Int Surg 2019;**104**:467–473
DOI: 10.9738/INTSURG-D-16-00018.1

Effects of Prophylactic Ozone Therapy on General Anesthesia and Surgical Stress Response: Neutrophil/Lymphocyte Ratio and Ischemia-Modified Albumin

Huseyin Toman[1], Hasan Sahin[1], Mesut Erbas[1], Hakan Turkon[2], Tuncer Simsek[1], Hasan Ali Kiraz[1], Muhammed Turgut Alper Özkan[3]

[1]*Department of Anesthesiology and Reanimation,* [2]*Department of Biochemistry,* [3]*Department of Cardiovascular Surgery, Canakkale, Turkey*

General anesthesia and surgical stress cause an acute endocrine, metabolic, and immunologic inflammatory response in organisms and an increase in neutrophil lymphocyte ratio (NLR) and ischemia-modified albumin (IMA) levels. Ozone, other than inhalation administration, reduces the release of antioxidants and some proinflammatory cytokines and has been shown to have an anti-inflammatory effect. Our aim is to research how the NLR and IMA response is affected in rabbits undergoing surgical intervention with general anesthesia given prophylactic with ozone therapy. We divided 12 New Zealand rabbits into 2 groups: group O was given 70 µg/mL 10 mL ozone by the rectal route in 6 sessions on alternate days, and group C was given air by the rectal route. The rabbits underwent surgical intervention under general anesthesia. Blood samples were taken at basal, preoperation, 30 minutes postanesthesia, and 24 hours postoperation and were examined for hemogram and IMA. At 24 hours postoperation, an increase in NLR was observed in both groups, more clearly in group C ($P < 0.05$). In both groups, comparisons within the groups showed a significant increase in NLR only at 24 hours postoperation compared to other times ($P < 0.05$).When IMA values were compared, differences between the groups were observed between preoperative values and those at the 30 minutes postanesthesia and 24 hours postoperation ($P < 0.05$). When general anesthesia and surgical stress response were evaluated using inflammatory parameters of both NLR and IMA, there

Corresponding author: Huseyin Toman, MD, Department of Anesthesiology and Reanimation, Canakkale Onsekiz Mart University, Faculty of Medicine, 17100, Canakkale, Turkey.
Tel.: +90-505-4505956; Fax: +90-286-2635956; E-mail: huseyintoman01@hotmail.com

was significantly less of an increase in levels in rabbits given ozone compared to the control group.

Key words: General anesthesia – Surgery response – Ozone – İschemic-modified albumin – Neutrophil lymphocyte ratio

General anesthesia and surgical stress cause an acute endocrine, metabolic, and immunologic inflammatory response in organisms.[1,2] With this inflammatory response, some sequelae elements, acute phase reactants, cellular mediators, and cytokines in blood increase or reduce in circulation. The physiologic response to stress of leukocytes in circulation increases neutrophil counts and causes a fall in lymphocyte numbers. Neutrophil relative lymphopenia is used as a simple marker of the inflammatory response as the neutrophil/lymphocyte ratio (NLR).[3] Ischemia-modified albumin (IMA) occurs more in ischemic situations and is a protein that increases in serum due to hypoxia, acidosis, free radical damage, and membrane disruption, as well as during the inflammatory response.[4]

Ozone therapy comprises a medical O_3/O_2 gas mix applied through a variety of pathways.[5] Ozone therapy has known antioxidant, antibacterial, immunomodulator, analgesic, anti-inflammatory, and many other beneficial effects.[5,6] Toxic if taken at high concentrations through inhalation pathways, ozone undertakes a trigger role in releasing inflammatory mediators.[7] When ozone therapy is administered, apart from by inhalation, it reduces the release of antioxidants like superoxide dismutase (SOD), as well as proinflammatory cytokines like interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, showing an anti-inflammatory effect.[5,8]

The aim of our study is to research how the NLR and IMA response is affected in rabbits undergoing surgical intervention with general anesthesia given prophylactic ozone therapy. We believe the data obtained will, at the same time, benefit humans undergoing operations under general anesthesia.

Materials and Methods

This study used 12 adult New Zealand white rabbits weighing 2.5 to 3.0 kg. Required permissions for experiments were granted by Çanakkale Onsekiz Mart University Animal Experiments Ethics Committee and the study was completed at Çanakkale Onsekiz Mart University Experimental Research Center. Before beginning the study, the rabbits were clinically examined for behavior, respiratory, and cardiovascular systems, and no negative results were found for animals included in the study.

During the experiment animals were given standard feed, and had continuous access to water. The temperature of the housing was 21 ± 2°C and light was regulated as 12 hours brightness and 12 hours darkness.

The twelve rabbits were randomly divided into 2 groups: (1) group O (n = 6): ozone group given O_2-O_3 mix; and (2) group C (n = 6): control group given air.

Methods

Before all procedures, the basal blood samples were obtained and IMA and hemogram values were recorded for both groups. We preferred the rectal insufflation of ozone therapy that is a commonly used route in experimental rabbit models. In group O, during 6 sessions on alternate days, 70 μg/mL 10 mL O_2-O_3 mix (Turkozon Medical Ozone Generator, Blue S, Ozon Sağ. Hiz. İç ve Dış Tic. Ltd. Şti, Istanbul) was slowly insufflated over 1 minute through the rectal pathway. For insufflation a 10-Fr nasogastric probe was cut to 5 cm, the catheter tip was filed to prevent irritation of the rectal mucosa, and it was inserted 4 cm into the rectum using lubricant. The same procedure was used to administer 10 mL of air to group C by the rectal pathway. The operation was initiated after nearly the 12th day of these procedures.

The rabbits were starved for 12 hours before the operation. To prevent heat loss during anesthesia, a 39°C blanket was laid on the ground. A duration of about 30 minutes general anesthesia was planned for the rabbits and 5 mg/kg xylazine (400 mg/20 mL Rompun, Bayer Healthcare LLC, Pittsburgh, Pennsylvania) and 30 mg/kg dose of ketamine HCl (100 mg/10 mL Ketasol %10, Schoeller Chemie Produkte GmbH, Vienna, Austria) were administered to the quadriceps femoris. Pedal and palpebral reflexes were checked and a 24-gauge catheter was inserted into the main auricular artery of the left ear for invasive blood pressure monitoring (PETAŞ KMA 800, Ankara, Turkey). The right ear had a 26-

gauge catheter inserted into the marginal vein. For hemogram and IMA, blood was taken into tubes. A different application method of ozone therapy, major autohemotherapy was administered only once. For this purpose 5 mL of blood was taken from the artery and treated with a 50 μg/mL 5 mL O_2-O_3 mix in a 10 mL heparin injector; the blood was then readministered to the rabbit through the vein over 3 to 4 minutes. The same procedure was completed on the control group using air. Care was taken to prevent the remaining gas in the injector from entering the venous system. Under sedation, all rabbits were given 4 μg/kg fentanyl (500 μg/10 mL Fentanyl-Janssen, Janssen-Cilac, Beerse, Belgium) and 2 mg/kg rocuronium bromide (50 mg/5 mL Esmeron, Organon Ilaç A.S. Istanbul, Turkey) intravenously to suppress respiration and subsequently anesthesia was induced in all rabbits. The rabbits were supported with an appropriate face mask and 100% O_2 through a Mapleson C (Armstrong Medical, Coleraine, Northern Ireland) pediatric ventilation cycle. When fully paralyzed, all rabbits had a V-gel Rabbit (R-3, Docsinnovent Ltd, London, UK) airway device increased by the same anesthetic expert. During this procedure, another anesthetic expert held the tongue laterally for easy insertion of the airway device.

Anesthesia maintenance and laparotomy

All experimental animals were linked to a pediatric Jackson Rees cycle using the anesthetic device (Anesthesia Machine with O_2 Flush Model M3000PK, Parkland Scientific, Inc, Coral Springs, Florida), combined with a pediatric ventilator (MPV-10 Infant Transport Ventilator, Bio-Med Devices, Guilford, Connecticut). To maintain anesthesia, 1.5 to 2 MAC isoflurane (100 mL inhalation Isoflurane USP, Adeka İlaç ve Kimyasal Urunler San. Ve Tic. A.S., Istanbul, Turkey) was given with a 50% oxygen and 50% air mix. The rabbits were ventilated with a respiration rate of 40/minute, 15 cmH_2O (tidal volume of about 10 mL/kg) pressure, and an inspiration/expiration rate of 1:2, appropriate for rabbit physiology. From the 5th minute of anesthesia, all rabbits were given a 6-cm incision and laparotomy for a true application of anesthesia and surgical procedure. The suturing was begun and this procedure continued until the 20th minute of anesthetic maintenance. Blood was taken in the 30th minute for hemogram and IMA and then the inhalation agent was ended. A 4 mg/kg dose of Sugammadex (200 mg/2 mL Bridion, Schering Plough Tıbbi Urunler Ticaret A.S., Istanbul, Turkey) was given intravenously to reverse muscle relaxation and the rabbits were extubated when sufficient spontaneous respiration was achieved. Respiration was supported for a while with the mask. The rabbits taken for operation were monitored for 1 day, and in the 24th hour blood was taken for hemogram and IMA.

Laboratory analysis

Rabbit blood samples were obtained before and after the end of the operation in each group of animals. Hematologic parameters, including white blood cells (WBC), neutrophils, and lymphocytes were analyzed on the same day by an analyzer (LH-780, Beckman Coulter, Inc, Brea, California). The blood samples taken were put into anticoagulants tubes and centrifuged at 4000 rpm for 10 minutes for analyses. The resultant serum samples were aliquoted and stored at −80°C until the IMA analysis. IMA levels were measured by using the colorimetric method discovered by Bar-Or *et al*.[9] The results were reported as absorbance units (ABSUs).

Statistical analysis

The statistical program (SPSS 16, SPSS Inc, Chicago, Illinois) was used for the obtained data, and significance level was assessed ($P < 0.05$). For group comparisons, NLR and IMA values in blood taken at basal, before anesthesia, 30 minutes after anesthesia, and 24 hours postoperation were compared using the Wilcoxon test and Mann-Whitney *U*-test.

Results

In the groups included in the study the mean weight of the rabbits were similar at 2.6 ± 0.4 and 2.5 ± 0.5 kg. During the experiment, the comparisons within the groups and between groups of hematocrit and platelet values on hemograms taken at basal, before anesthesia, 30 minutes postanesthesia, and 24 hours postoperation showed no significant difference. Again, a comparison of rabbits between the groups found no significant difference in WBC ($\times 10^3/\mu L$) values on hemograms at basal, before anesthesia, 30 minutes postanesthesia, and 24 hours postoperation; while in both groups the WBC values at 24 hours were observed to be significantly higher compared to the other times in the same group ($P < 0.05$; Table 1).

Table 1 White blood cells ($\times 10^3/\mu L$): time

	Basal	Preoperative	30 Minutes postanesthesia	Postop hour 24
Group O	4.95 ± 0.32	4.70 ± 0.28	4.86 ± 0.46	7.16 ± 0.29[a]
Group C	5.05 ± 0.42	5.10 ± 0.34	4.83 ± 0.24	8.01 ± 0.36[a]

[a]In both groups, intragroup WBC values at 24 hours were significantly higher than other times. However, there was no significant difference between groups O and C.

Comparison of NLR between the groups did not find a significant difference in values at basal, preoperation, and 30 minutes postanesthesia. In the postoperative 24th hour, there was an increase in NLR observed in both groups, most clearly in group C, and the comparison found a significant difference between the groups ($P < 0.05$). Comparisons within the groups found a significant increase in NLR in both groups only in the postoperative 24th hour compared to the other times ($P < 0.05$; Fig. 1).

When both groups were compared there was no significant difference found in basal IMA values, there were significant differences at preoperation, 30 minutes postanesthesia, and postoperative hour 24 ($P < 0.05$). A comparison of group O within the group found basal IMA was significantly different to preoperative and postoperative 24-hour values ($P < 0.05$), while there was no clear difference observed in the value at 30 minutes postanesthesia ($P > 0.05$). Again, within group comparison of group O found a significant difference in preoperative IMA values compared to the basal and 30 minutes postanesthesia values ($P < 0.05$), while there was no clear difference found compared to the postoperative 24th hour ($P > 0.05$; Fig. 2). Within group comparisons of group C found no significant difference between basal IMA values and preoperative IMA values ($P > 0.05$), but there was a significant difference observed compared to the 30th minute of anesthesia and 24-hour postoperative values ($P < 0.05$). Again, within group comparisons of group C found a significant difference only between preoperative IMA values and those at 30 minutes postanesthesia ($P < 0.05$; Fig. 2).

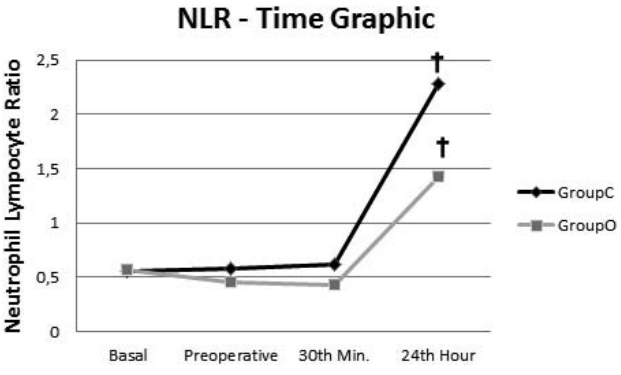

Fig. 1 Neutrophil/lymphocyte ratio: time graphic. †At postoperative hour 24, there was a significant difference in terms of NLR in comparison of group O with group C. Intragroup comparison of both groups showed a significant increase at postoperative hour 24 with respect to other times.

Discussion

This study determined that prophylactic ozone therapy clearly suppresses the inflammatory increase in NLR, WBC, and IMA due to general anesthesia and surgical stress responses. Ozone therapy provided an anti-inflammatory effect.

General anesthesia administration causes more hemodynamic and inflammatory responses compared to spinal and local anesthesia.[10] A stress response is created due to causes such as preoperative fear, painful medication injections, laryngoscopy, intubation, acidosis, hypothermia, hyperglycemia, and hypotension in general anesthesia.[11,12] In surgery a stress response may form due to incision, ischemia, and tissue damage.[13] This stress response, although a pathologic situation, begins with a variety of damaging stimuli and is a response group targeting the maintenance of the body's hemostasis, thus prolonging life.[14] During the general anesthesia and surgical

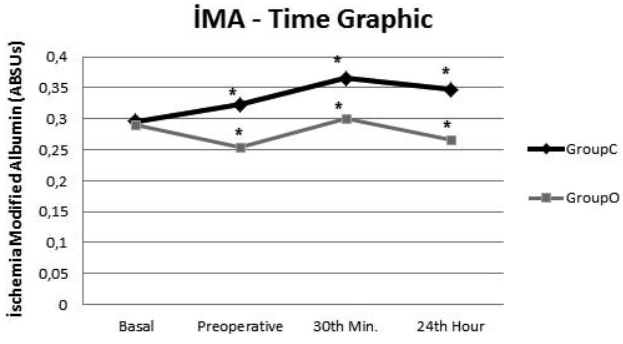

Fig. 2 Ischemia-modified albumin: time graphic. *When the 2 groups were compared, a significant difference was seen in terms of IMA values, at 30 minutes of preoperative anesthesia and at postoperative hour 24.

inflammatory response there is an increase in proinflammatory cytokines like IL-1, IL-6, and TNF-α, neutrophils, and some acute phase reactants.[11,16,17] The anti-inflammatory effect is inactivation of enzymes causing inflammation, or prevention of the release of a variety of mediators, through different pathways. Though not fully explained, ozone therapy has an anti-inflammatory effect on a variety of mechanisms.[5,6,8] Additionally, the anti-inflammatory effect provided by ozone therapy varies according to the number of sessions and concentration.[8,18] While insufficient effect is observed at low doses, at high doses unwanted toxic effects occur. To prevent these toxic effects a new generation of medical ozone generators limits the production of ozone.[18] According to the Madrid Declaration accepted by the International Scientific Committee of Ozone Therapy in 2010, rectally administered ozone for humans can have a dose interval of 10 to 60 μg/mL concentration and 30 to 150 mL volume.[19] As rabbit metabolism is faster than human metabolism, we administered an ozone therapy of 70 μg/mL concentration at 10 mL volume rectally.

Rectal insufflation of ozone therapy is easy, painless, less invasive, and less expensive.[20] Additionally, as opening a vein in rabbits, maintaining the integrity of the vein during ozone therapy, and obtaining sufficient venous blood for major autohemotherapy or minor autohemotherapy is difficult, we chose to use rectal insufflation. However, several studies have demonstrated the effectiveness of applying ozone rectally in human in the literature.[21,22] Because the evaluation of application routes of ozone therapy was not the objective of the present study, and also there wasn't any study comparing the effect of ozone therapy in different application on inflammatory response, we don't have opinion about the advantageous to give ozone rectally on other alternative measures for diminishing inflammatory response.

NLR increases with general anesthesia and surgical stress response.[23,24] A study by Blichert-Toft *et al*[23] found a progressive increase in leukocyte counts in the hours after surgical incision, while there was a slight increase in lymphocyte counts in the first hours followed by a fall. Rem *et al*[25] found significant lymphopenia in blood samples taken 6 and 9 hours after a skin incision in patients undergoing abdominal hysterectomy with general anesthesia, and showed that granulocyte amounts increased and continued to do so for 24 hours.[25] A gas mix of O_3/O_2, toxic when administered by inhalation, increases alveolar neutrophil amounts and reduces neutrophil amounts in peripheral blood.[26,27] Margalit *et al*[28] found no difference in neutrophil adhesion and chemotaxis in blood exposed to major autohemotherapy in the in vitro environment, but showed that, contrary to this, there was a reduction in neutrophil amount at ozone concentrations greater than 30 μg/mL. In our study, while the NLR values in both groups were similar until the 30th minute of the operation, it was high in blood taken 24 hours postoperation and this rate of increase was greater in those not given ozone therapy.

WBC increases with general anesthesia and surgical stress.[24] WBC is mainly formed of neutrophils; thus in infection and inflammatory situations, WBC increases with neutrophils.[25,29] Ozone therapy reduces the leukocyte amount in circulation.[27] In our study, WBC levels rose in both groups at 24 hours postoperation and this increase was more limited in rabbits given ozone therapy.

Though not very significant, general anesthesia administration increases IMA.[30] The surgical incision triggers a systemic inflammatory response, causing a release of oxidative stress markers. It is thought that this change in IMA levels causes a similar increase in inflammation to that occurring during inflammatory diseases, rather than ischemia and tissue damage.[31,32] Free radicals formed by tissue damage cause IMA to increase within minutes; it returns to normal values after remaining high for 6 to 12 hours.[32] In this study, the basal IMA levels in both groups were the same. In the preoperative period, the IMA values in the ozone therapy group fell, while we observed an increase in the rabbits not given ozone therapy. We believe this is due to the stress source of rectal air insufflation increasing IMA levels. In rabbits given ozone, the IMA reached peak levels in the 30th minute of the operation. IMA during the whole study did not rise above 0.4 ABSU, accepted as the level of ischemia. In the postoperative 24th hour, the IMA levels were on a downward trend. At all stages, ozone therapy application suppressed mediators occurring due to tissue damage and the systemic inflammatory response, we believe limiting the increase in IMA levels

This study on the efficacy of ozone therapy for the suppression of general anesthesia and surgical stress response is the first on this topic. In our study, the inflammatory parameters of both NLR and IMA—used to assess the general anesthesia and surgical stress response—rose by significantly less in the rabbits given ozone compared to the control group. We believe this is due to the anti-inflammatory efficacy of ozone therapy.

In conclusion, we observed that ozone therapy provided anti-inflammatory effect by suppressing NLR and IMA. We are of the opinion that this study provides an idea of the benefits of prophylactic ozone therapy for humans undergoing general anesthesia and surgical intervention. We believe further research and clinical studies are required.

Acknowledgments

This study was supported by Canakkale Onsekiz Mart University Scientific Research Project Found with TSA-2014-318 project number. The authors have no financial relationship with the companies that manufactured the materials used in this study. Conflict of interest: The authors declare that they have no conflict of interest.

References

1. Milosavljevic SB, Pavlovic AP, Trpkovic SV, Ilic AN, Sekulic AD. Influence of spinal and general anesthesia on the metabolic, hormonal, and hemodynamic response in elective surgical patients. *Med Sci Monit* 2014;20:1833–1840
2. Kehlet H. Manipulation of the metabolic response in clinical practice. *World J Surg* 2000;**24**(6):690–695
3. Tamhane UU, Aneja S, Montgomery D, Rogers EK, Eagle KA, Gurm HS. Association between admission neutrophil to lymphocyte ratio and outcomes in patients with acute coronary syndrome. *Am J Cardiol* 2008;**102**(6): 653–657
4. Chan MY, Pronovost PJ. Clinical utility of biomarkers in myocardial injury. *Curr Opin Anaesthesiol* 2004;**17**(1):49–55
5. Azuma K, Mori T, Kawamoto K, Kuroda K, Tsuka T, Imagawa T *et al*. Anti-inflammatory effects of ozonated water in an experimental mouse model. *Biomed Rep* 2014;**2**(5):671–674
6. Clavo B, Santana-Rodriguez N, Gutierrez, D, Lopez JC, Suarez G, Lopez L *et al*. Long-term improvement in refractory headache following ozone therapy. *J Altern Complement Med* 2013;**19**(5):453–458
7. Sunil VR, Francis M, Vayas KN, Cervelli JA, Choi H, Laskin JD *et al*. Regulation of ozone-induced lung inflammation and injury by the β-galactoside-binding lectin galectin-3. *Toxicol Appl Pharmacol* 2015;**284**(2):236–245
8. Chang JD, Lu HS, Chang YF, Wang D. Ameliorative effect of ozone on cytokine production in mice injected with human rheumatoid arthritis synovial fibroblast cells. *Rheumatol Int* 2005;**26**(2):142–151
9. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. *J Emerg Med* 2000;**19**(4):311–315
10. Kulacoglu H, Ozdogan M, Gurer A, Ersoy EP, Onder DA *et al*. Prospective comparison of local, spinal, and general types of anaesthesia regarding oxidative stress following Lichtenstein hernia repair. *Bratisl Lek Listy* 2007;**108**(8):335–339
11. Kurosawa S, Kato M. Anesthetics, immune cells, and immune responses. *J Anesth* 2008;**22**(3):263–277
12. Daabiss M, Hashish M. Effects of lornoxicam on the hemodynamic and catecholamine response to laryngoscopy and tracheal intubation. *Eur J Clin Pharmacol* 2011;**67**(8):783–786
13. Agirbasli M, Nguyen ML, Win K, Kunselman AR, Clark JB, Myers JL *et al*. Inflammatory and hemostatic response to cardiopulmonary bypass in pediatric population: feasibility of seriological testing of multiple biomarkers. *Artif Organs* 2010; **34**(11):987–995
14. Rosenfeld BA, Faraday N, Campbell D, Dise K, Bell W, Goldschmidt P. Hemostatic effects of stress hormone infusion. *Anesthesiology* 1994;**81**(5):1116–1126
15. Norman JG, Fink GW. The effects of epidural anesthesia on the neuroendocrine response to major surgical stress: a randomized prospective trial. *Am Surg* 1997;**63**(1):75–80
16. Watt DG, Horgan PG, McMillan, DC. Routine clinical markers of the magnitude of the systemic inflammatory response after elective operation: a systematic review. *Surgery* 2015;**157**(2): 362–380
17. Erskine R, Janicki PK, Ellis P, James MF. Neutrophils from patients undergoing hip surgery exhibit enhanced movement under spinal anaesthesia compared with general anaesthesia. *Can J Anaesth* 1992;**39**(9):905–910
18. van Hoof HJ, Zijlstra FJ, Voss HP, Garrelds IM, Dormans JA, van Bree L *et al*. The effect of ozone exposure on the release of eicosanoids in guinea-pig BAL fluid in relation to cellular damage and inflammation. *Mediators Inflamm* 1997;**6**(5-6):355–361
19. Spanish Association of Medical Professionals in Ozone Therapy. Madrid declaration on ozone therapy. Available at: http://aepromo.org/en/madrid-declaration-on-ozone-therapy-2nd-edition/. Accessed October 10 2015
20. Guanche D, Zamora Z, Hernández F, Mena K, Alonso Y, Roda M *et al*. Effect of ozone/oxygen mixture on systemic oxidative stress and organic damage. *Toxicol Mech Methods* 2010;**20**(1): 25–30
21. Hidalgo-Tallon J, Menendez-Cepero S, Vilchez JS, Rodriguez-Lopez CM, Calandre EP. Ozone therapy as add-on treatment in fibromyalgia management by rectal insufflation: an open-label pilot study. *J Altern Complement Med* 2013;**19**(3):238–242
22. Martinez-Sanchez G, Al-Dalain SM, Menendez S, Re L, Giuliani A, Candelario-Jalil E *et al*. Therapeutic efficacy of ozone in patients with diabetic foot. *Eur J Pharmacol* 2005; **523**(1–3):151–161
23. Blichert-Toft M, Christensen V, Engquist A, Fog-Moller F, Kehlet H, Madsen SN *et al*. Influence of age on the endocrine metabolic response to surgery. *Ann Surg* 1979;**190**(6):761–770
24. Moldal ER, Kirpensteijn J, Kristensen AT, Haga HA, Nodtvedt A, Eriksen T. Evaluation of inflammatory and hemostatic

surgical stress responses in male cats after castration under general anesthesia with or without local anesthesia. *Am J Vet Res* 2012;**73**(11):1824–1831

25. Rem J, Brandt MR, Kehlet H. Prevention of postoperative lymphopenia and granulocytosis by epidural analgesia. *Lancet* 1980;**1**(8163):283–284
26. Barreno RX, Richards JB, Schneider DJ, Cromar KR, Nadas AJ, Hernandez CB *et al*. Endogenous osteopontin promotes ozone-induced neutrophil recruitment to the lungs and airway hyperresponsiveness to methacholine. *Am J Physiol Lung Cell Mol Physiol* 2013;**305**(2):L118–L129
27. Bosson JA, Blomberg A, Stenfors N, Helleday R, Kelly FJ, Behndig AF *et al*. Peripheral blood neutrophilia as a biomarker of ozone-induced pulmonary inflammation. *PLoS One* 2013;**31**; 8(12):e81816
28. Margalit M, Attias E, Attias D, Elstein D, Zimran A, Matzner Y. Effect of ozone on neutrophil function in vitro. *Clin Lab Haematol* 2001;**23**(4):243–247
29. Ozaki Y, Ohashi T, Niwa Y. Oxygen radical production by neutrophils from patients with bacterial infection and rheumatoid arthritis. Measurement of hydrogen peroxide may most accurately represent enhancement of oxygen radical production during infection. *Inflammation* 1986; **10**(2):119–130
30. Kosucu M, Coskun I, Eroglu A, Kutanis D, Mentese A, Karahan SC *et al*. The effects of spinal, inhalation, and total intravenous anesthetic techniques on ischemia-reperfusion injury in arthroscopic knee surgery. *Biomed Res Int* 2014;2014: 846570
31. Toker A, Karatas Z, Altın H, Karaarslan S, Cicekler H, Alp H. Evaluation of serum ischemia modified albumin levels in acute rheumatic fever before and after therapy. *Indian J Pediatr* 2014;**81**(2):120–125
32. Alvarado A. A practical score for the early diagnosis of acute appendicitis. *Ann Emerg Med* 1986;**15**(5):557–564