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The perioperative effect of anesthetic drugs on the immune response in total intravenous anesthesia in patients undergoing minimally invasive gynecological surgery

MARIUS BOGDAN NOVAC[1)], LIDIA BOLDEANU[2)], LUCIANA TEODORA ROTARU[3)], ANDA LORENA DIJMĂRESCU[4)], MIRCEA-SEBASTIAN ŞERBĂNESCU[5)], LUCREŢIU RADU[6)], SIMONA DANIELA NEAMŢU[7)], ALINA MARIA VÎLCEA[8)], MIHAELA NICULESCU[9)], CECIL SORIN MIREA[10)], MIHAIL VIRGIL BOLDEANU[11,12)], MARIA MAGDALENA MANOLEA[4)]

[1)]*Department of Anesthesiology and Intensive Care, University of Medicine and Pharmacy of Craiova, Romania*
[2)]*Department of Microbiology, University of Medicine and Pharmacy of Craiova, Romania*
[3)]*Department of Emergency Medicine and First Aid, University of Medicine and Pharmacy of Craiova, Romania*
[4)]*Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania*
[5)]*Department of Medical Informatics and Biostatistics, University of Medicine and Pharmacy of Craiova, Romania*
[6)]*Department of Hygiene, University of Medicine and Pharmacy of Craiova, Romania*
[7)]*Department of Hematology and Immunology, University of Medicine and Pharmacy of Craiova, Romania*
[8)]*Department of Dermatology, University of Medicine and Pharmacy of Craiova, Romania*
[9)]*Department of Anatomy, University of Medicine and Pharmacy of Craiova, Romania*
[10)]*Department of Surgery, University of Medicine and Pharmacy of Craiova, Romania*
[11)]*Department of Immunology, University of Medicine and Pharmacy of Craiova, Romania*
[12)]*Medico Science SRL – Stem Cell Bank Unit, Craiova, Romania*

Abstract

Background: The specific mechanism of action of each anesthetic drug on the immune system is still incompletely known. It is important to know how the various anesthetics used in minimally invasive surgery (MIS) act on the inflammatory response because the choice of the anesthetic agent can influence the patient's immune system. *Aim*: Evaluation of the effect of anesthetic drugs used for total intravenous anesthesia (Propofol and Midazolam) on the inflammatory response after minimally invasive gynecological surgery. *Patients, Materials and Methods*: The inflammatory response in 20 female patients who underwent minimally invasive gynecological surgery under which intravenous anesthesia was performed. Depending on the combination of anesthetics used, we subdivided the study group into two groups, Group 1 consisting of the patients (n=10) who were given for total intravenous anesthesia, the combination with Midazolam+Fentanyl, and Group 2 (n=10) the patients who received the combination of Propofol+Fentanyl, respectively. Surgical interventional procedures included day surgery: diagnostic and operative hysteroscopy, endometrial ablation, surgical treatment of vulvar disorders. Serological profiling of patients was performed by dosing the serum concentration of nucleotide-binding domain (NOD) and leucine-rich repeat protein 3 (NLRP3) inflammasomes, interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), IL-10 before and two hours after the surgical procedure. *Results*: In our study, we found that in both groups of patients (Midazolam+Fentanyl – Group 1, Propofol+Fentanyl – Group 2), NLRP3 and cytokines concentrations in the serum were higher after MIS than those before MIS. *Conclusions*: It appears that both Midazolam and Fentanyl and Propofol and Fentanyl have an immunomodulatory action due to the anti-inflammatory effect of both anesthetics. Therefore, anesthesiologists must choose an anesthetic method that uses individualized anesthetic agents, depending on the patient's immune status and disease.

Keywords: Propofol, Midazolam, minimally invasive surgery, cytokines, inflammatory response.

Introduction

The beneficial or harmful effects of anesthetic drugs on the immune system have been known for over a century [1]. But, despite all the advances made in immunology and anesthesiology, the specific mechanism of action of each anesthetic drug on the immune system is still incompletely known. Anesthetic drugs act on each subpopulation of immune cells, inducing changes in neutrophils, mononuclear phagocytes, lymphocytes, natural killer (NK) cells, T-lymphocytes, B-lymphocytes, but also on cytokines.

Cytokine synthesis occurs through cellular activation, some being considered as regulators of innate immunity, others as regulators of the adaptive immune response, they act in postoperative immunosuppression when there is an imbalance between pro- and anti-inflammatory mediated responses [2].

It is important to know how the various anesthetics used in minimally invasive surgery (MIS) act on the inflammatory response because the choice of the anesthetic agent can influence the patient's immune system. It should also be noted that surgical stress is added to the effect of anesthetic

drugs. It induces, through the neuroendocrine system, as well as through activation/inhibition of proinflammatory and anti-inflammatory cytokines an immunosuppressive effect in the perioperative period [3].

Potential danger associated with this immunosuppression relate to an increased risk of infection and tumor metastasis (which should be considered when MIS in a malignant process), while the anti-inflammatory effects of some anesthetics may lead to benefits in the conditions in which we have systemic and local inflammation. Given that immune suppression and the presence of excessive inflammatory mediators expose the patient to several perioperative complications, anesthetists should select anesthetic techniques and anesthetic drugs, considering both the clinical situation and the patient's immune status, morbidity, and optimal prognosis. Different anesthetic techniques can influence the stress response, especially by activating intraoperative and postoperative cytokines, with changes in postoperative evolution. But, for an immunocompetent patient, the immunosuppressive effect of anesthetic drugs should not have significant consequences, especially when it comes to MIS and short-lived surgery.

Propofol are the most commonly used anesthetic drugs for sedation, as well as for total intravenous anesthesia in minimally invasive gynecological surgery. Midazolam, a commonly used benzodiazepine, acts by regulating the proinflammatory function of macrophages [4]. As an anesthetic drug, Midazolam is used in premedication and procedural sedation. Midazolam can minimize the pro-inflammatory response to anesthesia [5]. It seems that Midazolam can induce apoptosis and can suppress the progression of cancer cells, having antitumorigenic properties, which should be considered in choosing the anesthetic drug [6].

Propofol is a frequently used intravenous anesthetic drug, causing a rapid induction of anesthesia, but also a rapid recovery after anesthesia. Information about the effect of Propofol on cytokines is still controversial, but most studies agree that this anesthetic drug has antioxidant and anti-inflammatory properties [7–9]. Opioids affect the synthesis of cytokines and immunoglobulins, as well as the activation of NK cells and phagocytosis, with immuno-modulatory effects [10, 11].

But most studies have found that synthetic opioids (Fentanyl, used in our study as a narcotic analgesic agent) used in general anesthesia, cause only temporary immuno-modulatory effects [1, 2, 12].

Studies in recent years have used frequently, as an index to assess the immune response in the inflammatory process, immunocytes. It has also been observed that different rates of immunocytes can be used to assess the extent of inflammation, such as neutrophil-to-lymphocyte (NLR) count ratio, platelet-to-lymphocyte (PltLR) count ratio and monocyte-to-lymphocyte (MLR) count ratio, as well as mean platelet volume-to-platelet (MPV/Plt) count ratio [13–16]. These indices are not only affected by surgical stress, but also by the type of used anesthesia [17].

Studies on the mechanisms involved in innate immunity have shown the involvement of multiprotein complexes (inflammasomes). Inflammasomes are protein complexes with key roles in intracellular signaling (mediation or even involvement in the transmission of intracellular signaling), as well as involvement in the activation of cysteine-dependent aspartate-directed protease-1 (caspase-1), which will lead to the conversion of inactive precursors of interleukin (IL)-1β (pro-IL-1β) and IL-18 (pro-IL-18), in active forms [18, 19].

Aim

This study aimed to evaluate the effect of anesthetic drugs used for total intravenous anesthesia (Propofol and Midazolam) on the inflammatory response after minimally invasive gynecological surgery. For this purpose, the serum concentrations of IL-6 a proinflammatory cytokine, IL-10 an anti-inflammatory cytokine, tumor necrosis factor-alpha (TNF-α), the inflammasome nucleotide-binding domain (NOD) and leucine-rich repeat protein 3 (NLRP3) and the rates of immunocytes (NLR, PltLR and MLR) were investigated.

Patients, Materials and Methods

Subjects and clinical assessment

This prospective study included the evaluation through a panel of biomarkers of the evolution of the immune response in 20 female patients who underwent minimally invasive gynecological surgery under which intravenous anesthesia was performed.

The patients included in this study were diagnosed in the OpenMed Private Hospital and in the Department of Gynecology of the Filantropia Municipal Clinical Hospital, Craiova, Dolj County, Romania.

The introduction of the cases in the study was based on certain criteria. The inclusion criteria were over 18 years of age, obtaining informed consent to participate in the study, *American Society of Anesthesiologists* (ASA) grade I or II, operation duration less than 60 minutes, the type of intervention to be minimally invasive.

Exclusion criteria were predicted duration of a surgery over 60 minutes, allergy to anesthetic drugs used, possible alteration of the immune response by the presence of genetic diseases, liver or kidney diseases, endocrinopathy, diabetes mellitus, morbid obesity, analgesic or immunosuppressive therapy with any indication, the patient's refusal to sign informed consent.

Depending on the combination of anesthetics used, we subdivided the study group into two groups, Group 1 consisting of the patients (n=10) who were given for total intravenous anesthesia, the combination with Midazolam+ Fentanyl, and Group 2 (n=10) the patients who received the combination of Propofol+Fentanyl, respectively.

Group 1 anesthesia was induced by administration of Fentanyl 3 μg/kg, three minutes before the administration of Midazolam, then Midazolam – a target-controlled infusion 0.15–0.2 mg/kg, continued with 0.03–0.1 mg/kg to maintain the effect.

Group 2 anesthesia was induced by administration of Fentanyl 3 μg/kg, three minutes before the administration of Propofol, then Propofol – a target-controlled infusion of Propofol 1.5 mg/kg/h to 4.5 mg/kg/h, then 0.1–0.2 mg/kg/min to maintain the effect. Spontaneous respiration, administration of 98% O_2 on the nasal cannula, 4 L/min.

Surgical interventional procedures included day surgery: diagnostic and operative hysteroscopy, endometrial ablation.

Even if we cannot eliminate surgery stress to have only the effect of anesthetics on the inflammatory response, we wanted to introduce in the study only the cases subjected to MIS with as little tissue trauma as possible.

We performed a histopathological (HP) study on endometrial fragments obtained during the hysteroscopy and endometrial ablation procedure. Depending on the results obtained, we classified the lesions in simple and complex endometrial hyperplasia, with and without atypia and in endometrial carcinoma. HP aspects were compared and interpreted with the other investigations, to follow a possible association between systemic inflammation and local inflammation.

Intraoperative monitoring was standard, with electrocardiogram, peripheral oxygen saturation (SpO_2), noninvasive arterial pressure.

Sample collection

Blood samples were obtained before anesthetic induction and two hours after the end of the surgical procedure, from all subjects into tubes without additives (the vacutainer from Becton Dickinson) by venous approach, in the morning before meals. The tubes were kept in upright positions for 30 minutes at room temperature (RT) to allow clot to form, then centrifuged at 3000×g for 10 minutes. After the clot was removed, the sera were distributed in several cryotubes and stored at temperatures below -20°C until evaluation. At the time of processing the samples, we left the cryotubes to accommodate at RT, not allowing the remaining samples to re-freeze.

Peripheral venous blood was collected into separator vacutainers with Ethylenediaminetetraacetic acid (EDTA) anticoagulant and was used to perform complete blood counting (CBC) cells: neutrophils, basophils, eosinophils, lymphocytes, monocytes, red blood cells, platelets.

Immunological investigations

Immunological investigations were performed with the support of the Department of Immunology, University of Medicine and Pharmacy of Craiova. The technique used was represented by enzyme-linked immunosorbent assay (ELISA), the sandwich variant, quantitative, respecting the working instructions specified in the kits by the manufacturer. The ELISA method was performed with a standard optical analyzer, at 450 nm wavelength.

Specifically-designed commercial test kits were used for each of the mediators: NLRP3 (Cat# OKEH03368, assay range: 0.312–20 ng/mL) – Aviva Systems Biology, San Diego, USA; TNF-α (Cat# BMS223-4, assay range: 7.8–500 pg/mL), IL-6 (Cat# BMS213-2, assay range: 1.56–100 pg/mL), IL-10 (Cat# BMS215-2, assay range: 3.15–200 pg/mL) – Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA.

HP investigations

Tissue fragments were fixed in 10% neutral buffered formalin for 24 hours before routine embedding in paraffin. For fixation, the tissue fragments were cut into the multiple longitudinal slices of 1–2 cm and kept in 10% neutral buffered formalin for several days. After fixation, tissue samples were taken from representative areas. For the HP study, there were used the Hematoxylin–Eosin (HE).

Complete blood counting (CBC)

To differentiate and count the neutrophils, basophils, eosinophils, lymphocytes, monocytes, red blood cells and platelets, we used an automatic hematology analyzer, able to give us an extended leukocyte formula 5 diff, by flow cytometry and Coulter's principle. Using these determinations, we calculated the different rates of immunocytes: NLR, MLR and PltLR.

Ethical issue

All stages of our study were conducted in accordance with both the ethical standards for human studies recommended by the responsible institutional committees and the Helsinki Declaration of 1975, revised in 2008. Also, to carry out this project, we have the agreement of the Ethics, Academic and Scientific Deontology Committee of the University of Medicine and Pharmacy of Craiova (Approval No. 135/17.09.2021).

Statistical analysis

The patients' information obtained from medical documents were uploaded and filtered, to be further statistically processed, in Microsoft Excel files (Data Analysis package). As a program dedicated to statistical processing, we used the free trial version of GraphPad Prism 5. Significant differences between the means of the parameters investigated in the groups included in the study were analyzed with the Mann–Whitney *U*-test (for the analysis of two differences) and the Kruskal–Wallis test (for the analysis of several differences). The values of the tests that have $p \leq 0.05$ were considered statistically significant, the tests were on two-sided.

Results

Clinical features of the enrolled patients

The two groups of patients were similar, the demographic data being homogeneous, depending on age or urban/rural areas, we did not register statistically significant differences (Table 1).

Table 1 – ***Demographics and clinical characteristics of enrolled patients***

Characteristics	Midazolam+Fentanyl Group 1 (*n*=10)	Propofol+Fentanyl Group 2 (*n*=10)
Age (years) (mean ± SD)	42.89±7.52	42.67±6.65
BMI (kg/m^2) (mean ± SD)	25.70±2.07	26.20±2.04
Urban/rural areas, *n*	5/4	4/5
ASA grade, *n* (%):		
▪ ASA I	7 (70%)	8 (80%)
▪ ASA II	3 (30%)	2 (20%)
HP lesions, *n* (%):		
▪ Endometrial hyperplasia without atypia	5 (50%)	6 (60%)
▪ Endometrial hyperplasia with atypia	4 (40%)	3 (30%)
▪ Endometrioid endometrial carcinoma	1 (10%)	1 (10%)

ASA: *American Society of Anesthesiologists*; BMI: Body mass index; HP: Histopathological; *n*: No. of cases; SD: Standard deviation.

Evaluating the medical comorbidities of the patients before the anesthesia, by using the *ASA Physical Status Classification* level, we noticed that in both analyzed groups ASA I predominated (Group 1 – seven cases, 70%; Group 2 – eight cases, 80%).

Depending on the minimally invasive gynecological surgery, in Group 1 (the patients who received the combination of Midazolam+Fentanyl), in most cases (eight cases, 80%) were performed the diagnosis and operative hysteroscopy (four cases, 40%) and the surgical treatment of vulvar disorders (four cases, 40%), respectively. Instead, patients from Group 2 (the patients who received the combination of Propofol+Fentanyl) benefited from the diagnosis and operative hysteroscopy in 60% of the cases.

Analyzing the mean rates of immunocytes, we did not find statistically significant differences in the two investigated groups. The combinations used, Midazolam+Fentanyl and Propofol+Fentanyl, respectively, did not cause statistically significant changes in these rates of immunocytes after performing minimally invasive gynecological surgery.

In Midazolam+Fentanyl group, when we compared the mean rates of immunocytes between after performing the MIS *vs* before performing MIS, we obtained: NLR (2.49±0.27 *vs* 2.40±0.32, p=0.622), PltLR (123.00±50.85 *vs* 126.00±54.98, p=1.000), MLR (0.21±0.04 *vs* 0.19±0.11, p=0.342) (Table 2). And in the Propofol+Fentanyl group, when comparing the mean of the immunocytes rates, after performing the MIS *vs* before performing the MIS, we found: NLR (2.29±0.73 *vs* 1.98±0.87, p=0.426), PltLR (152.40±51.13 *vs* 127.90±68.93, p=0.472), MLR (0.24±0.07 *vs* 0.20±0.08, p=0.141).

HP analysis

HP analysis of the obtained tissue fragments revealed HP aspects characteristic for endometrial hyperplasia. But the correlation with non-specific inflammatory indices showed that local inflammatory changes, manifested by the more or less frequent presence of lymphocytes on HP preparations, are not expressed in the serum values of these inflammatory markers (Figures 1–5).

NLRP3 and cytokines concentrations

In our study, we found that in both groups of patients (Midazolam+Fentanyl – Group 1, Propofol+Fentanyl – Group 2), NLRP3 and cytokines concentrations in the serum were higher after MIS than those before MIS (Tables 3 and 4).

Analyzing the results obtained for Group 1, the patients who received the combination Midazolam+Fentanyl, we observed that the serum concentrations of NLRP3, TNF-α and IL-10 have higher values, statistically significant differences, after MIS than those determined before this: NLRP3 [16.93 ng/mL, 95% confidence interval (CI): 14.66–19.19, p=0.049], TNF-α (19.54 pg/mL, 95% CI: 17.10–22.07, p=0.044), and IL-10 (21.78 pg/mL, 95% CI: 19.28–24.29, p=0.035) (Table 3).

In the analysis of Group 2 (the patients who received the combination of Propofol+Fentanyl), we found that only the serum concentrations of NLRP3 (16.11 ng/mL, 95% CI: 15.00–17.21, p=0.037) and IL-10 (17.37 pg/mL, 95% CI: 15.30–19.45, p=0.043), have statistically significant differences, after performing the MIS *vs* before performing MIS (Table 4).

In Figures 6–9, we highlighted the NLRP3, TNF-α, IL-6, and IL-10 concentrations in serum of patients who underwent MIS, in the two groups enrolled in the study.

Table 2 – ***The mean rates of immunocytes in the two investigated groups***

Characteristics	Midazolam+Fentanyl Group 1 (n=10)		p-value	Propofol+Fentanyl Group 2 (n=10)		p-value
	Before MIS	*After MIS*		*Before MIS*	*After MIS*	
	Mean ± SD	*Mean ± SD*		*Mean ± SD*	*Mean ± SD*	
NLR	2.49±0.27	2.40±0.32	0.622	2.29±0.73	1.98±0.87	0.426
PltLR	123.00±50.85	126.00±54.98	1.000	152.40±51.13	127.90±68.93	0.472
MLR	0.21±0.04	0.19±0.11	0.342	0.24±0.07	0.20±0.08	0.141

MIS: Minimally invasive surgery; MLR: Monocyte-to-lymphocyte count ratio; NLR: Neutrophil-to-lymphocyte count ratio; PltLR: Platelet-to-lymphocyte count ratio; SD: Standard deviation.

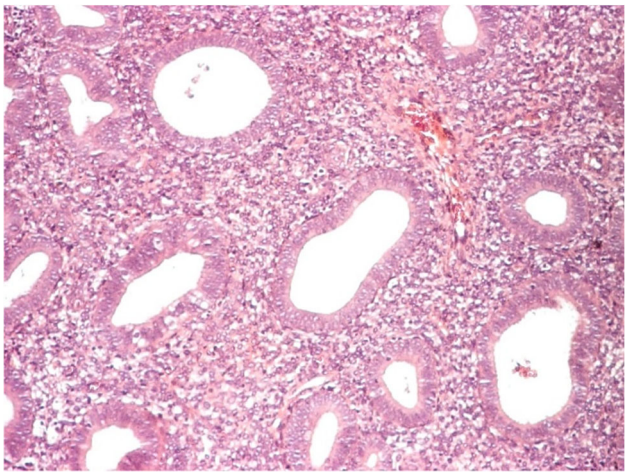

Figure 1 – ***Simple endometrial hyperplasia without atypia with moderate diffuse lymphocytic infiltrate. Hematoxylin–Eosin (HE) staining, ×200.***

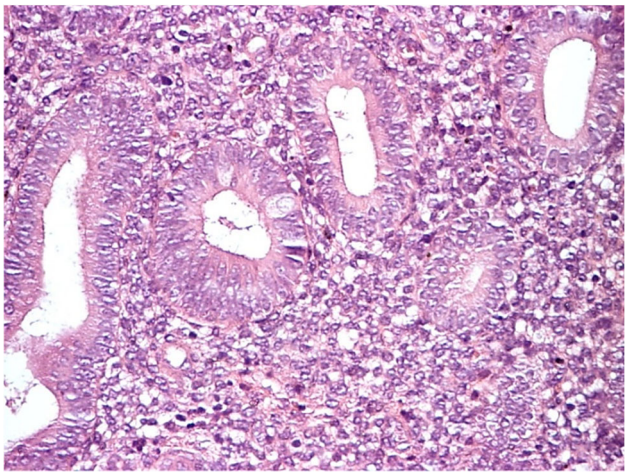

Figure 2 – ***Simple endometrial hyperplasia with atypia. HE staining, ×200.***

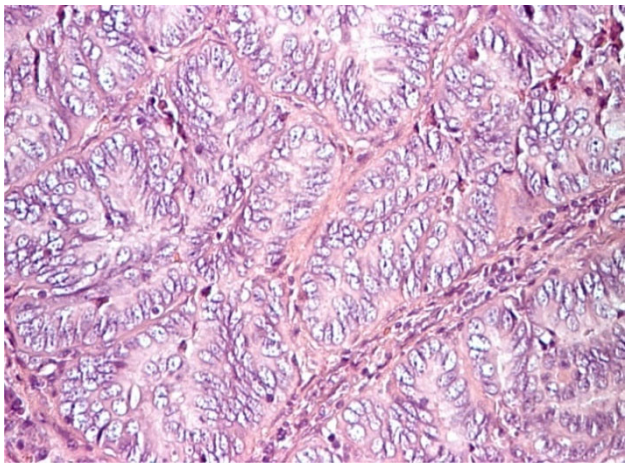

Figure 3 – *Complex endometrial hyperplasia without atypia, periglandular lymphocyte band. HE staining, ×200.*

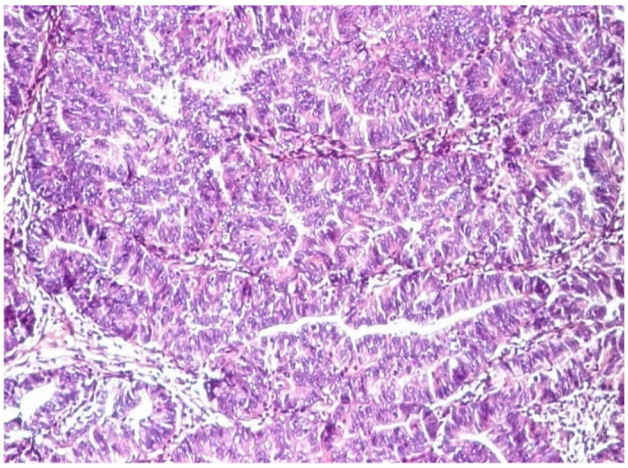

Figure 4 – *Complex endometrial hyperplasia with atypia, periglandular lymphocytic infiltrates with occasional intraepithelial extension. HE staining, ×200.*

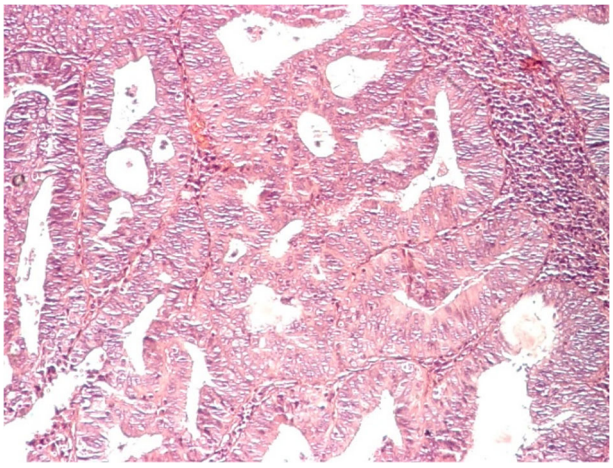

Figure 5 – *Well-differentiated endometrioid adenocarcinoma, rich periglandular lymphocytic infiltrate. HE staining, ×100.*

Table 3 – *NLRP3 and cytokines concentrations in the serum of patients for Group 1, the patients who received the combination Midazolam+Fentanyl*

Parameter	Midazolam+Fentanyl Group 1 (*n*=10)				*p*-value
	Before MIS		*After MIS*		
	Mean ± SD	*95% CI*	*Mean ± SD*	*95% CI*	
NLRP3 [ng/mL]	13.74± 2.35	12.06–15.42	16.93± 3.17	14.66–19.19	*0.049**
TNF-*α* [pg/mL]	15.77± 4.12	12.82–18.71	19.54± 3.54	17.10–22.07	*0.044**
IL-6 [pg/mL]	11.63± 3.75	8.95–14.31	13.13± 3.72	10.46–15.79	0.436
IL-10 [pg/mL]	18.12± 3.12	15.89–20.35	21.78± 3.50	19.28–24.29	*0.035**

CI: Confidence interval; IL: Interleukin; MIS: Minimally invasive surgery; NLRP3: Nucleotide-binding domain (NOD) and leucine-rich repeat protein 3; SD: Standard deviation; TNF-*α*: Tumor necrosis factor-alpha. *Statistically significant.

Discussions

In our study, we wanted to see what is the effect of general total anesthesia with Propofol and Midazolam on the inflammatory response, only in minimally invasive gynecological interventions, in day surgery. Even in this type of intervention, surgical stress cannot be eliminated, as it is involved in increasing the tendency towards inflammation and reducing the number of lymphocytes in the post-intervention period, increases the chance of developing infections [20].

Table 4 – *NLRP3 and cytokines concentrations in the serum of patients for Group 2, the patients who received the combination Propofol+Fentanyl*

Parameter	Propofol+Fentanyl Group 2 (*n*=10)				*p*-value
	Before MIS		*After MIS*		
	Mean ± SD	*95% CI*	*Mean ± SD*	*95% CI*	
NLRP3 [ng/mL]	14.82± 1.54	13.72–15.91	16.11± 1.54	15.00–17.21	*0.037**
TNF-*α* [pg/mL]	12.83± 4.79	9.39–16.26	16.43± 4.66	13.09–19.76	0.089
IL-6 [pg/mL]	12.34± 4.51	9.11–15.56	14.89± 4.12	11.94–17.84	0.143
IL-10 [pg/mL]	14.58± 3.00	12.43–16.73	17.37± 2.89	15.30–19.45	*0.043**

CI: Confidence interval; IL: Interleukin; MIS: Minimally invasive surgery; NLRP3: Nucleotide-binding domain (NOD) and leucine-rich repeat protein 3; SD: Standard deviation; TNF-*α*: Tumor necrosis factor-alpha. *Statistically significant.

Published studies have shown that NLR and PltLR can be considered reliable markers of systemic inflammation [21]. Studies to date have investigated more types of anesthetic agents, finding that total intravenous anesthesia influences less serum growth of immune mediators than other types of anesthesia [22]. Maybe that's why in our study analyzing mean rates of immunocytes we did not find statistically significant differences depending on the type of anesthetic used in minimally invasive gynecological surgery.

Kubyshkin *et al.* showed the role that the inflammatory process has in the progression of the degree of endometrial hyperplasia, the inflammatory changes being more accentuated in the presence of the malignant transformation of the endometrial hyperplasia with atypia [23]. It has also been shown that inflammatory markers have higher values in endometrial hyperplasia with atypia than in those without atypia [24]. But in our study, we did not find an accord between local and systemic inflammatory changes.

Surgical stress can cause an immunosuppressive status, on which anesthetics and analgesic drugs act by directly affecting immunocompetent cells, by increasing the inflammatory response [25].

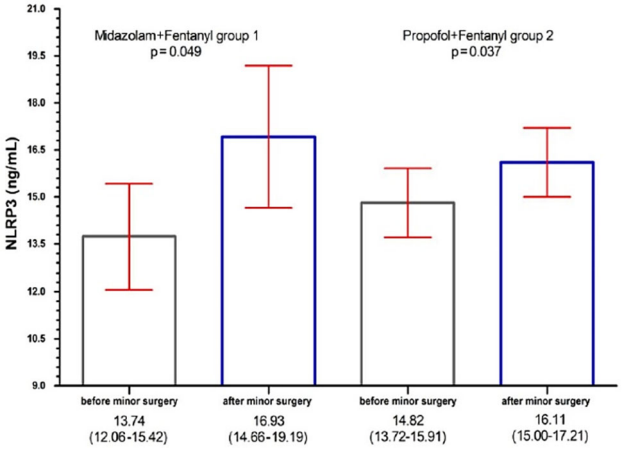

Figure 6 – ***NLRP3 concentrations in serum of patients who underwent minimally invasive surgery: bar per column represent the concentrations of NLRP3 [ng/mL]; red horizontal lines represent the 95% confidence interval (CI). NLRP3: Nucleotide-binding domain (NOD) and leucine-rich repeat protein 3.***

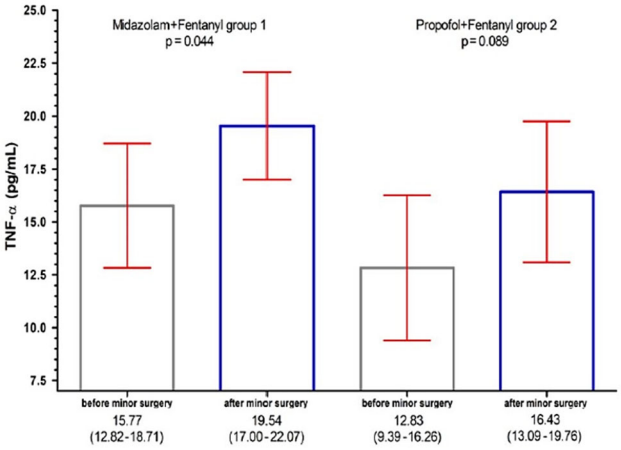

Figure 7 – ***TNF-α concentrations in serum of patients who underwent minimally invasive surgery: bar per column represent the concentrations of TNF-α [pg/mL]; red horizontal lines represent the 95% confidence interval (CI). TNF-α: Tumor necrosis factor-alpha.***

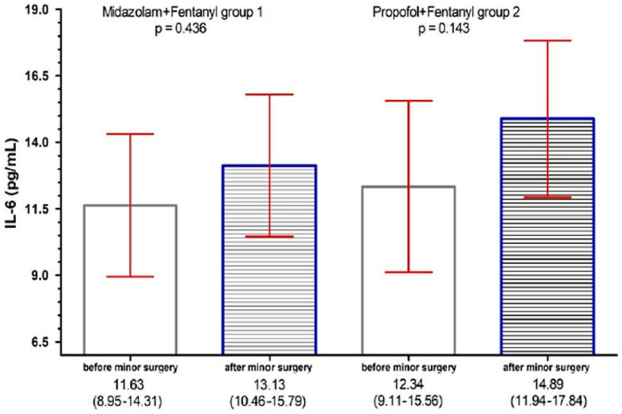

Figure 8 – ***IL-6 concentrations in serum of patients who underwent minimally invasive surgery: bar per column represent the concentrations of IL-6 [pg/mL]; red horizontal lines represent the 95% confidence interval (CI). IL-6: Interleukin-6.***

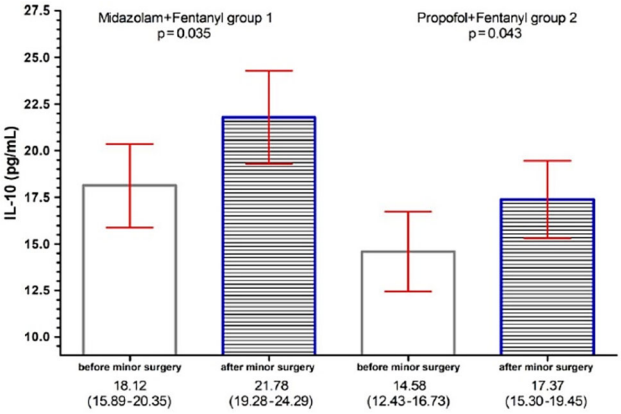

Figure 9 – ***IL-10 concentrations in serum of patients who underwent minimally invasive surgery: bar per column represent the concentrations of IL-10 [pg/mL]; red horizontal lines represent the 95% confidence interval (CI). IL-10: Interleukin-10.***

Activation of inflammatory mediators is also done by inflammasomes, which are an intracellular multiprotein complex known as a modulator of inflammation [26].

We observed that the serum concentration of NLRP3 inflammasomes in the study performed, in both groups of patients (Midazolam+Fentanyl and Propofol+Fentanyl), were higher after a MIS than before. The increase of NLRP3 inflammasome in both groups of anesthetics used shows that there is still a high inflammatory response during MIS.

NLRP3 inflammasome is structured by NLRP3 protein, procaspase-1 and another protein that has a domain in which it recruits caspase and is associated with apoptosis, called ASC [27]. Procaspase-1, the inactive form, is activated in caspase-1, which is responsible for activating the inactive forms of IL-1β (pro-IL-1β) and IL-18 (pro-IL-18) in their active, mature forms, which trigger the inflammatory response [28]. As Kelley *et al.* [29], the NLRP3 inflammasome can be activated by a multitude of stimuli, the activation of NLRP3 inflammasomes being a mechanism of self-defense against invasive factors and stress. The activation of NLRP3 results in the production of proinflammatory cytokines, cytokines having a key role in the action of anesthetic drugs and for systemic inflammatory responses [30].

But how NLRP3 responds to signaling stimuli and how NLRP3 inflammasome formation is initiated is not fully understood to date. Ma *et al.* [31], in an animal study, founded that in the case of neuroinflammation, when there is excessive activation of NLRP3 in the cerebral cortex, Propofol can reduce the inflammatory response, thus reducing brain damage. Midazolam also decreases NLRP3-mediated proinflammatory cytokines secretion, according to the study of Feng *et al.* [32].

The immune response is affected in anesthesia by inhibiting/releasing cytokines that can affect the inflammatory response, along with the body's metabolic and immune response to surgical stress, influencing the profile of inflammatory factors and especially of cytokines [12, 33–35].

The increase in serum concentration of NLRP3 in both groups studied should be reflected by the increase in the proinflammatory cytokines IL-6, TNF-α, cytokines studied by us in both groups, Midazolam anesthesia, and Propofol anesthesia. But the results of our study showed

that only in the case of the Midazolam group, the pro-inflammatory proteins TNF-α, not IL-6, showed a statistically significant increase, while in the Propofol group we did not have this increase.

Serum vascular endothelial growth factor (VEGF) has a strong angiogenic effect, this effect causing neovascularization and endothelial regeneration induced by ischemia. VEGF promotes angiogenesis in neoplasms and has an important role in pathogenesis, progression and neoplastic metastasis [36–38]. Low levels of VEGF in neoplasm may reduce the risk of invasion, proliferation and metastasis [39, 40].

These results are partially in contradiction with the studies of Helmy & Al-Attiyah [41], Lu *et al.* [42], Xiao *et al.* [43], which show that Midazolam significantly reduces the level of proinflammatory cytokines after administration.

A recent 2021 study by Lotfy *et al.* [5] on a population of pediatric patients with intra-abdominal infection showed that the infusion of Midazolam and not Propofol decreases serum cytokine levels. A series of studies by Lisowska *et al.* [9] and Marik [44] have shown that Propofol decreases serum concentrations of IL-1, TNF-α, and IL-6 and increases the concentration of IL-10, so this anesthetic drug may have antioxidant and anti-inflammatory properties.

This situation was encountered in the study, because in the Propofol group we did not find statistically significant serum values of the TNF-α, and IL-6. In the study of Ke *et al.* [45], focused on the comparison between total anesthesia with Propofol *vs* inhalational technique, it has been shown that in the case of total anesthesia with Propofol, IL-6 and TNF-α on the one hand but also IL-10 showed significantly elevated plasma concentrations during surgery. But, at the end of the intervention, the plasma values of IL-6, TNF-α decreased considerably, while the level of IL-10 remained at high values in the anesthetic technique with Propofol compared to the inhalation technique. It has been suggested that total intravenous anesthesia with Propofol and Remifentanil inhibits the inflammatory response. This finding is in contradiction with the study of Mazoti *et al.* [46] who found no difference in plasma cytokine profiles in Isoflurane *vs* Propofol anesthesia in patients who underwent minimally invasive procedures.

On the other hand, the study conducted by Fekkes *et al.* [47] shows that an increase in IL-6 during surgery could be determined by the duration and extent of the surgical procedure so that Propofol would not influence the IL-6 response.

However, the increase in serum IL-10 in both the Midazolam group and the Propofol group shows that the two anesthetics confer an anti-inflammatory effect.

Research in recent years, included more and more, investigation of NLR, MLR, PltLR ratio and systemic immune-inflammation index (SII=NLR×platelets) as new biological markers, useful in assessing the inflammatory condition of the organism, but also the possibility of assessing the activity of the disease. Several scientific reports have highlighted the usefulness of NLR and PltR as important non-invasive tests in monitoring immune status in patients with autoimmune diseases, neoplasms, rheumatoid arthritis, osteoarthritis, myocardial infarction [48–55].

We also set out in this study to investigate whether these ratios change before or after the minimally invasive intervention are performed, or if after administration of the two combinations of anesthetics (Midazolam+Fentanyl and Propofol+Fentanyl) significant changes occur. We observed that in both groups of patients (Midazolam+ Fentanyl and Propofol+Fentanyl), did not encounter statistically significant differences after performing the MIS *vs* before performing MIS.

Given that in the study we collected data from an only healthcare provider, that we have a small sample size group of patients, the retrospective and descriptive nature of the study, makes this study have limitations and the results highlighted in our study do not have a statistically significant power. Additional studies are needed in which to reproduce the results obtained on a larger population sample, both in our reference center and at national level in the other university centers. It is difficult to perform a systemic comparative analysis due to the heterogeneity of the type of parameters studied: surgical mode, pediatric or adult populations, the type of disease for which the surgical procedure was performed, the type of hospitalization, *in vitro* or *in vivo* research, etc. Therefore, the results obtained by us in the performed study are valid only for the minimally invasive gynecological surgery studied, having no other research focused on this type of surgical procedure.

Conclusions

It has long been suspected that anesthetic drugs may affect the functionality of the immune system, but these effects are only minor and temporary in a patient with a healthy immune system. It appears that both Midazolam+ Fentanyl and Propofol+Fentanyl groups have an immunomodulatory action due to the anti-inflammatory effect of both anesthetics. Therefore, anesthesiologists must choose an anesthetic method that uses individualized anesthetic agents, depending on the patient's immune status and disease.

Conflict of interests

The authors declare that they have no conflict of interests.

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Authors' contribution

Anda Lorena Dijmărescu and Marius Bogdan Novac equally contributed to the manuscript.

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Corresponding authors

Mihail Virgil Boldeanu, Associate Professor, MD, PhD, Department of Immunology, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone +40724–515 810, e-mail: mihail.boldeanu@umfcv.ro, boldeanumihailvirgil@yahoo.com

Mircea-Sebastian Şerbănescu, Associate Professor, MD, PhD, Department of Medical Informatics and Biostatistics, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone +40745–766 610, e-mail: mircea_serbanescu@yahoo.com

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