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Adaptative Immune Response In Female Cats Undergoing Ovariohysterectomy With Total Intravenous Or Dissociative Anesthesia

Andriele da Costa Poerschke

andriele_cp@hotmail.com

Universidade do Estado de Santa Catarina

Márcio Machado Costa

Federal University of Uberlândia

André Vasconcelos Soares

Universidade Federal de Santa Maria

Tais Julia Sala

Associação Nacional de Clínicos Veterinários de Pequenos Animais

Luísa de Oliveira

Universidade Regional do Noroeste do Estado do Rio Grande do Sul

Karina Deckmann

Universidade Federal de Santa Maria

Leonardo José Gil Barcellos

Universidade de Passo Fundo

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Abstract

Sixteen healthy female cats were selected for the study to evaluate the adaptive immune response following ovariohysterectomy by quantifying lymphocytes and adenosine deaminase (ADA) activity in serum and lymphocytes. Cats were divided into two groups using either total intravenous anesthesia (TIVA) or dissociative anesthesia protocols and correlated with pain scores. Animals in the first group received a combination of 12.5 $\mu g.kg^{-1}$ dexmedetomidine, 0.25 $mg.kg^{-1}$ butorphanol, and 5 $mg.kg^{-1}$ tiletamine-zolazepam (GDTZB) by intramuscular (IM) injection. In comparison, female cats of the second group received dexmedetomidine at a dose of 5 $\mu g.kg^{-1}$ and butorphanol at a dose of 0.3 $mg.kg^{-1}$ via IM injection as preanesthetic medication (PAM); they were then induced with propofol at a dose of 2 $mg.kg^{-1}$ and maintained with propofol via continuous infusion at a rate of 0.4 $mg.kg.min^{-1}$ (GTIVA). Blood samples were collected at three time points (D0, D6, and D24) corresponding to preoperative, 6, and 24 hours postoperatively to quantify lymphocytes and assess serum and lymphocyte ADA activity. Postoperatively, patients were monitored and assessed hourly for the need for analgesic rescue. The postoperative pain was quantified on D6 and D24 using three distinct pain scales. No discernible variations were observed in lymphocyte count, ADA activity, and pain levels between GDTZB and GTIVA. Prior to surgery, the GDTZB felines required oxygen supplementation due to their low partial saturation, as indicated by pulse oximetry results. Neither of the two protocols had an adverse effect on the patient's adaptive immune response, and no cat required post-operative analgesic rescue.

Introduction

The combined administration of dexmedetomidine, tiletamine-zolazepam, and butorphanol can be an effective strategy for achieving rapid anesthesia (Grimm et al. 2017; Rydén et al. 2021) and consistent anesthesia planning (Cagle et al. 2017). In contrast, propofol-based total intravenous anesthesia (TIVA) is known for promoting better cardiopulmonary stability (Ilkiw and Pascoe 2003) and quicker recovery time (Oliveira et al. 2007).

It is established that anesthetic drugs may impair the inflammatory response, thereby disrupting functions of immune cells (Kurosawa and Kato, 2008), and influence the transmission of nerve impulses to induce analgesia

(McBride et al. 1996). Nonetheless, producing preventive analgesia is crucial for proper postoperative pain management (White 2017).

Adenosine deaminase (ADA) has been examined as a potential inflammatory biomarker in numerous feline diseases (Kahraman and Gökce 2020; Hankanga et al. 2007; Altuğ et al. 2007, Biezus et al. 2017). However, no research has explored its activity in other immunosuppressive conditions such as during anesthetic procedures. Thus, here we aimed to compare the adaptive immune response of cats undergoing ovariohysterectomy with total intravenous anesthesia and dissociative anesthesia protocols, while valuating postoperative pain.

Materials and Methods

Animals

Sixteen female cats meeting the following inclusion criteria were selected for elective ovariohysterectomy: an age range of six months to three years, having already completed their first estrus cycle, no specific breed, and a minimum weight of 2 kg. Prior to selection, all cats underwent clinical and laboratory assessments to verify their health status. They were classified as ASA I, according to the American Society of Anesthesiologists classification, and any cats with abnormalities were excluded. Furthermore, the study only included animals with docile behavior that could be managed.

The experimental animals were split into two groups in a 1:1 ratio, utilizing covariate adaptive randomization with the Pocock and Simon approach, which analyzes covariate imbalances to minimize bias (Kang et al. 2008). As such, covariates such as age, weight, race, total leukocyte and lymphocyte counts were factored in during group randomization. The sample calculation utilized an alpha error of 0.05, a test power of 0.90, a clinically meaningful difference of 20% (4.61 points on 23.07 UI/L) and an assumed standard deviation (SD) of 2.05 points on 23.05 UI/L in serum ADA activity (Altuğ et al. 2009). Therefore, considering the number of groups and the number of time measurements, a minimum of 16 cats were needed, 8 in each group.

Study Model

The felines were transported to the veterinary clinic two days prior to the surgical procedure to acclimate to the surroundings and decrease potential stress. The cats were housed in a separate area of the clinic to ensure a peaceful and noise-free environment and were given access to air conditioning. Each cat underwent clinical evaluation, weight assessment, and was placed in an individual cage equipped with water, food, litter box, and bedding. Solid and water fasting were instituted eight and four hours before the surgical procedure, respectively.

The 16 cats were distributed into two groups of eight. In the first group, named GDTZB, cats received an anesthetic protocol consisting of 12.5 $\mu g.kg^{-1}$ of dexmedetomidine (Dexdomitor®, Zoetis, SP, Brazil), 0.25 $mg.kg^{-1}$ of butorphanol (Butorfin®, Vetnil, SP, Brazil), and 5 $mg.kg^{-1}$ of tiletamine-zolazepam (Zoletil®, Virbac, Sp, Brazil) intramuscularly (IM). Ten minutes after application, the cats were accessed in one of the cephalic veins using a 24G catheter and received fluid therapy with Ringer's lactate at a rate of 3 $ml.kg.min^{-1}$.

In the second group, named GTIVA, cats received pre-anesthetic medication (PAM) consisting of dexmedetomidine at a dose of 5 $\mu g.kg^{-1}$ and butorphanol at a dose of 0.3 $mg.kg^{-1}$, administered IM. The felines were given venous access using a 24G catheter in one of their cephalic veins. They were then induced with anesthesia utilizing propofol (Provive®, União Química, SP, Brazil) at 2 $mg.kg^{-1}$, and maintenance was achieved with a continuous propofol infusion at a rate of 0.4 $mg.kg.min^{-1}$. Afterwards,

the cats were intubated using an endotracheal tube no. 3.0 or 3.5 in order to deliver 100% oxygen therapy at a rate of 200 ml.kg.min^{-1} for the Baraka system without gas rebreathing.

The surgical preparation was identical for both groups. The cats were placed in a dorsal decubitus position, and the operative field was shaved and disinfected with 2% chlorhexidine solution for degerming purposes. Subsequently, 0.5% alcoholic chlorhexidine solution was applied to the area. The surgical procedure utilized for ovariohysterectomy involved the conventional method with retro-umbilical access through the midline. Ovaries were exposed and ligatures were applied using a three-clamp technique. Synthesis occurred in layers, beginning from the muscles, and ending at the skin.

Anesthetic monitoring was performed utilizing a multi-parameter monitor equipped with pulse oximetry, electrocardiography, plethysmography, thermometer, and non-invasive blood pressure monitoring. This allowed for observation of partial oxygen saturation (SpO2), heart rate (HR), pulse, respiratory rate (f), body temperature (TºC), as well as systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP). All parameters were measured and recorded at five-minute intervals. The ideal surgical plan was identified as Plan II/Stage 3 based on objective parameters such as eyeball rotation, palpebral and pupillary reflexes, according to the Guedel's classification.

Prior to the surgical procedure, all patients received dipyrone 20 mg.kg^{-1} and cephalothin 30 mg.kg^{-1} intravenously (IV), in addition to the drugs mentioned above. Furthermore, post-operative meloxicam 0.1 mg.kg^{-1} was administered subcutaneously (SC). If rescue analgesia was required during the operation, fentanyl was administered intravenously at a dose of 2.5 µg.kg^{-1}. Tramadol hydrochloride was utilized as needed for post-operative pain management at a subcutaneous dose of 3 mg.kg^{-1}.

To evaluate post-operative pain, two evaluators who were blind to the anesthetic protocols utilized the UNESP-Botucatu Multidimensional Pain Scale (EMAD U-B), the Glasgow Composite Pain Scale for Felines (Glasgow CMPS-F), and the University of Colorado's Acute Pain Scale for Felines.

The felines underwent assessment hourly following surgical conclusion, while implementation of the post-operative pain scale occurred at D6 and D24 (equivalent to 6 hours and 24 hours after the surgical process, respectively). Biological samples were gathered at D0 (pre-surgery), D6, and D24. After the evaluation of pain, whole blood samples with anticoagulant (EDTA) were obtained via the jugular vein for the purpose of blood count and lymphocyte separation. Additional samples without anticoagulant were collected for the determination of adenosine deaminase activity through serum extraction by centrifugation at 2000 rpm for five minutes. The collected serum samples were divided into three Eppendorf tubes and stored at -70°C for subsequent processing.

The blood samples with EDTA were utilized for the blood count. An aliquot was obtained for the blood smear and microhematocrit using a microcapillary. The text was free from grammatical errors, spelling mistakes, and punctuation errors and adhered to conventional academic structure and formatting with clear, objective, and value-neutral language. The hematology analyzer determined parameters, including

erythrocyte indices such as VCM, CHCM, RDW, and PDW, or they were calculated according to Schalm and Jain (1986).

Statistics

The impact of the two anesthetic protocols on serum ADA and lymphocyte levels was analyzed using a Generalized Estimating Equation (GEE), with groups and time as fixed variables, and repeated measures within the model, respectively. The results achieved a significant difference level of less than 5% probability ($p < 0.05$). The statistical analyses were conducted using version 4.05 of the R program. The graphic composition was carried out using the program Graphpad Prism, version 8.0.1.

Results

Four felines were excluded from the study due to their aggressive behavior and inability to handle them. The average weight of the animals in the GBTZB group was 2.930 ± 0.313 kg, while in the GTIVA group it was 2.810 ± 0.390 kg. Additionally, the average age of the cats in the GDTZB and GTIVA groups was 14.5 ± 8.8 months and 11.1 ± 2.47 months, respectively. All cats were non-breed and had undergone at least one estrus.

The marginal means of hematocrit (ht), total leukocytes, band neutrophils, segmented neutrophils, eosinophils, monocytes, and basophils were estimated in a mixed model using GDTZB and GTIVA D0 - D6 and GDTZB and GTIVA D0 - D24 as fixed effects (Fig. 1). No statistical differences ($p > 0.05$) in hematological parameters were observed between the two groups at the designated time intervals. Furthermore, the proposed mixed model evaluated the estimated marginal means of lymphocytes, serum ADA, and lymphocyte ADA (Fig. 2) and showed no significant difference ($p > 0.05$).

The transanesthetic parameters analyzed were heart rate (HR), body temperature (HT), partial oxygen saturation (SpO2), and mean arterial pressure (MAP). Despite observing high blood pressure values in relation to baseline values, there was no significant difference between the two groups ($p > 0.05$).

Two of the cats of the GTIVA group required an increased propofol infusion rate due to a shallower anesthetic plane; they also required intraoperative analgesic reinforcement with a bolus of fentanyl 2.5 $\mu g.kg^{-1}$, IV. Conversely, two other cats required a decreased propofol infusion rate due to a deeper anesthetic plane.

All cats in the GDTZB group required oxygen supplementation via mask as they presented with a SpO2 level between 86% and 90% before surgery.

The pain scales used to evaluate analgesia in the postoperative period, D6 and D24, showed no significant difference between the groups ($p > 0.05$). Furthermore, none of the cats required analgesic rescue.

Discussion

At least to our knowledge, this study is the first to explore ADA as a potential biomarker for immunosuppression related to anesthetic-surgical procedures in cats. It also compares the adaptive immune response in cats under two different anesthetic protocols.

Hematological parameters were evaluated for GDTZB and GTIVA at specific time intervals, with no significant differences observed. Similarly, cats anesthetized with the combination of dexmedetomidine, ketamine, and butorphanol experienced fewer clinical, physiological, hematological, and biochemical alterations when compared to cats anesthetized with either the combination of dexmedetomidine and ketamine or the combination of ketamine and midazolam (Pal et al. 2022).

In dogs, propofol has displayed immunosuppressive effects during continuous infusion, causing a reduction in lymphocyte count, regardless of whether or not it is administered with tramadol (Costa et al. 2013). Conversely, propofol continuous infusion for anesthesia maintenance during ovariohysterectomy in canines premedicated with dexmedetomidine and butorphanol exhibited lower immunosuppressive potential when compared to maintenance with ketamine or isoflurane (Bayan et al. 2022).

Increased ADA activity in the serum of symptomatic FeLV-infected cats plays a vital role in compromising the immune response and contributing to the inflammatory process (Biezus et al. 2017). However, here we did not find significant difference in the mean levels of ADA in serum and lymphocytes.

Postoperative pain scales showed no difference in scores among the proposed experimental groups. However, during the surgical procedure, two patients receiving GTIVA required analgesic rescue, and all GDTZB patients required supplemental oxygen. In dogs, administering anesthesia with dexmedetomidine combined with tiletamine-zolazepam and butorphanol or with dexmedetomidine combined with ketamine and butorphanol resulted in hypoventilation and respiratory acidosis, necessitating oxygen supplementation to prevent hypoxia (Krimins et al. 2012; Pal et al. 2022). In cats, the combination of dexmedetomidine and propofol, given through continuous infusion, has demonstrated effectiveness in promoting analgesia and cardiorespiratory stability. Furthermore, it reduces the required propofol dose, which in turn minimizes the resulting respiratory depression (Sanches et al. 2022).

Dogs administered butorphanol either before or after undergoing an ovariohysterectomy demonstrated a swift elevation in pain score within one hour following the surgery, decreasing gradually up to 24 hours later (Gültiken et al. 2022). Conversely, cats sedated via a continuous infusion of propofol necessitated more intraoperative analgesic intervention when compared to cats pre-medicated with dexmedetomidine and maintained with a continuous infusion of dexmedetomidine and propofol (Sanches et al. 2022). Thus, it can be concluded that certain GTIVA cats required rescue analgesia, potentially resulting from the lower dose of dexmedetomidine administration. Besides, dissociative anesthetics such as tiletamine can prompt superficial sleep and favorable somatic analgesia (Dugassa and Fromsa 2018), validating

the need for transoperative analgesia and even an augmentation in the propofol infusion rate in certain GTIVA cats.

Although the study's animal sample size was not large enough to meet the necessary calculations, the collected data remains concise and applicable to the feline population.

No significant differences in adaptive immune response between the GDTZB and GTIVA groups were detected, suggesting that both anesthetic protocols did not cause immunosuppression in healthy female cats undergoing ovariohysterectomy. Our findings reinforce that both anesthetic protocols are effective in achieving appropriate levels of anesthesia, providing good postoperative pain relief, and supporting a smooth recovery from anesthesia.

Further research is required to make a more extensive and nuanced comparison between different anesthetic protocols. This investigation should include the analysis of biomarkers associated with ADA to elucidate its use and its relationship with immune response in the context of anesthesia.

Declarations

Author contribution

André Vasconcelos Soares: Investigation, Methodology, Visualization.

Andriele da Costa Poerschke: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing.

Karina Bueno Deckmann: Investigation, Methodology, Visualization.

Leonardo José Gil Barcellos: Conceptualization, Data curation, Formal analysis, Visualization, Writing.

Luísa de Oliveira: Investigation, Methodology, Visualization.

Márcio Machado Costa: Conceptualization, Data curation, Formal analysis, Software, Visualization, Writing.

Taís Julia Sala: Data curation, Investigation, Resources, Supervision.

Ethics approval

The authors affirm that the journal's ethical policies, as delineated on the author guidelines page, have been adhered to. The present study utilizes client-owned felines as subjects. Written informed consent was obtained from the cat owners for the performance of procedures and the utilization of clinical data and images for the purposes of publication. The study was conducted subsequent to the receipt of approval from the Ethics Committee For The Use of Animals of the University of Passo Fundo (CEUA - UPF n. 026/2022).

Consent to participate

All authors confirm their participation in the study.

Data availability

This manuscript does not report data generation or analysis.

Competing interests

The authors declare no competing interests.

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Figures

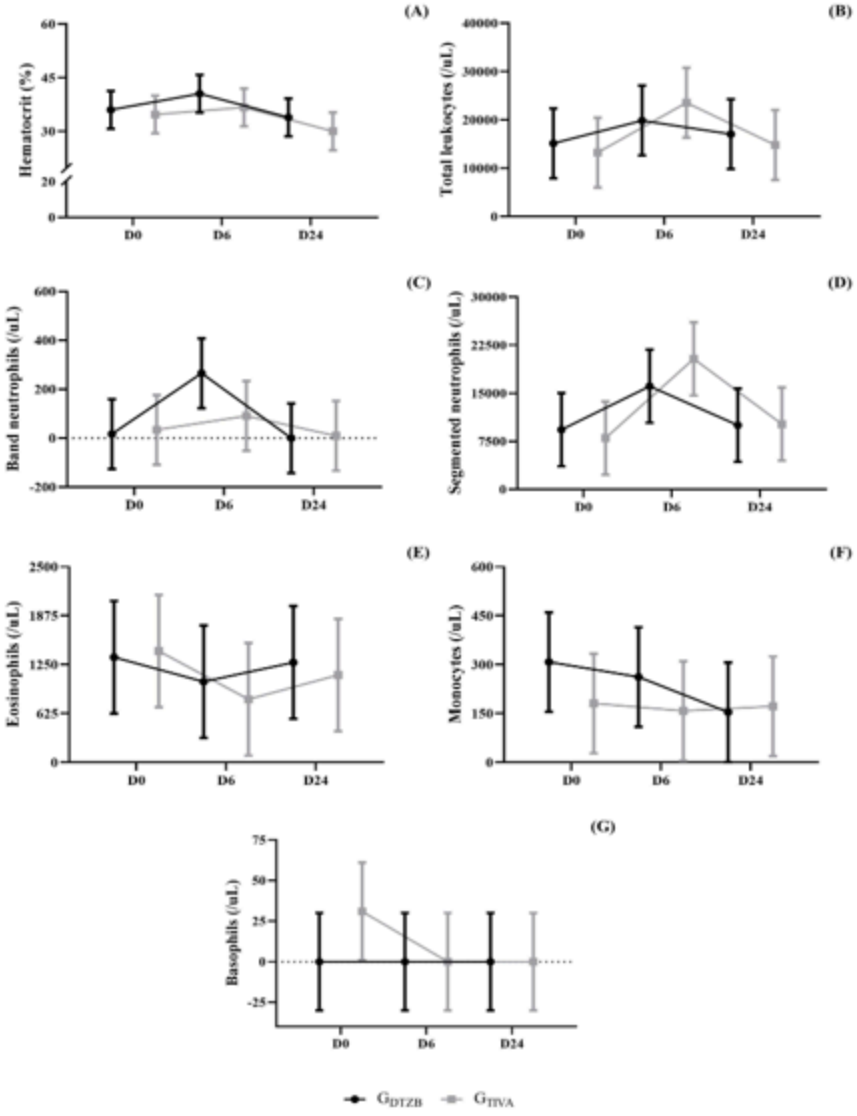


Figure 1. Estimated marginal means (•,■) and 95% asymptotic confidence intervals (⊤,⊥) of the hematological parameter of female cats undergoing ovariohysterectomy using either total intravenous anesthesia (TIVA) or dissociative anesthesia protocol: (A) hematocrit; (B) total leukocytes; (C) band neutrophils; (D) segmented neutrophils; (E) eosinophils; (F) monocytes; (G) basophils.

Figure 1

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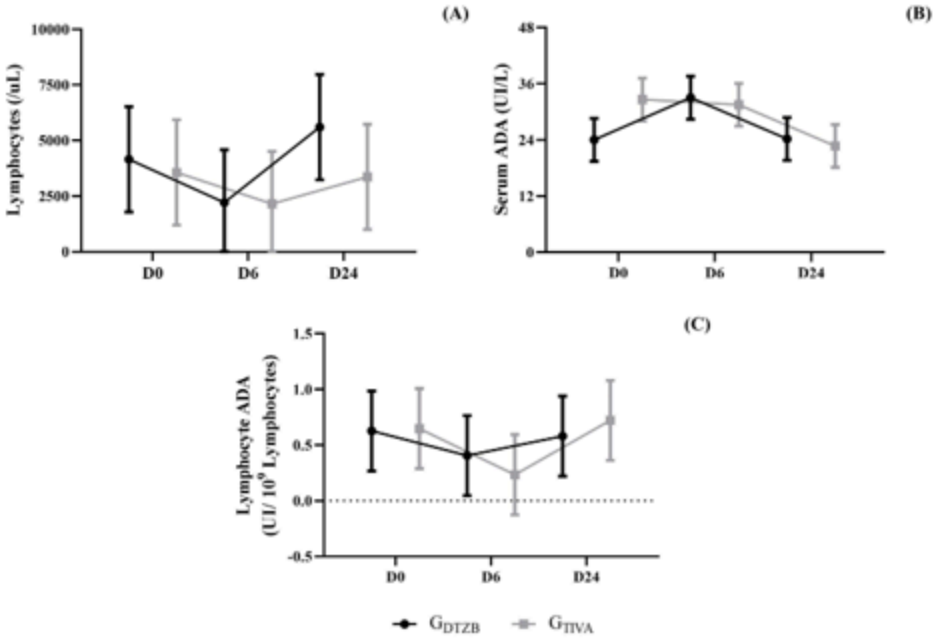


Figure 2. Estimated marginal means (•,▪) and 95% asymptotic confidence intervals (┬,┴) of adenosine deaminase (ADA) activity in female cats undergoing ovariohysterectomy using either total intravenous anesthesia (TIVA) or dissociative anesthesia protocol: (A) serum ADA; (B) lymphocytes; (C) lymphocytes ADA.

Figure 2

See image above for figure legend