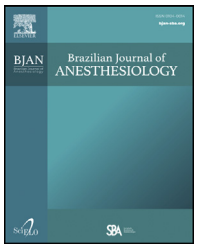
Sociedade Brasileira de Anestesiologia

Brazilian Journal of ANESTHESIOLOGY



ORIGINAL INVESTIGATION

Influence of exogenous opioids on the acute inflammatory response in the perioperative period of oncological surgery: a clinical study

Odirlei João Titon, Joana Perotta Titon, Janaína Carla da Silva, Mariane Okamoto Ferreira, Matheus Ricrado Garbim, Daniel Rech, Janoário Athanazio de Souza, Carolina Panis *

Universidade Estadual do Oeste do Paraná (Unioeste), Francisco Beltrão, PR, Brazil

Received 19 February 2021; accepted 5 September 2021
Available online 5 October 2021

KEYWORDS
Opioids;
Cancer;
Cytokines;
Anesthesia

Abstract
Background: Recently, opioids have been related to trigger changes in cytokine release and tumor angiogenesis processes, influencing tumor growth, metastasis, and recurrence.
Methods: This is a prospective randomized clinical study to test whether if exogenous opioids used in the anesthesia during cancer surgery can affect the systemic inflammatory and immunological patterns. Patients were randomly allocated to the OP (opioid-inclusive) or OF (opioid-free) anesthesia group. A total of 45 patients were selected, being carriers of prostate, stomach, pancreas, bile ducts, breast, colon, lung, uterus, kidneys, or retroperitoneum tumors. Plasma levels of IL-4, IL-12, IL-17A, and TNF-α, and their oxidative stress profile before and after surgery were evaluated in both groups. In vitro tests were performed by using healthy donor blood incubated with each isolated drug used in patients' anesthesia for 1 hour, the same cytokines were measured in plasma.
Results: There was a significant reduction in lipid peroxidation in both groups. Patients from OF group had a significant consumption of IL-12 in the perioperative period. The other cytokines evaluated did not vary. It was also observed a significant correlation between IL-12 and TNF-α levels in the OF-post group. Except for atracurium, all tested drugs led to a reduction in IL-12 levels.
Conclusions: This study demonstrated that there is a reduction of IL-12 in the OF-post patients, suggesting acute consumption and that this seems to be a general mechanism of anesthetic drugs, as demonstrated *in vitro*. Also, these findings bring us to reflect if IL-12 changes may influence the disease progression and recurrence.

* Corresponding author.
E-mail: carolpanis@hotmail.com (C. Panis).

https://doi.org/10.1016/j.bjane.2021.09.011

Introduction

Cancer is the second main group of diseases responsible for the death of adults in the world.[1] In most solid cancers, surgery represents the greatest chance of cure or increased disease-free survival.[2–5] The surgical procedure causes pain and activates the inflammatory cascade, releasing pro-inflammatory cytokines. It must be considered that the time of surgery increases the patient's vulnerability to local tumor recurrence and maximizes the risk of hematological spread of micrometastasis.[2,3,5–8]

The surgical procedure triggers an increase in pro-inflammatory mediators as interleukin-1β (IL-1β), interleukin-6 (IL-6), and C-reactive protein (CRP). The microenvironment generated by surgical trauma is also rich in reactive oxygen species (ROS), which can induce cellular processes favorable to tumor growth and metastasis. Oxidative stress activates the inflammatory pathways that lead to the transformation of a normal cell into a tumor cell, and changes in its survival, proliferation, chemoresistance, radioresistance, invasion, angiogenesis, and the survival of tumorigenic stem cells.[2,9]

In this context, anesthesia and analgesia are performed in a multimodal way, at different points to block the transmission of pain and inflammatory pathways. Among the therapeutic classes, opioids are often used both as adjuvants in epidural and spinal anesthesia (intrathecal administration), and in general anesthesia. The pharmacodynamics of opioid drugs is related to their binding to opioid receptors, mainly the mu (μ) class, present in the central nervous system (CNS). Tumor cells are thought to express such receptors, and its use in oncological patients seems to favor tumor angiogenesis, tumor growth and spreading of micrometastasis.[10,11] Therefore, the use of opioids in cancer surgery is doubtful because the association with the possibility of disease metastasis and recurrence was observed.[12]

In vitro studies show that opioids as morphine can influence the migration and proliferation of endothelial cells.[12] Also, morphine, methadone, and buprenorphine significantly decrease the cytotoxic activity of NK cells,[13] which is dependent on IL-12. Morphine promotes tumor progression and reduces patient survival due to the increase of tumor angiogenesis, peri-tumor lymphangiogenesis, mast cell activation, and the liberation of higher levels of cytokines and substance P in tumors.[11,14] A study by Khabbazi et al. (2015) shows that morphine prevents the increase in IL-4-induced MMP-9 and IL-4-induced M2 macrophage activation, leading to high tumor aggressiveness.[15]

Considering that the perioperative period for cancer patients is a multifactorial and complex scenario,[16] more studies are necessary to understand the influence of anesthetic drugs on the production of inflammatory mediators that could help to eliminate tumors. In this context, the balance among the pro- and anti-inflammatory cytokine profiles (Th1/Th2/Th17) may be affected by opioids, and its relationship with clinicopathological features of cancer is still poorly understood.

To contribute to the comprehension of the influence of opioid-free anesthesia in the cytokine profile of cancer patients, we investigated its influence on the acute inflammatory response induced using opioids in the immediate pre- and postoperative period of patients with different types of cancer undergoing surgery to remove the tumor. This study aims to quantify the levels of cytokines of the Th1/Th2/Th17 immune profiles (IL-12 and TNF-α for Th1, IL-4 for Th2, and IL-17A for Th17) in the peripheral blood of patients before and after surgery, with and without the use of opioids; establish the profile of blood lipid peroxidation in these patients; and, by *in vitro* investigations, assess if there is any change due to the use of opioids, as well as its putative mechanism of action. Considering that opioids can affect the production of inflammatory mediators under normal conditions, we hypothesized that their use in cancer patients could affect negatively their postoperative levels.

Methods

Design of the study

This is a prospective clinical study, randomized to use or not opioids in the anesthetic technique. It was conducted at the Francisco Beltrão Cancer Hospital (CEONC) from July 2019 to October 2020. Patients were randomly divided into two groups, OP (anesthesia with the use of exogenous opioid) or OF (anesthesia without exogenous opioid, i.e., opioid-free), using the electronic random number table method. They received more epidural general anesthesia with the use of opioids or more epidural general anesthesia free of opioids, respectively. After being processed by the Human Research Ethics Committee (CEP) of the Western Paraná State University (UNIOESTE) – Francisco Beltrão, Brazil, it was approved under CAAE number 15251519.1.0000.0107.

The Consolidated Standards of Reporting Trials (CONSORT) flow diagram is presented in Figure 1.

All adult patients attended at the hospital with an indication for oncological surgery according to the previous evaluation of the surgeons who attend at that institution in the period (July 2019 to October 2020) were included. The sample size was calculated considering the population attended by the 8th State Regional of Health (324,178 people that can be sent to Ceonc if they have cancer), the cancer incidence estimated by the National Cancer Institute, in Paraná, for 2020 (13,990 cases per 11 million people, representing a maximum percentage of 3% of cancer in the studied population), and a confidence level of 95%. By including these data in the general formula to estimate the sample size ($n = N.Z2.p.(1-p)/Z2.p.(1-p) + e2.N-1$), in which n: sample size, N: population, Z: normal variable, *p*: real probability of the event, e: sample error), the minimum number of individuals for the study was 45. In total, 47 adult patients of both sexes were selected for surgery to remove a tumor mass located in the prostate, stomach, pancreas, bile ducts, breast, colon, lung, uterus, kidneys, and retroperitoneum. Of the 47 patients who participated in the research, 2 were excluded due to lack of material on the day of the surgical procedure. Thus, there were 23 patients in the OP group and 22 in the OF group. All the 45 patients were followed up until the end of the study and had their biological samples analyzed.

All patients underwent preanesthetic consultation for the free decision to participate or not in the study, preserving the confidentiality of the information obtained, as well as

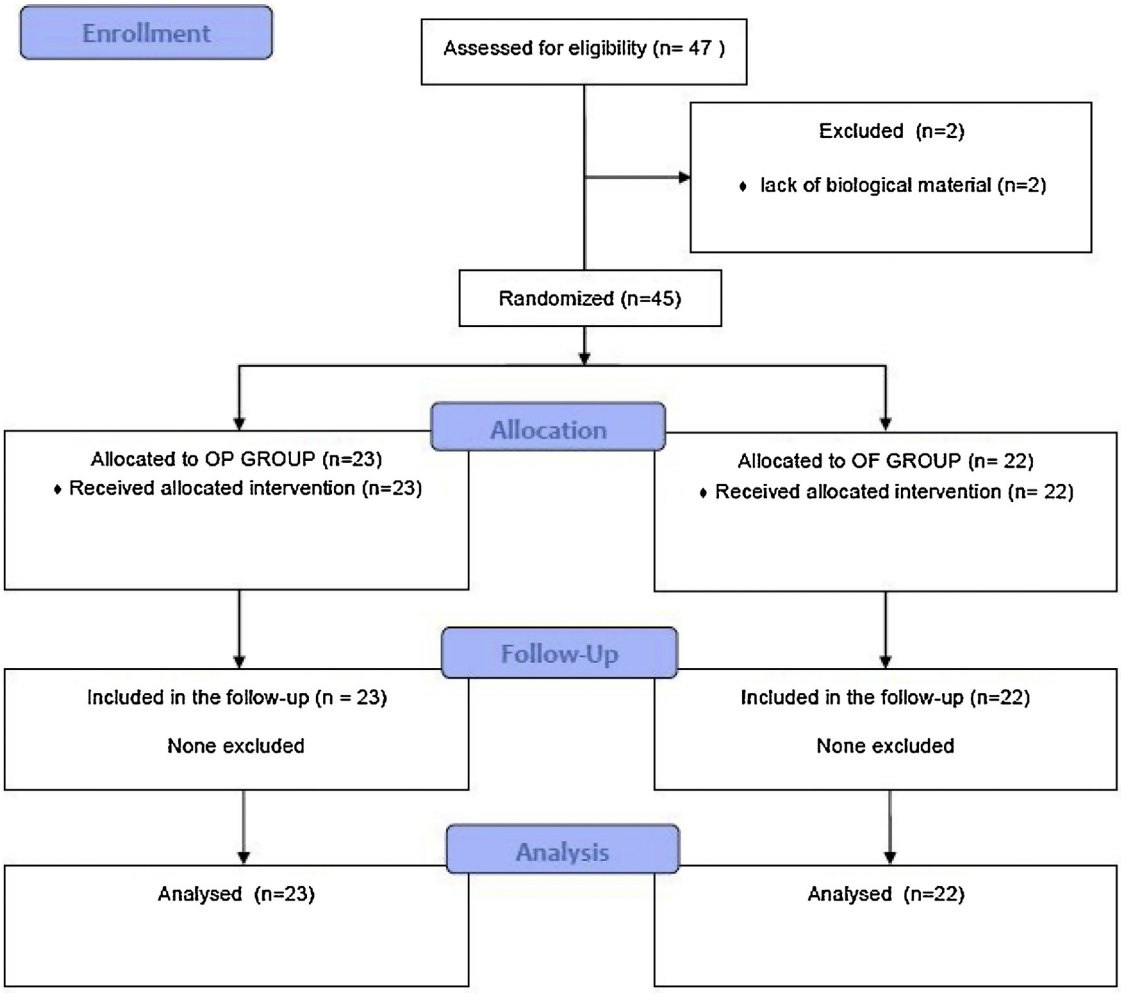


Figure 1 The Consolidated Standards of Reporting Trials (CONSORT) flow diagram for patients selection, allocation, follow-up and analysis.

the identity of the participating patients. After due clarification on the study, everyone who signed the informed consent form regarding anesthesia and the study participated in the research. At the moment of the preanesthetic evaluation, both evaluating physician, patient, and the person responsible for the analysis had no information on whether the sample came from a patient in the OF or OP group. The group to which the patient was allocated was revealed on the day of surgery only to the anesthetist responsible for the anesthetic procedure.

The exclusion criteria were patients under the age of 18, patients with allergies to the medications in the treatment protocols, patients who had previously used opioids or corticosteroids in the last 6 months, patients using immunosuppressive therapy or had autoimmune diseases, patients with chronic or acute pain related or not to cancer, patients with contraindication or refusal to epidural block, and patients who needed transfusion of blood products in the perioperative period. The preanesthetic evaluation was also excluded if patients had severe life-threatening systemic disorders, with or without surgery, and dying patients with little chance of survival. No patient suffered any loss in terms of postoperative pain control due to their allocation in the OF or OP group, with their intensity being routinely assessed and treated, if necessary, through multimodal therapy. All patients were assessed for the Visual Analogue Scale (VAS) for pain, before and after the anesthetic procedure, so the analgesia was effective in both groups.

For laboratory analysis, a sample of peripheral venous blood anticoagulated with heparin was obtained in two moments. The first sample (T1) was collected from the venous system, in the operating room, through the peripheral venous device before installing the line to infuse the solutions and drugs. The caliber of the peripheral venous device was chosen according to the size of the surgery and the patient's anatomy, using 20G, 18G, 16G, or 14G devices. The second collection (T2) of blood was carried out in the operating room, in the immediate postoperative period, as part of the routine postoperative laboratory control of the institution. After T2 collection, patients were transferred to the intensive care unit (ICU) or surgical ward of the hospital unit, according to the anesthesiologist's clinical indication, where they received postoperative care. Samples were immediately sent to the lab, centrifuged (3500 rpm, 5 minutes), and the plasma obtained was frozen until analysis.

Paired comparative analyzes were performed among the peripheral blood samples before and after anesthesia for each group to obtain an overview of the inflammatory profile of the patients. The following names were used for statistical analysis of the results: without preanesthesia opioid (OF-Pre), without postanesthesia opioid (OF-Post), with preanesthesia opioid (OP-Pre), and with postanesthesia opioid (OP-Post).

Data taken from medical records and perioperative records of nursing and anesthesiologists were: number of medical records, age, weight, height, sex, education, marital status, city where the patient live, current and previous illnesses, medications in use, smoking, alcoholism, ASA (|American Society of Anesthesiologists) physical status, duration of surgery (min), volume of estimated blood loss (mL), number of leukocytes. These data were stored in a database for further studies and further analysis.

Anesthetic procedure

In the operating room, standard monitoring was performed with a Dixtal 2010® multiparametric monitor (Dixtal Biomedical, Brazil) and the neuromuscular block was controlled through the sequence of four stimuli with the Watch® SX train-of-four (TOF) monitor (Organon, USA).

Hydration and replacement of losses were performed with an isotonic crystalloid solution (0.9% sodium chloride and/or lactated Ringer) and, when necessary, by blood transfusion.

All patients received dipyrone 30 mg.kg^{-1} intravenously (IV) and antibiotic prophylaxis according to the surgical site and the guidance of the Hospital Infection Control Commission (CCIH) of the institution. The prophylaxis for stress ulcers was by antagonism of the H2 receptor with 50 mg IV of ranitidine hydrochloride.

Conscious sedation for epidural puncture was performed with midazolam 0.05 mg.kg^{-1} IV with oxygen supplementation 3–4 L.min^{-1} in a face mask with reservoir. After installed anxiolysis and immediately before peripheral venipuncture, baseline blood pressure was determined by the average of three successive measurements.

Epidural anesthesia was conducted in the thoracic region with the patient positioned in sitting position. It was performed using aseptic technique. We injected 1% of lidocaine and epinephrine in the skin, throughout the path of the puncture and, if necessary, we inserted a catheter for anesthetic complementation.[17] A syringe was prepared with saline solution for the OF group or with 2 mg morphine for the OP group, with a standard volume of 2 mL.

General anesthesia was combined with epidural block to facilitate airway control and for better perioperative comfort for the patient due to the surgical positioning, surgical time, and standardization of anesthesia.

General anesthesia was administered using the total venous technique, with induction performed with propofol 1.5–2.5 mg.kg^{-1}, lidocaine 1.5 mg.kg^{-1}, magnesium sulfate 25 mg.kg^{-1}, and atracurium 0.3–0.6 mg.kg^{-1}, with tracheal intubation being performed after obtaining no response to the sequence of four stimuli (TOF 0/4). An additional syringe for anesthetic induction was prepared with saline solution for the OF group or with fentanyl 2 μg.kg^{-1} for the OP group, with a standard volume of 5 mL.

Maintenance of the anesthetic plan was performed with lidocaine 1.5 mg.kg^{-1}.h^{-1}, propofol 100–200 μg.kg^{-1}.min^{-1} and, when TOF presented two or more responses to the sequence of four stimuli (TOF $\geq$ 2), a complementary dose of atracurium was administered to maintain adequate muscle relaxation. Older adults with comorbidities need lower doses, therefore their doses were titrated to avoid hypotension during the induction and maintenance of general anesthesia.

At the end of the procedure, the patients were extubated in the operating room and sent to the ICU or surgical ward for immediate postoperative care. During the 24 hours following surgery, the patients were followed up by the anesthesiology service and evaluated for vital signs and anesthetic-surgical complications.

Evaluation of systemic inflammatory status

The circulating levels of interleukin 4 (IL-4), interleukin 12 (IL-12 p70), interleukin 17A (IL-17A), and tumor necrosis factor-alpha (TNF-α), and the lipid peroxidation profile of plasma were evaluated. The levels of cytokines were measured using commercial kits (Invitrogen, USA) using the enzyme immunoassay technique (ELISA), following the analysis protocol recommended by the manufacturer. The kits have a sensitivity of 4 pg.mL^{-1}. The values obtained were used to calculate cytokine ratios (Th1/Th2, Th1/Th17, and Th2/Th17).

We also evaluated the oxidative stress profile of patients, by analyzing their lipid peroxidation status and the antioxidant capacity of plasma. The analysis of lipoperoxidation levels was obtained from the determination of hydroperoxide levels using the chemiluminescence technique induced by t-butyl, after standardization of the test in the laboratory to adjust the analytical technical conditions. For this assessment of plasma lipoperoxidation, 125 μL of the sample was added in 865 μL of 10 mM monobasic phosphate buffer and pH 7.4 in 0.9% NaCl with incubation at 37 °C for 5 minutes. To trigger the reaction, a 10-μl aliquot of the t-butyl solution was added. The reaction reading was performed in a GloMax® 20/20 luminometer (Promega, USA) in the protocol of one reading per second, for 60 minutes, where the qualitative photon emission curve, measured in relative units of light (URL), was evaluated. Concerning the antioxidant capacity of samples, TRAP provides an overview of the antioxidant non-enzymatic defenses of plasma. The compound 2,2'-Azobis (ABAP) reacts with lipids present in the plasma to form lipoperoxides. This reaction emits photons in low quantities, undetectable by spectrophotometry. To amplify this reaction, luminol was added. It is a compound more unstable than lipoperoxides, capable of capturing the unpaired electrons of these lipoperoxides, amplifying the light emission and signal emission to the luminometer. The greater the quantity of antioxidants present in the sample, the greater the delay in the rise of the ABAP curve, which corresponds to the impediment in the formation of lipoperoxides due to the level of low molecular weight antioxidants. The samples were diluted 1:50 in 980 μL of 1 M glycine buffer, pH 8.6, added with 50 μL of

luminol solution (0.0398 mg.mL^{-1}) and 50 μL of ABAP solution (54.24 mg.mL^{-1}) to start the reaction. The results were analyzed using the OriginLab 7.5 software and expressed in nM trolox, based on the ABAP curve inhibition profile of a standard solution of the water-soluble vitamin E analogue (Trolox 0.5 mg.mL^{-1}). Both methods to evaluate oxidative stress were previously published by Herrera et al. 2014.[31]

In vitro study

Aiming to investigate the individual mechanisms triggered by anesthetic drugs that resulted in altered levels of IL-12 and oxidative stress, each drug was tested individually. For this, 1 mL of healthy donor blood (n = 10) was incubated for 1 hour with each of the following drugs and concentrations: propofol (3 μg.mL^{-1}), fentanyl (2.5 ng.mL^{-1}), lidocaine (1.5 μg.mL^{-1}), magnesium sulphate (5 mg.dL^{-1}) and atracurium (0.29 μg.mL^{-1}). The concentrations were estimated based on the therapeutic plasmatic concentration estimated for each drug, as used in the anesthetic protocol of cancer patients. After incubation, samples were centrifuged at 5000 rpm for 5 minutes, and analyzed in the cytokine ELISA kit and oxidative stress protocol, as described above.

Statistical analysis

From the data obtained and properly tabulated in Microsoft Excel® spreadsheets, an analysis of the association of sociodemographic data concerning the different treatments was made: without the use of opioids (OF) and with the use of opioids (OP). This was performed using the Chi-square test of independence, followed by the post-test of adjusted residuals, which allows the identification of which categories the variables have a statistical association.

For each cytokine, to assess their interaction concerning time and treatments, the ANOVA test for repeated measures was applied, followed by the Tukey-HSD test, in case of statistical significance ($p < 0.05$). For this test to be applied, the statistical assumptions of normality (Shapiro-Wilk test) and homoscedasticity of the data (Cochran test) were evaluated. Finally, to assess the existence of an association among the different variables, the PERMANOVA test was applied, followed by the Principal Coordinate Analysis (PCoA) to visualize them. Statistical analyzes were performed using XLStat Version 19.4 (ADDINSOFT, 2018), STATISTICA 7.0 (StatSoft, 2004), and the computer program R (R Development Core Team, 2019), and we assumed a significance level of $p < 0.05$ in all analyzes.

Results

Table 1 shows the clinicopathological profile of the patients. The population showed no difference between the OF and OP groups concerning most sociodemographic data.

As shown in Figure 2, we first investigated by principal component analysis if there was any association among the studied variables. In the analysis of each cytokine separately, in interaction with the different groups (OP/OF) and different times (pre and postoperative), we observed that the cytokines did not show statistical significance. However, when observing the pre- and postoperative periods, in both groups there was a significant reduction in oxidative damage ($F = 20.56$; $p = 0.000$). When analyzing all variables together, we observed an association between them concerning time (pre- or postanesthesia) ($F = 3.11$; $p = 0.022$). However, this association was not seen concerning the groups ($F = 2.40$; $p = 0.101$).

Since the principal component analysis pointed to no association among the variables measured in the plasma, we started to analyze each component individually.

All data concerning the inflammatory profile of patients (oxidative stress profile + cytokines), pre- and postanesthesia, are shown in Table 2 as the minimum and maximum values for each parameter.

Regarding oxidative stress profile, there was a reduction in the generation of lipid peroxidation in the postoperative period, regardless of the use or not of opioids (Fig. 3A, OF PrexPost: $p = 0.0197$; OP Pre × Post, $p = 0.0016$). The antioxidant capacity of plasma was augmented after anesthesia, regardless of the use of opioids (Fig. 3B, OF PrexPost: $p = 0.0033$; OP Pre × Post, $p = 0.0024$).

Concerning cytokines, there was no significant variation in IL-4 (Fig. 4A, $p > 0.05$), IL-17A (Fig. 4B, $p > 0.05$) and TNF-α (Fig. 4C, $p > 0.05$) in the pre- and postoperative periods. Regarding IL-12 (Fig. 4D), there was a significant reduction in the OF group postanesthesia (OF-Pre x Post: $p = 0.0250$).

There was a correlation between the values observed in IL-12 and TNF-α in the OF group. ($R = 0.5089$, $p = 0.051$). There was no correlation between the results of cytokines or lipoperoxidation with the clinicopathological data in Table 1 (Chi-square test, $p > 0.05$).

The in vitro study (Fig. 5) demonstrated that all anesthetic drugs are capable to significantly reduce IL-12 levels in the blood (71.6 ± 20.42 pg.mL^{-1} for propofol, 88.83 ± 9.57 pg.mL^{-1} for fentanyl, 63.3 ± 0.64 pg.mL^{-1} for lidocaine, 76 ± 4 pg.mL^{-1} for magnesium sulfate), excepting for atracurium (173.1 ± 26.17 pg.mL^{-1}), when compared with untreated control blood (123.9 ± 19.15 pg.mL^{-1}, $p < 0.05$). *In vitro* evaluation of oxidative stress parameters shown no difference among groups, and the results were greatly variable (data not shown in graphs).

Discussion

Although anesthesia is a fundamental piece of oncological surgery, the use of opioids is doubtful. Evidence concerning its possible relationship with cancer recurrence and spreading has raised the need of understanding its influence on the inflammatory scenario of operated cancer patients. Considering this, we performed our study aiming to investigate the acute impact of opioids on the inflammatory systemic profile of cancer patients. To reach this goal, we evaluated two groups of cancer patients before and after anesthesia considering the presence or absence of opioids. Our results showed a reduction in the systemic lipid peroxidation profile from both groups, revealing its antioxidant potential against oxidative stress generation. Further, we also demonstrated that opioid-free anesthesia has a significant influence on IL-12 circulating levels, suggesting that this set of drugs may induce its acute consumption, which could be a benefit when

Table 1 Absolute frequency (AF) and relative frequency (FR%) of sociodemographic data and clinical variables data in association with the use or not of opioids.

Variable	Category	On opioids use (OP)		Opioids-free (OF)		*p*-value
		AF	FR%	AF	FR%	
Gender	Male	7	30.43	13	59.09	0.053
	Female	16	69.57	9		40.91
Age (years)	<40	4	17.39	3	13.64	0.380
	50	4	17.39	9		40.91
	60	11	47.83	7		31.82
	≥70	4	17.39	3		13.64
Education	Up to 5 years of study	12	52.17	17	77.27	0,152
	From 6 to 10 years of study	4	17.39	3		13.64
	More than 10 years of study	7	30.43	2		9.09
BMI	Underweight (BMI < 18.5)	2	9.09	1	4.76	0.780
	Normal (18.5 ≥ BMI ≤ 24.9)	12	54.55	12		57.14
	Overweight (25 ≥ BMI ≤ 29.9)	5	22.73	4		19.05
	Obese (30 ≥ BMI ≤ 34.9)	1	4.55	3		14.29
	Extremely obese (BMI ≥ 35)	2	9.09	1		4.76
Marital status	Single	0	0.00	4	18.18	0.007
	Married	14	60.87	12		54.55
	Divorced	1	4.35	5		22.73
	Widowed	8	34.78	1		4.55
SAH	Yes	10	43.48	8	36.36	0.626
	No	13	56.52	14		63.64
Diabetes mellitus	Yes	2	8.70	2	9.09	0.962
	No	21	91.30	20		90.91
CAD	Yes	0	0.00	0	0.00	–
	No	23	100.00	22		100.00
Dyslipidemia	Yes	2	8.70	3	13.64	0.598
	No	21	91.30	19		86.36
Medicines	Yes	18	78.26	16	72.73	0.665
	No	5	21.74	6		27.27
Smoking	Yes	6	26.09	7	31.82	0.671
	No	17	73.91	15		68.18
Alcoholism	Yes	4	17.39	1	4.55	0.170
	No	19	82.61	21		95.45
Tumor location	Prostate	3	13.04	6	28.57	0.125
	Stomach	5	21.74	3		14.29
	Pancreas	0	0.00	1		4.76
	Bile ducts	2	8.70	0		000
	Breast	7	30.43	1		4.76
	Colon	1	4.35	5		23.81
	Lung	1	4.35	1		4.76
	Uterus	1	4.35	2		9.52
	Kidney	3	13.04	1		4.76
	Retroperitoneum	0	0.00	1		4.76
ASA	I	8	34.78	4	18.18	0.449
	II	14	60.87	17		77.27
	III	1	4.35	1		4.55
Surgery duration	Up to 60 min	4	17.39	3	13.64	0.380
	60 to 120 min	14	60.87	10		45.45
	More than 120 min	5	21.74	9		40.91
Blood loss	Up to 100 ml	5	22.73	5	23.81	0.978
	From 100 to 200 ml	8	36.36	7		33.33
	More than 200 ml	9	40.91	9		42.86
Leukocytes/μL	Less than 450	4	21.05	1	5.56	0.151
	Between 4500 and 1100	15	78.95	15		83.33
	More than 1100	0	0.00	2		11.11

BMI, body mass index; SAH, systemic arterial hypertension; ASA, physical status classification system by the American Society of Anesthesiologists; CAD, coronary artery disease (CAD).

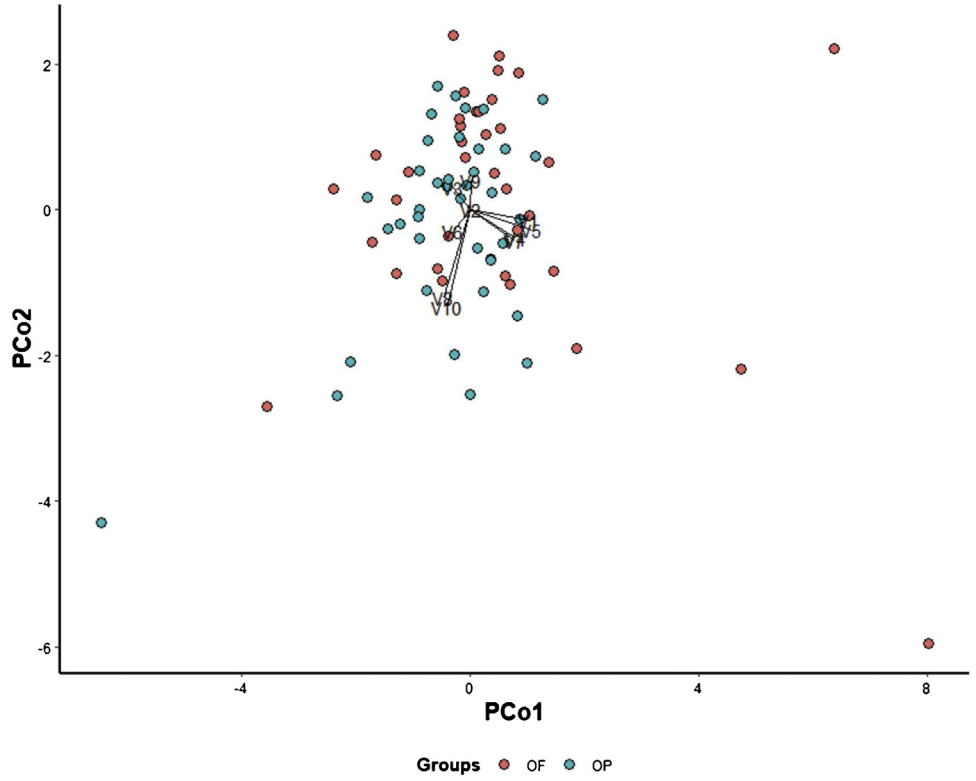


Figure 2 Study of association of the variables investigated in the study through the analysis of main components (PCo). Red dots indicate opioid-free patients and blue dots represent those who received opioids in the anesthetic procedure. V1 = IL-4; V2 = IL-12; V3 = IL-17; V4 = TNF; V5 = RatioTh2/Th17; V6 = RatioTh1/Th2; V7 = RatioTh1/Th17; V8 = Lipidperoxidation; V9 = Antioxidantcapacity; V10 = Stress index.

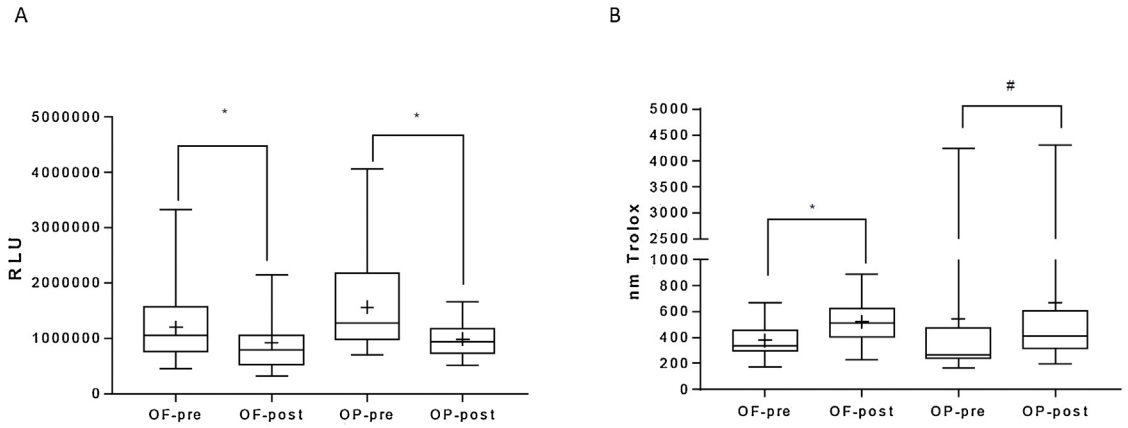


Figure 3 Comparative analysis of the plasma lipoperoxidation profile (A) and antioxidant capacity (B) of cancer patients undergoing anesthesia with (OP) or without (OF) opioid, pre and post anesthesia.
The data are represented in boxplots (min-max). + indicates the average value of each group. * indicates statistical difference ($p < 0.05$), Student' t test or # Wilcoxon test. RLU = relative unities of light.

considering that this cytokine activates the main immune defense against tumors, the natural killer cells (NK cells).

Studies involving opioids and cancer in the perioperative period have mainly reported the relationship between opioids and VEGF production, and their influence on cellular immunity.[15,18–20] Other researchers evaluate the presence of the opioid receptor in cancer, relating it to metastasis, disease aggressiveness, and tumor growth.[10,21–23] Opioids interfere with both cellular and humoral immunity, significantly decreasing the cytotoxic activity of NK cells.[18] They stimulate the apoptosis of macrophages and T cells, as well as the migration and proliferation of tumor cells *in vitro*.[14,24] Opioids inhibit the production of pro-inflammatory cytokines by monocytes and the transcription of interleukin 2 (IL-2) in activated T lymphocytes[25] and increase the production of IL-4.[15] In our study, there was no variation in IL-4 levels,

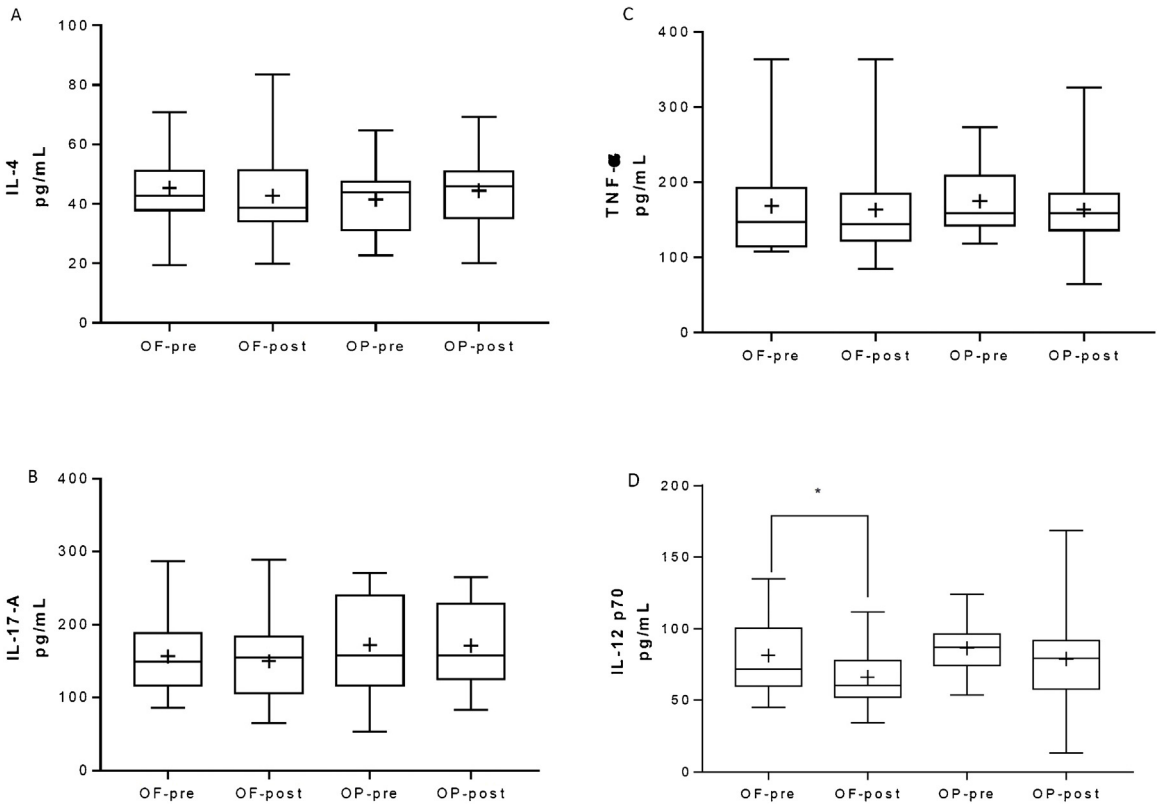


Figure 4 Plasma levels of interleukin4 (IL-4), interleukin17A (IL-17A), tumor necrosis factor alpha (TNF-α) and interleukin12 (IL-12) of cancer patients undergoing anesthesia with (OP) or without (OF) opioid, pre- and postanesthesia.
The data are represented in box plots (min-max). + indicates the average value of each group. * indicates statistical difference ($p < 0.05$), Student's t test or # Wilcoxon test.

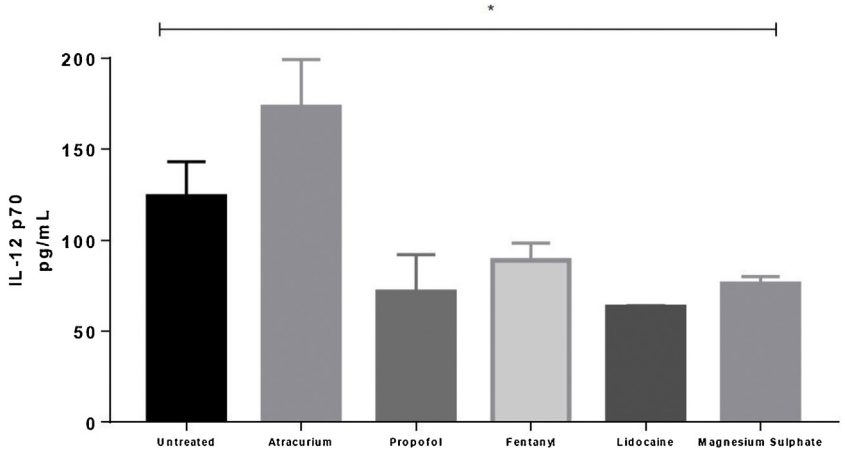


Figure 5 In vitro interleukin12 levels in plasma samples from healthy donnors individually challenged with each of the anesthetic drug employed in cancer patients anesthesic procedure.
Total blood samples were incubated for 1 hour with each individual drug. After this, samples were centrifuged and IL-12 levels measured by ELISA. The data are represented as mean ± standard errors of the means. * indicates statistical difference ($p < 0.05$), ANOVA test with Bonferroni's test as post-hoc.

regardless of the use of opioids, because the assessed time was possibly too short to see any influence of anesthesia on the healing and repair process, of which IL-4 is a pivotal mediator. Also, there was no variation in the levels of IL-17A and TNF-α, regardless of the use of opioids, probably because it does not vary, or it would take more time to allow this cytokine to have its synthesis or consumption altered. In the study conducted by Maher et al. (2019), morphine did not interfere with the concentration of IL-1, IL-2, IL-4, IL-10, IL-17a, TNF-α, IFγ and increased the concentration not

statistically significantly. From IL-6,[18] similarly to our data, in which IL-4, IL-17A, and TNF-α remained stable in both groups after anesthesia.

On the other hand, IL-12 showed a significant reduction in the post-surgical period in the OF group. We hypothesize that this cytokine may be acutely consumed by NK cells, which could become activated, and act in the recognition of tumor antigens that were exposed during the surgical process. Therefore, this process could benefit opioid-free patients in the future, corroborating with literature findings that support less metastasis and disease recurrence when cancer patients do not receive opioids during surgery anesthesia.[10,21–23] We also observed a correlation between IL-12 and TNF-α levels in plasma, suggesting that the production of one cytokine also leads to the production of the other. Likewise, consumption of one leads to consumption of the other.

To better understand this phenomenon, we performed an *in vitro* study, isolating each anesthetic drug applied in cancer patients' surgery, and challenging healthy donor's blood cells with them. We demonstrated that blood cells could acutely consume IL-12 after the anesthetic drug challenge, excepting for atracurium, demonstrating that regardless of being or not an opioid, these drugs can influence this cytokine. Fentanyl has been reported as an anti-inflammatory drug with immediate effects in healthy people,[26] but no information was found concerning its effect on IL-12 levels. The same has been reported to propofol, which acts as an anti-inflammatory by suppressing the cyclooxygenase activity and prostaglandin E production, without impacting IL-12 levels on dendritic cells in an experimental model.[27] Lidocaine seems to be similar since can act as an anti-inflammatory by regulating IL-6 levels operative time and postoperative in healthy people,[28] but no information about its influence on IL-12 was reported. Therefore, our findings of IL-12 seem a novelty, which aligns with the anti-inflammatory effect of anesthesia reported in the literature.

Lipid peroxidation levels, a measurement directly linked to oxidative stress, was also observed as reduced after the anesthetic and surgical procedure, regardless of the use of opioids. Despite data suggesting opioids as ROS inducers in non-cancer individuals,[29] our finding in cancer patients suggests that possibly some of the anesthetic drugs used in the patients may have some antioxidant effect, as demonstrated in our results. Indeed, this information is well known to propofol, which acts as an antioxidant, and has the effect of reducing ROS.[30] Anyway, this information reinforces that some combined anesthetic drugs can influence the immediate inflammatory response of patients in different ways, since oxidative stress constitutes a relevant arm of inflammation.

Concerning the limitations of our study, it is important to consider the time between the collection of blood samples, which lacks a long-term follow-up. It was possible to identify only the changes that immediately occurred after the surgical event, and it was not possible to conclude about the long-term changes or the clinical consequences. However, these patients will be followed up by the research group for further cytokine assessments in addition to the clinical consequences as relapses and metastases. Other limitations include the distinct types of cancer in the evaluated groups, anesthetist unblinding, and patients' emotional distress. It is possible that these factors also affected the cytokine profile, mainly if considering the wide dispersion observed for some cytokines – especially to the not significant ones.

In conclusion, this study demonstrated that in different topographies of cancers, in the perioperative period, the anesthetic schedule may influence the acute inflammatory response by leading to a consumption of IL-12 when opioids are not used. Further, our data showed that oxidative stress production is also affected in such patients and provides information about how anesthetic drugs can regulate IL-12 levels individually and acutely in healthy cells. These findings concerning IL-12 are novelty and may have some association with tumor progression and the appearance of future metastasis in cancer patients undergoing opioids.

Funding

Fundação Araucária – Programa de Pesquisa Básica, Unioeste, EDITAL Nº 016-2016/PRPPG.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Ministério da Saúde (Brasil). Protocolos Clínicos e Diretrizes Terapêuticas em Oncologia. Brasília: Ministério da Saúde; 2014. Available from: http://bvsms.saude.gov.br/bvs/publicacoes/protocolos_clinicos_diretrizes_terapeuticas_oncologia.pdf [Accessed February 09, 2019].
2. Bajwa SJ, Anand S, Kaur G. Anesthesia and cancer recurrences: the current knowledge and evidence. J Cancer Res Ther. 2015;11:528–34.
3. Le-Wendling L, Nin O, Capdevila X. Cancer Recurrence and Regional Anesthesia: The Theories, the Data, and the Future in Outcomes. Pain Med. 2016;17:756–75.
4. Tavare AN, Perry NJ, Benzonana LL, et al. Cancer recurrence after surgery: direct and indirect effects of anesthetic agents. Int J Cancer. 2012;130:1237–50.
5. Tedore T. Regional anaesthesia and analgesia: relationship to cancer recurrence and survival. Br J Anaesth. 2015;115:ii34–45.
6. Cakmakkaya OS, Kolodzie K, Apfel CC, et al. Anaesthetic techniques for risk of malignant tumour recurrence. Cochrane Database Syst Rev. 2014:CD008877.
7. Cronin-Fenton DP, Heide-Jørgensen U, Ahern TP, et al. Opioids and breast cancer recurrence: A Danish population-based cohort study. Cancer. 2015;121:3507–14.
8. Kim R. Effects of surgery and anesthetic choice on immunosuppression and cancer recurrence. J Transl Med. 2018;16:8.
9. Lukoseviciene V, Tikuisis R, Dulskas A, et al. Surgery for triple-negative breast cancer- does the type of anaesthesia have an influence on oxidative stress, inflammation, molecular regulators, and outcomes of disease? J Buon. 2018;23:290–5.
10. Cheng S, Guo M, Liu Z, et al. Morphine promotes the angiogenesis of postoperative recurrent tumors and metastasis of dormant breast cancer cells. Pharmacol. 2019;104:276–86.
11. Nguyen J, Luk K, Vang D, et al. Morphine stimulates cancer progression and mast cell activation and impairs survival in transgenic mice with breast cancer. Br J Anaesth. 2014;113:i4–13.

12. Afsharimani B, Cabot P, Parat MO. Morphine and tumor growth and metastasis. Cancer Metastasis Rev. 2011;30:225–38.
13. Maher DP, Walia D, Heller NM. Suppression of Human Natural Killer Cells by Different Classes of Opioids. Anesth Analg. 2019;128:1013–21.
14. Aich A, Gupta P, Gupta K. Could Perioperative Opioid Use Increase the Risk of Cancer Progression and Metastases? Int Anesthesiol Clin. 2016;54:e1–16.
15. Khabbazi S, Goumon Y, Parat MO. Morphine Modulates Interleukin-4- or Breast Cancer Cell-induced Pro-metastatic Activation of Macrophages. Sci Rep. 2015;5:11389.
16. Sekandarzad MW, van Zundert AAJ, Lirk PB, et al. Perioperative Anesthesia Care and Tumor Progression. Anesth Analg. 2017;124:1697–708.
17. Baumunk D, Strang CM, Kropf S, et al. Impact of thoracic epidural analgesia on blood loss in radical retropubic prostatectomy. Urol Int. 2014;93:193–201.
18. Maher DP, Walia D, Heller NM. Suppression of Human Natural Killer Cells by Different Classes of Opioids. Anesth Analg. 2019;128:1013–21.
19. Moss J, Rosow CE. Development of peripheral opioid antagonists' new insights into opioid effects. Mayo Clin Proc. 2008;83:1116–30.
20. Singleton PA, Lingen MW, Fekete MJ, et al. Methylnaltrexone inhibits opiate and VEGF-induced angiogenesis: role of receptor transactivation. Microvasc Res. 2006;72:3–11.
21. Lennon FE, Mirzapoiazova T, Mambetsariev B, et al. Overexpression of the μ-opioid receptor in human non-small cell lung cancer promotes Akt and mTOR activation, tumor growth, and metastasis. Anesthesiology. 2012;116:857–67.
22. Gupta K, Kshirsagar S, Chang L, et al. Morphine stimulates angiogenesis by activating proangiogenic and survival-promoting signaling and promotes breast tumor growth. Cancer Res. 2002;62:4491–8.
23. Tripolt S, Neubauer HA, Knab VM, et al. Opioids drive breast cancer metastasis through the δ-opioid receptor and oncogenic STAT3. Neoplasia. 2021;23:270–9.
24. Sen S, Koyyalamudi V, Smith DD, et al. The role of regional anesthesia in the propagation of cancer: A comprehensive review. Best Pract Res Clin Anaesthesiol. 2019;33:507–22.
25. Gach K, Wyrębska A, Fichna J, et al. The role of morphine in regulation of cancer cell growth. Naunyn Schmiedebergs Arch Pharmacol. 2011;384:221–30.
26. Kwon Y, Hwang SM, Jang JS, et al. Effects of a Preoperative Transdermal Fentanyl Patch on Proinflammatory Cytokine and Pain Levels During the Postoperative Period: A Randomized Controlled Trial. Surg Laparosc Endosc Percutan Tech. 2019;29:339–43.
27. Inada T, Kubo K, Ueshima H, et al. Intravenous anesthetic propofol suppresses prostaglandin E2 production in murine dendritic cells. J Immunotoxicol. 2011;8:359–66.
28. Kościelniak-Merak B, Batko I, Kobylarz K, et al. Intravenous, Perioperatively Administered Lidocaine Regulates Serum Pain Modulators' Concentrations in Children Undergoing Spinal Surgery Pain. Med. 2020;21:1464–73.
29. Zahmatkesh M, Kadkhodaee M, Salarian A, et al. Impact of opioids on oxidative status and related signaling pathways: An integrated view. J Opioid Manag. 2017;13:241–51.
30. Wei J, Luo J, Lv X. How does the anesthetic agent propofol affect tumors? Int J Clin Exp Med. 2017;10:5995–6003.
31. Herrera AC, Victorino VJ, Campos FC, Verenitach BD, Lemos LT, Aranome AM, Oliveira SR, Cecchini AL, Simão AN, Abdelhay E, Panis C, Cecchini R. Impact of tumor removal on the systemic oxidative profile of patients with breast cancer discloses lipid peroxidation at diagnosis as a putative marker of disease recurrence. Clin Breast Cancer. 2014;14(6):451–9, http://dx.doi.org/10.1016/j.clbc.2014.05.002. Epub 2014 Jun 25. PMID: 25077997.