesthesia alone ( $p = 0.012$ ) (EXADAKTYLOS, BUGGY et al, 2006 [25]).

Biki et al showed that in radical prostatectomy, using epidural anesthesia and analgesia was associated with a lower risk of biochemical cancer recurrence (57%,  $p=0,012$ ) (BIKI, MASCHA et al, 2008 [12]).

Christopherson et al demonstrated that epidural supplementation was associated with enhanced survival of patients without metastases before 1.46 years ( $p=0,012$ ) (CHRISTOPHERSON, JAMES et al, 2008 [26]).

Lusty et al advocate for the beneficial roles of regional anesthesia in urological surgery, with an improvement of the oncological and immunological responses after surgery potentially through a decreased use of opioids and the direct anti-inflammatory effect of the local anesthetics (LUSTY, HOSIER et al, 2019 [27]).

Regarding the influence of opioids on the activity of NK cells, Wodehouse et al. found the superiority of non-opioid epidural analgesia at clinical dose compared with Morphine by preserving the activity of NK cells (WODEHOUSE, DEMOPOULOS et al, 2019 [28]). In our study, the use of Ropivacaine, a non-opioid epidural analgesia, which may partially explain the trend of the NKCC in the GAE group.

Wang et al. suggest that a combination technique of anesthesia may attenuate the intraoperative stress response and postoperative pain in patients undergoing radical esophagectomy (WANG, YIN et al, 2019 [29]).

However, other studies failed to show any benefits for regional anesthesia/analgesia regarding recurrence and mortality rate after surgery for colorectal, cervical cancers and prostate cancer, even though it reduced the need for postoperative systemic opioid administration (GOTTSCHALK, FORD et al, 2010 [30]), (ISMAIL, HO et al, 2010 [31]).

Regarding the NK cell evaluation in patients with pulmonary carcinoma, Chen J. et al, showed at 24 h after surgery that NK cells values were decreased in both groups, with immunological function recovered in the epidural technique group at 72 h after surgery ( $p<0.05$ ) (CHEN, LUO et al, 2017).

Contrary to the above, Gao H. stated the better effects of general anesthesia on survival rates in colorectal cancers with liver metastases, underlining the importance of cancer type in analyzing the effects of anesthetic techniques (GAO, MENG et al, 2019 [32]).

Still, there is an increasing interest in the opioid-free anesthesia in cancer patients, a procedure known for its effects on tumor cells, but is under debate (MALO-MANSO, RAIGON-PONFERRADA et al, 2019 [33]).

## Conclusions

Our study reveals that combined general anesthesia with postoperative epidural analgesia demonstrated a favorable impact on immune function by preserving NK cells cytotoxicity compared with general anesthesia and postoperative opioids analgesia in patients undergoing colorectal cancer surgery.

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