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Original Article

Can peri-surgical electroacupuncture relieve immunity suppression? A pilot study in dogs

V. Rabbogliatti [a], F. A. Brioschi [a], A. Pecile [a,] *, V. Bronzo [a], V. Martini [a], P. Dall'Ara [a], G. Ravasio [a], D. Groppetti [a]

[a] *Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, via dell'università 6, 26900 Lodi (LO), Italy.*

* Corresponding author. Tel: +39 02503 34563, via dell'università 6, 26900 Lodi (LO), Italy
E-mail address: alessandro.pecile@unimi.it (A. Pecile).

Abstract:

General anesthesia and surgical stress can suppress the immunological response by acting both directly on the immune system and indirectly on the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Disturbance of the immune system during the perioperative period can lead to complications such as wound-healing disorders and infections up to sepsis. Effectiveness of acupuncture in regulating the immune function by increasing leukocyte numbers and inhibiting inflammatory response has been proven. This study aimed to explore the impact of electroacupuncture (EAP) on the dynamic balance of the immune system and immune cell populations in dogs undergoing surgery. Twelve healthy bitches scheduled for elective ovariectomy were divided into two groups according to whether (EAP, n=6) or not (CTR, n=6) a peri-operative electroacupuncture treatment was performed. Levels of leukocytes (neutrophils, monocytes, T- and B-cells) and immunoglobulins M (IgM) and A (IgA) were measured in blood samples collected before (T0), 1 hour (T1) and 2.5 hours (T2) after anesthesia induction.

Leukocytes count decreased from T0 to T1 in both groups and restored within 1.5 hours in EAP group whereas remained significantly lower in CTR group ($P<0.02$). In particular, neutrophils and monocytes increased in dogs receiving EAP ($P<0.01$) while T-cells decreased in CTR group ($P<0.04$) at T2. B-cells and cytotoxic T-cells decreased in EAP dogs ($P<0.04$) at T2. No differences in helper T-cells, IgM and IgA levels were recorded between groups and over time. Our results

suggest a modulatory effect of EAP on the immune system which is early expressed on neutrophils, monocytes and T-cells.

Keywords: Dogs; Electroacupuncture; Immunoglobulins; Leukocytes; Lymphocytes

Introduction

The innate immunity is the first defense of the organism, it acts immediately after an aggression and involves both cellular and molecular defenses. In particular, phagocytic cells such as neutrophils, monocytes and macrophages play a crucial role, whereas complement, interferon, lysozyme and defensins are some examples of molecules (Tizard 2017). The innate immunity uses primitive nonspecific recognition systems to bind, neutralize and destroy pathogens (Kurosawa and Kato 2008). The adaptive or acquired immunity is responsible for a targeted response to antigens through the activation of B- and T-cells and the synthesis of proteins such as antibodies and cytokines (Vivier and Malissen 2005; Kurosawa and Kato 2008; Li et al., 2013a). Adaptive response comprises humoral and cellular components (Li et al., 2013a). Humoral immunity is mediated by antibodies (immunoglobulins) secreted by plasma cells. In particular, immunoglobulins M (IgM) are produced in response to the first antigenic stimulation and are soon replaced by immunoglobulins G (IgG) or A (IgA) (Poli et al., 2017; Tizard 2017). Cell-mediated immunity involves helper T-cells and cytotoxic T-cells. Helper T-cells play a central role in facilitating and driving the activity of other immune cells through the release of cytokines and chemokines, either improving antibody production or cell-mediated responses depending on the antigen nature and localization. Cytotoxic T-cells act by killing cells expressing non-self antigens, including tumor cells and virus-infected cells (Kurosawa and Kato 2008; Poli et al., 2017; Tizard

2017). These cells have been reported to increase during the surgical trauma period (Navarro et al., 1988).

Surgical tissue injury and exposure to anesthetic drugs during the perioperative period can affect the immune system (Cardinale et al., 2011). The first pro-inflammatory response to surgical stress triggers the innate immune system eliciting an increase in monocytes, neutrophils and macrophages (Cook et al., 2007; Cardinale et al., 2011). Since an excessive reaction may be harmful, a compensatory immunosuppressive response occurs, the extent and duration of which depends on the surgery magnitude (Hogan et al., 2011). It mainly involves cells of the adaptive immune system and mediators such as cytokines, chemokines and other molecules, leading to a decrease in circulating lymphocytes (Cook et al., 2007; Cardinale et al., 2011; Hogan et al., 2011). Apparently, T-cells are the most affected, whereas B-cells numbers change little (Hogan et al., 2011). A progressive suppression of the immune response during the first week after surgical trauma contributes to the development of sepsis and the multiorgan dysfunction syndrome (Li et al., 2013a).

Evidence suggests that acupuncture can regulate the immune system (Takahashi et al., 2009; Cho et al., 2017). In humans, electroacupuncture (EAP), an application of electrical current on acupuncture needles, is reported to be effective in enhancing immune function by alleviating immunosuppression of patients during (Li et al., 2013a) and after surgery (Cho et al., 2017; Li et al., 2013a). Similarly, in laboratory species, EAP resulted in a decrease in lymphocyte apoptosis induced by surgical trauma (Wang et al., 2005) and in a restoration of suppressed lymphocyte proliferation (Cheng et al., 1997; Li et al., 2013a). Electroacupuncture appears to reduce immunosuppression of both humoral and cellular components by contrasting the decrease in IgM and IgA levels as soon as 2 hours after anesthesia (Li et al., 2013a).

The present study aimed to explore the effect of EAP on the immune system and immune cell populations in healthy dogs undergoing ovariectomy. In particular, leukocytes count (WBC) and neutrophils, monocytes, T- (cytotoxic and helper) and B-cells, and IgM and IgA levels were evaluated at three time points within 2.5 hours from induction of anesthesia in dogs treated with peri-operatively EAP and compared with those of untreated dogs. Furthermore, a complete blood cell count (CBC) was performed at each time, also red cells and platelet parameters were evaluated.

We expected that EAP could restrain the immunosuppression that develops during the perioperative period by harmonizing cellular and humoral immune responses.

Materials and Methods

This study was approved by the Institutional Ethical Committee for Animal Care of Università degli Studi di Milano, Italy (OPBA_56_2023). Written consent acquisition by the owner was mandatory to participate to the research.

Animals

Twelve healthy bitches (ASA status I–II) undergoing elective ovariectomy were randomly (www.randomizer.org) assigned either to the electroacupuncture (EAP, n=6) or to control (CTR, n=6) group. Bitches were both purebred dogs (American Staffordshire n=3; Bouledogue n=1; Hound n=1; Schnauzer n=1) and mongrels (n=6), aged from 9 months to 2 years (1.4±0.4), weighted 7.8 kg to 25 kg (18.2±5.5) and had body condition score (BCS) 2/5 to 3/5 (2.6±0.5).

Dogs with ASA status > II, aged more than 3 years, that underwent previous anesthesia and surgery and that were administered pharmacological therapy within 6 months prior to ovariectomy, or that were otherwise unhealthy were excluded from the present study.

Anesthetic protocol

Anesthetic protocol was the same in both groups (EAP and CTR). Dogs were fasted for 8 hours, and water was withheld for 2 hours before the beginning of the study. Dogs were premedicated with intramuscular methadone (Semfortan, Dechra Veterinary Products, Italy) at 0.3 mg/kg. After 20 minutes, an intravenous (IV) catheter was aseptically placed into a cephalic vein and general anesthesia was induced with IV propofol (Proposure; Merial Italia S.p.A., Italy) at 2.5 mg/kg, in combination with dexmedetomidine (Dexdomitor, Vetoquinol S.r.l., Italy) administered IV at a dose of 2 mcg/kg, as co-inducer and supplementary analgesic drug. After orotracheal intubation, general anesthesia was maintained with isoflurane (Isoflo, Esteve S.p.A., Italy) in oxygen (100%) titrated to effect in order to obtain a plane of anesthesia that maintained a ventral eye globe position, absence of palpebral reflex and relaxed jaw tone. During anesthesia, IV lactated Ringer's solution (Ringer Lattato; Fresenius Kabi, Italy) was given at 5 mL/kg/h and IV cefazolin (Cefazolina, Teva, Italy) 25 mg/kg was administered 20 minutes before surgery. During the anesthesiologic and surgical periods, the animals were continuously monitored for respiratory rate, heart rate, electrocardiogram, oxyhemoglobin saturation, end-tidal CO2 concentration and non-invasive arterial blood pressure using a multiparameter monitor (Datex Ohmeda S5, GE Healthcare, Italy). Post-operative pain was further controlled by a non-steroidal anti-inflammatory drug, meloxicam (Metacam; Boehringer Ingelheim, Germany), administered subcutaneously at 0.2 mg/kg upon awakening, according to standard practice. Surgical and anesthetic procedures were performed routinely by the same operators and their duration was recorded.

Electroacupuncture protocol

In EAP group, acupoints stimulation began 20 minutes after general anesthesia induction, i.e. 15 minutes before the start of surgery, and lasted until the last skin suture was placed. An electronic acupunctoscope (WQ-6F(57–6F), Beijing Haidian, China) was used. Dogs in CTR group were kept inducted as long as those in the EAP group; for both groups surgery started 35 minutes

after general anesthesia induction. Selected acupoints were Kong Zui or Lung 6 (LU6), Hegu or Large intestine 4 (LI4), Zusanli or Stomach 36 (ST36), Xuan Zhong or Gall bladder 39 (GB39), Ge Shu or Bladder 17 (BL17), and Shenshu or Bladder 23 (BL23).

Needles (0.30 x 50 mm; Hwato, GMT2000, Laveno, Italy) were inserted at a depth of 15-20 mm. A frequency of 16 Hz and 0.4 V was applied to all acupoints except BL17 and BL23 stimulated with 43 Hz and 0.1 V.

Sampling and analysis of immune cells

Three blood samples were taken in both groups at the time of anesthesia induction (T0), 1 h after T0 (T1), i.e. 40 min from the start of EAP and 25 min after the start of surgery, and 2.5 hours after T0 (T2), i.e. after the end of the surgery. The blood samples were divided into two aliquots of 1.5 mL each: one was put in EDTA-containing tubes for hematological and flow cytometric analysis, the other was centrifugated at 4 °C for 5 min and the serum used for immunoglobulin titration. For each sample, a complete blood cell count (CBC) was performed with an automated hematology analyzer equipped with the veterinary software (Sysmex XN-V, Sysmex corporation, Kobe, Japan) and blood smear was prepared to perform leukocyte differential and platelet estimation. In order to further quantify leukocyte subclasses, flow cytometry (FC) was performed on each sample. All samples were processed according to already published protocols (Meazzi et al., 2022), acquired with a Bricyte E6 flow cytometer (Mindray, Shenzhen, China) and analyzed with the specific software MRflow (Mindray) by a single experienced operator (VM). A panel of five antibodies was applied, including anti-CD11b (neutrophils and monocytes), anti-CD5 (T-cells), anti-CD8 (cytotoxic T-cells), anti-CD4 (Helper T-cells), and anti-CD21 (B-cells) (Meazzi et al., 2022). Both CBC and FC were performed within few minutes from sampling.

Serum IgM and IgA levels were determined using specific ELISA kits (BT LAB Bioassay Technology Laboratory, Jiaxing, Zhejiang, China) based on the sandwich approach. Expressed as coefficient of variability (CV), the declared intra-assay precision was <8% and the declared inter-assay precision was <10%.

Statistical analysis

The number of animals included in the study (n=12 that is, n=6 dogs per each group EAP and CTR) represents the minimum number of replications necessary to highlight a statistically significant effect of the treatment. The sample size was calculated with G*Power 3.1.9.4.

Descriptive statistics are expressed as means (± sd). Data were analyzed using a commercial statistical software (IBM SPSS, 28.0) comparing analyzed different parameters in the experimental groups at each time point with non-parametric test U- Mann-Whitney, since data were not normally distributed (Shapiro-Wilk test). The effect of time in treated and control groups on different measured parameters was assessed with a non-parametric Friedman ANOVA test and pairwise comparison. Statistical significance was accepted at $P<0.05$.

Results

All surgeries and recoveries had a regular course. No peri-operative complications occurred in any dog. Dogs in the two groups had similar age (EAP: 1.6±0.5 years; CTR: 1.5±0.3), body weight (EAP: 17.8±3.1 kg; CTR:18.5±7.4) and BCS (EAP: 2.5±0.5; CTR: 2.6±0.5) as well as the same surgical (EAP: 40.8±11.9 min; CTR: 40.8±13.6 min) and anesthetic, i.e. from induction to extubation, (EAP: 86.7±4.5 min; CTR: 84.7±16) times. No statistical differences in these parameters were recorded between the groups.

Hematological parameters concerning erythrocytes (RBC) and platelets (PLT) showed no differences between groups and time points (Table 1). Conversely, leukocytes count (WBC) was significantly lower in CTR than EAP group at all time points ($P \leq 0.03$). They decreased soon after induction (at T1) in both groups but restored at T2 in EAP group while remained significantly lower than T0 in the CTR group (Fig. 1; $P<0.02$). Myeloid cells (neutrophils and monocytes) increased at T2 compared to T0 in dogs undergoing EAP ($P=0.005$) but not in CTR group (Fig. 2). On the contrary, 2.5 hours after induction T-cells decreased in CTR group ($P<0.04$) but not in EAP group (Fig. 3). Cytotoxic T-cells ($P=0.03$) and B-cells ($P<0.04$) decreased in EAP group at T2 compared to T0 but not in CTR group (Figs. 4 and 5). Helper T-cells did not show variations between groups and over time. No significant differences in IgM and IgA concentrations were recorded between groups and over time. However, a decreasing trend of IgM levels was observed in EAP compared to CTR group (Fig. 6). On the contrary, EAP dogs tended to have higher IgA values than CTR dogs even without statistical significance (Fig. 7).

Discussion

Immunosuppression due to surgery and anesthesia is a current and debated topic with many aspects still unknown in both human and veterinary medicine. Most publications report medium- and long-term effects on the immune system (days, weeks) (Mizutani et al., 1996; Cook et al., 2007; Zhou et al., 2022), while few studies target the very early perioperative period (Navarro et al., 1988; Lachmann et al., 2018). The present study focused precisely on this period which may account for some unexpected results. We only included elective ovariectomy as a routine surgery performed on healthy dogs that allows for standardized clinical trials. In fact, the surgical technique together with its duration and extent are closely related to the degree of immunosuppression (Hogan et al., 2011). Moreover, inclusion of dogs with different diseases and undergoing different surgical procedures would have biased the results. In our study the two groups (EAP and CTR) were equivalent in terms

of surgical and anesthesiologic procedures and exposition besides in size, age, body weight and BCS, making them suitable to compare and evaluate the effects of electroacupuncture.

To date, the detailed mechanisms of action of EAP are still unclear. Acupuncture is reported to be effective in regulating nonspecific immune function (Peng 2008) as well as cellular and humoral immunity (Liu et al., 2010; Matsubara et al., 2010). In general, acupuncture immunomodulation acts by stimulating the somatic-autonomic-immune reflexes that include the somatic-sympathetic-splenic reflex, the somatic-sympathetic-adrenal reflex, the somatic-vagal-splenic reflex and the somatic-vagal-adrenal reflex leading to a systemic involvement (Pan et al., 2021). Furthermore, the stimulation of peripheral nerve due to the insertion of an acupuncture needle induces a traumatic inflammation also responsible for a local immunomodulation at the acupoint level (Cabioğlu and Cetin 2008).

The most intriguing aspect of acupuncture recently arose in the literature is its bidirectional effect aimed to maintain the homeostasis by balancing hyper- and hypo-functional states (Pan 2019; Lee et al., 2021). It refers to the ability of acupuncture to act by both activating and inhibiting the same mechanism that relies on the body's self-healing (Pan et al., 2021). This is a unique aspect of acupuncture that no specific drug has achieved so far.

An initial pro-inflammatory effect is reported to occur as a result of surgical stress and tissue damage, which is followed by a compensatory immunosuppressive response aiming to facilitating the resolution of inflammation and protecting against excessive systemic consequences of the primary insult (Alazawi et al., 2016; Lachmann et al., 2018). The decreasing trend of WBC observed in both EAP and CTR groups within 1 hour from general anesthesia induction can be interpreted as an early sign of immunosuppression. The latter appeared to persist until the end of monitoring, i.e. 2.5 hours after induction, in CTR ($P<0.02$) but not in EAP group, with the number

of leucocytes significantly lower than pre-surgically. Furthermore, the percentage of neutrophils and monocytes increased in EAP dogs after 1.5 hours (P=0.005) while remained low in CTR dogs. Innate immunity is described to restore early during postoperative recovery taking 2 to 3 days for neutrophils and monocytes, respectively (Lachmann et al., 2018). EAP seems to be able to shorten this time.

In accordance with a suppression of acquired immunity lasting several days postoperatively (Lachmann et al., 2018) and characterized by apoptotic reduction in the number of T-cells (Alazawi et al., 2016), we recorded a decrease in T-cells in the CTR group (P<0.04). The concentration of T-cells in the EAP group, however, remained unchanged over time, suggesting a possible counteracting action due to EAP treatment. On the other hand, B-cells and cytotoxic T-cells decreased in EAP dogs (P<0.04) but not in CTR group. Since acquired immune cells are reported not to recover until 5 days from surgical insults (Lachmann et al., 2018), it is possible that the effect of EAP may be noticeable after such period, not earlier. Moreover, a modulatory action of EAP on the type 1 (Th1) and type 2 (Th2) T helper cells is reported (Yamaguchi et al., 2007; Silvério-Lopes and da Mota 2013; Dai et al., 2018;; Wang et al., 2023). EAP can both stimulate and downregulate Th1 and Th2, that are responsible for pro-inflammatory and anti-inflammatory responses, respectively (Park et al., 2004; Lin et al., 2014; Pan et al., 2021; Wang et al., 2023). In the present study we have not performed a distinction between Th1 and Th2 T helper cells, therefore it is not possible to argue about them. Finally, EAP stimulation at Zusanli (ST36) acupoint in rats increases lymphocyte proliferation after surgery (Cheng et al., 1997; Cao 2001; Wang et al., 2009) while in mice suppresses Th2 cytokine IL-4 (Park et al., 2014). To the authors' knowledge, to date no studies in dogs have been published on this aspect, and a species-specific modality of EAP can only be speculated.

The few data in the literature on immunoglobulin are conflicting. Some authors reported no differences in IgM and IgA concentrations between groups and over time (Cabioğlu et al., 2007) while a study on the effect of EAP on postoperative immunoinflammatory response in human patients undergoing craniotomy showed that the blood IgA decreased significantly in control group 4 hours after induction of anesthesia and one day after surgery, but no significant differences were noted between control and treated groups (Li et al., 2013b). The same research group soon after noted that electroacupuncture was able to alleviate intraoperative immunosuppression in the same type of human patients: in that case, both IgM and IgA decreased significantly in control group compared with treated groups 2 and 4 hours after induction of anesthesia, while no significant differences between groups were noted for IgG (Li et al., 2013a). In our study, dogs treated with EAP showed a decreasing trend of IgM levels and an increasing trend of IgA levels compared to the CTR group. It should be noted that all dogs except one in the CTR group resulted below the expected values for IgM in canine species (0.7-2.7 mg/mL) (Tizard 2017). On the contrary, IgA levels were always within the expected range (0.2-2.5 mg/mL) in both groups (Tizard 2017). The degree of dogs immunization, and particularly few contacts with new antigens never encountered before (which would have resulted in the production of IgM), could have affected our results, but these are aspects that we did not take into account in this study.

At last, even anesthetic drugs can have an impact on the immune function such as opioids which are mediated indirectly by activation of the HPA axis and sympathetic nervous system, and by a direct effect on many subtypes of immune cells (Hogan et al., 2011). Recent review studies underlined conflicting conclusions reporting immunosuppressive, immunostimulatory, or dual mechanisms for opioids (Liang et al., 2016). Morphine is known to suppress a variety of immune functions including T lymphocytes proliferation (Liang et al., 2016). The use of methadone to premedicate dogs in our study can justify the significant decrease in T-cells observed in the CRT

group. Conversely in EAP group, endogenous endorphins releasing due to EAP stimulation may have acted as competitive agonists thus reducing the suppressive effect of methadone.

The small sample size of our caseload is a limitation that cannot be neglected before generalizing these findings. In addition, although we included a standardized population in order to avoid biases, it is possible that different anesthetic or surgical procedures might bring to different results. Moreover, we monitored hematological and immune parameters only for 2.5 hours after induction, and effects on longer periods could be quite different.

Conclusions

The results of this exploratory study suggest that EAP may influence the immune response in dogs undergoing elective ovariectomy. EAP appears to reduce the immunosuppression through a modulatory effect which is expressed early on neutrophils, monocytes and T-cells. EAP also seems to shorten the time of immune system restoration after surgery. EAP due to a non-pharmacological and non-invasive approach, is an attracting and promising therapy to reduce immunosuppressive perioperative risk in dogs. In any case, a larger-scale randomized controlled trial also including a longer postoperative period is advisable to confirm these promising results.

Conflict of interest statement

None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Figure legends

Fig. 1. Leukocytes count in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P<0.02.

Fig. 2. Concentrations of myeloid cells in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.005.

Fig. 3. Concentrations of the T-cells in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.038.

Fig. 4. Concentrations of the cytotoxic T-cells in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.03.

Fig. 5. Concentrations of the B-cells in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.036.

Fig. 6. Concentrations of IgM in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0.

Fig. 7. Concentrations of IgA in EAP and CTR groups over time (mean ± sd). T0: time of anesthesia induction, T1: 1 h after T0; T2: 2.5 hours after T0.

Table 1. Hematological parameters in EAP and CTR groups at each time point

	RBC x106/µL			**WBC** X103/µL			**Hb** g/dL			**Ht** %			**PLT** x103/µL			**MCH** pg			**MCHC** g/dL			**MCV** μ^3			**RDW** %			**MPV** μ^3		
ID.	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
EAP1	5.8	6.4	6.8	14.8	14.0	18.5	12.9	14.2	15.2	37.6	41.9	44.4	385.1	243.2	372.1	22.2	22.2	22.3	34.1	33.9	34.5	64.4	65.4	64.2	16.9	18.4	18.7	10.3	10.5	10.1
EAP2	6.2	5.3	5.5	18.4	16.4	14.2	15.1	19.2	19.5	44.1	51.5	58.8	438.3	362.2	422.5	24.5	24.4	24.5	35.2	35.9	35.2	71.6	72.2	69.4	19.2	16.3	13.2	11.1	11.1	9.6

EAP3	7.5	6.1	6.5	1	1	1	1	1	1	5		4	2	2	2	2	2	2	3	3	3	6	7		2	1	1	1	1		
				3	3	2	8	4	5	0	4	5	1	3	3	4	4	4	6	3	4	7	1	7	0	7	8	1	2	1	
				.	.	.	.	.	.	.	4	.	3	6	3	.	.	.	.	.	.	.	.	0	.	.	.	.	.	2	
				5	7	6	3	8	7	6		6				4	1	1	2	6	4	4	8		3	9	5	7	4		
EAP4	7.6	5.6	7.4	1	1	1	1	1	1	4	3	4	2	1	2		2	2	3	4	3		6	6	1	1	1	1	9	1	
				4	4	3	7	5	6	9	8	7	1	9	3	2	7	3	5	0	4	6	9	7	9	5	8	0	.	1	
				.	.	.	.	.	.	.	.	.	1	0	7	3	.	.	.	.	.	5	.	.	.	.	.	.	7	.	
				8	5	4	5	6	4	4	8	8					9	3	4	2	3		3	8	8	6	9	5		8	
EAP5	7.2	6.6	6.1	1	1	1	1		1	4	4	4	2	1	1		2	2		3	3	3	6	6	6	2	1	1	1	1	1	
				9	6	5	6	1	4	6	1	1	0	9	8		2	3	2	5	3	3	4	8	8	0	8	8	1	1	1	
				.	.	.	.	4	.	.	.	.	1	2	4		.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	
				5	2	2	4		1	1	6	7					9	2		6	7	8	4	9	1	2	2	1	6	4	6	
EAP6	7.2	5.9	7.3	1	1	1	1	1	1	4	4	4	1	1	1		2	2	2	3	3	3		6	6	2	1	1	1	1	1	
				5	3	2	6	3	6	1	0	9	6	3	6		3	3	3	5	4	4	6	8	8	0	8	9	3	2	2	
				.	.	.	.	.	.	.	.	.	4	8	3		.	.	.	.	.	.	6	.	.	.	.	.	.	.	.	
				4	7	3	8	8	9	5	2	5					3	5	3	4	3	2		6	2	9	2	9	5	6	4	
CTR1	7.9	6.9	7.7	1	1	1	1	1	1	5	4	5	3	1	2		2	2	2	3	3	3	6	6	6	2	1	2	1	1	1
				3	0	1	9	6	8	3	7	2	1	7	7		4	4	4	6	5	5	7	9	7	0	8	0	1	1	1	
				.	.	.	.	.	.	.	.	.	1	2	8		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
				1	2	9	6	9	8	5	9	4					6	6	5	6	3	9	1	7	3	1	7	1	3	9	3	
CTR2	8.7		7.1	1	1	1	1	1	1	5	4	4	1	1	1		2	2	2		3	3	6	6		2	2	2	1		1
				3	1	0	9	7	7	4	7	6	7	7	6		4	4	4	3	5	6	7	7	6	1	0	0	2	1	3	
				.	.	.	.	.	.	.	.	.	0	4	4		.	.	.	6	.	.	.	.	6	.	.	.	.	3	.	
				1	6	2	6	1	2	4	7	7					4	3	3		8	8	7	8		1	2	1	9		1	
CTR3	6.7	5.4	5.7	1				1	1	4	3	4	2	2	1		2	2	2	3	3	3	7	7	7	1	1	1	1	1	1
				1	8			1	3	3	7	9	0	3	1	3	3	4	3	3	3	2	1	2	0	9	6	7	2	2	2
				.	.	8		.	.	.	.	.	.	0	2	9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
				4	2			1	2	9	4	1					8	1	3	4	2	9	2	6	7	1	3	8	3	6	3	
CTR4	6.8	6.4	6.6	1	1	1	1	1	1		4	4	2	2	2		2	2	2	3			6	6			1	1	1		1
				2	0	0	5	4	4	4	2	1	7	8	5		2	2	2	4	3	3	4	5	6	2	9	9	1	1	1	
				.	.	.	.	.	.	4	.	.	6	8	8		.	.	.	.	4	6	.	.	2	0	.	.	.	2	.	
				7	6	9	3	3	8		1	1					5	3	3	8			6	7			6	6	6		2	
CTR5	7.7	6.2	6.5	1			1	1	5	4	4	1	1	1	1		2	2	2	3	3	3	6	6	6	1	1	1	1		
				2	1	1	8	6	1	2	5	5	9	9	9		3	3	3	4	5	5	8	7	7	9	8	8	2	1	1	
				.	3	0	.	.	6	.	.	.	3	2	9		.	.	.	.	.	.	.	.	.	.	.	.	.	3	3	
				7			3	2		7	9	6					7	9	7	7	3	1	4	6	7	9	8	3	8			
CTR6	6.8	5.4	5.6	1	1	1	1	1	1	4	4	4	2	3	2		2	2	2		3	3	7	7	7	2	1	1	1	1	1
				4	2	1	7	4	4	7	1	1	8	1	8		5	5	5	3	4	4	0	3	4	0	7	6	1	1	1	
				.	.	.	.	.	.	.	.	.	4	0	7		.	.	.	6	.	.	.	.	.	.	.	.	.	.	.	
				5	1	4	2	4	1	8	6	3					4	4	3		6	1	5	4	1	4	8	8	5	7	2	

RBC: total red blood cell count, WBC: total white blood cell count, Hb: hemoglobin, Ht: hematocrit, PLT: platelet count, MCH: mean cell hemoglobin, MCHC: MCH concentration, MCV: mean cell volume, RDW: red cell distribution width, MPV: mean platelet volume.

T0: at the time of induction of anesthesia, T1: 30 min after T0, T2: 2 hours after T0

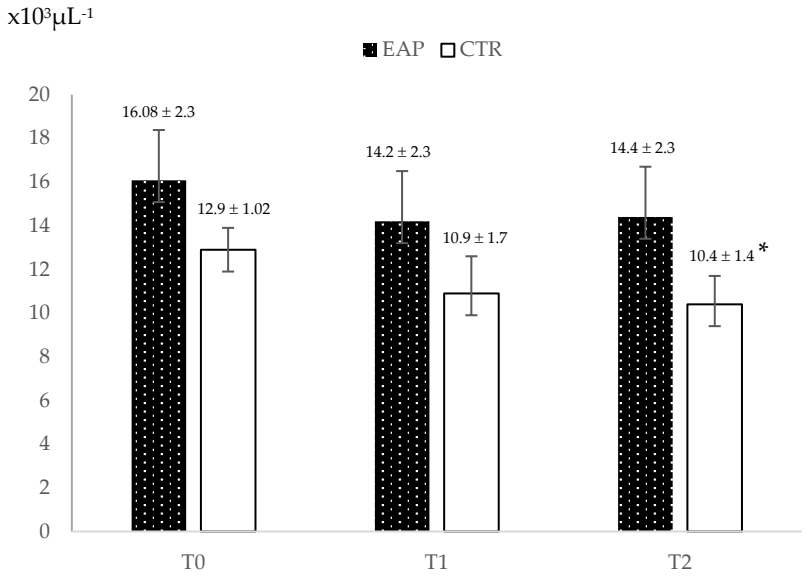

Figure 1. Leukocytes count in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P<0.02.

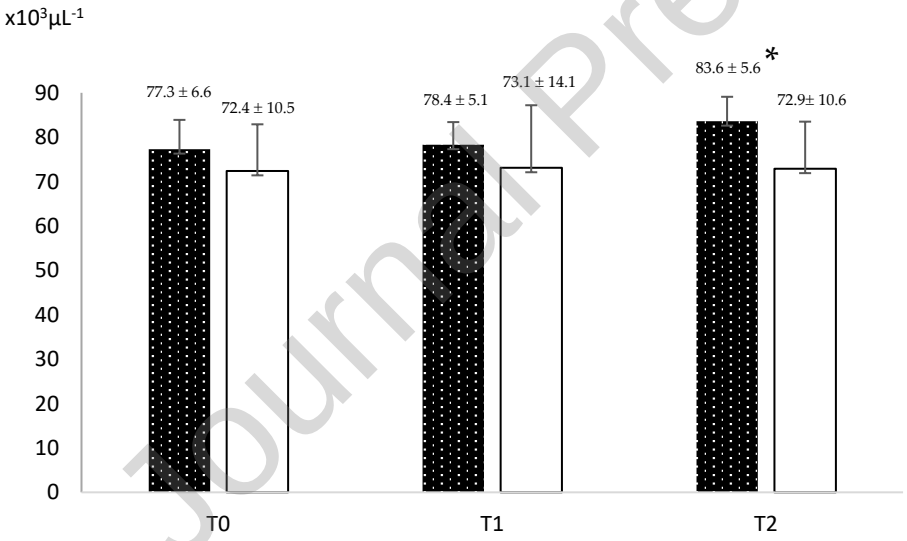

Figure 2. Concentrations of myeloid cells in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.005.

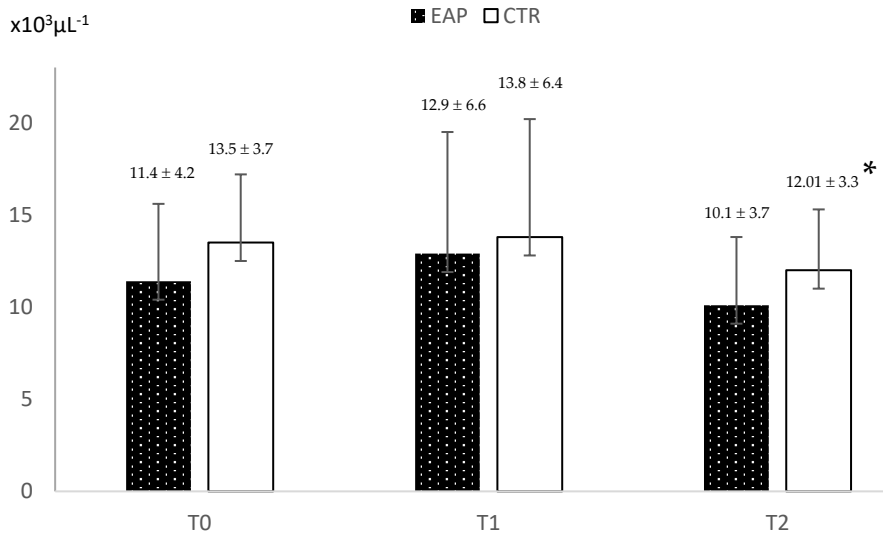

Figure 3. Concentrations of the T-cells in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.038.

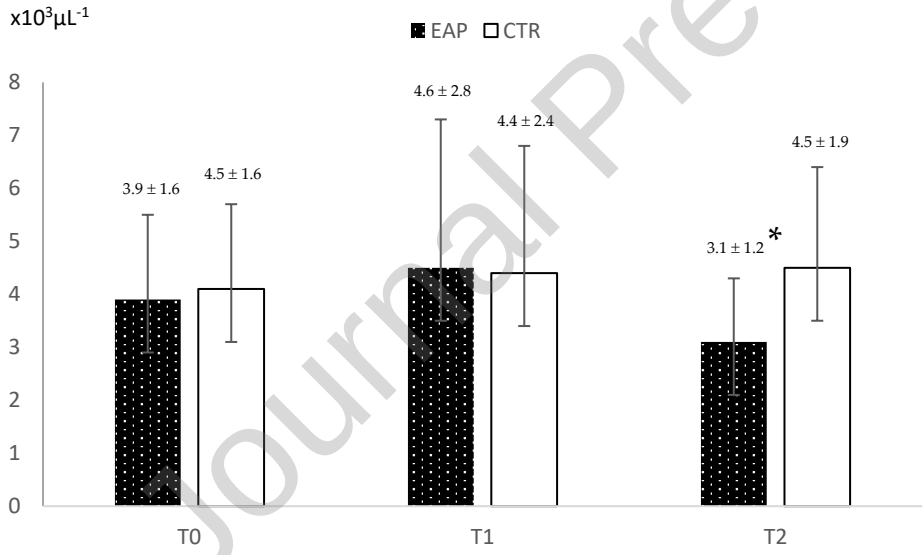

Figure 4. Concentrations of the cytotoxic T-cells in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.03.

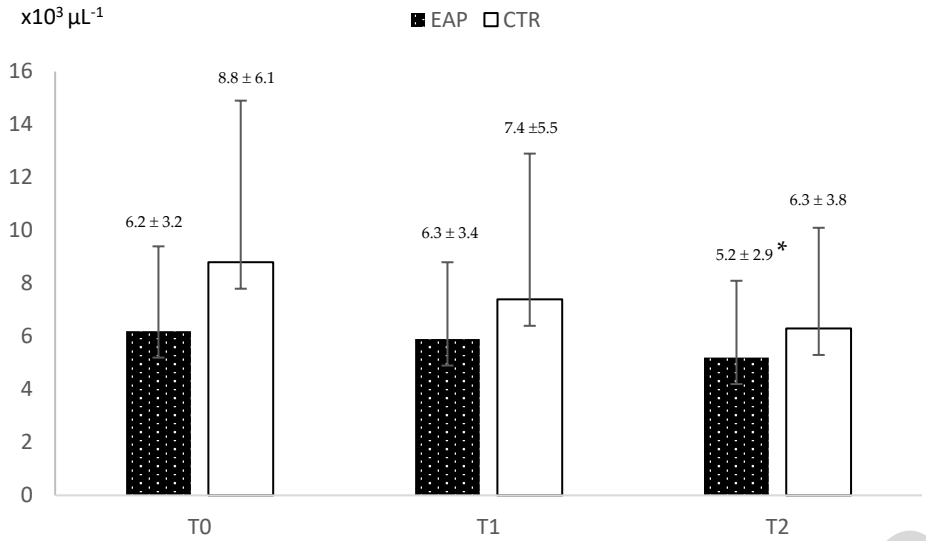

Figure 5. Concentrations of the B-cells in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.036.

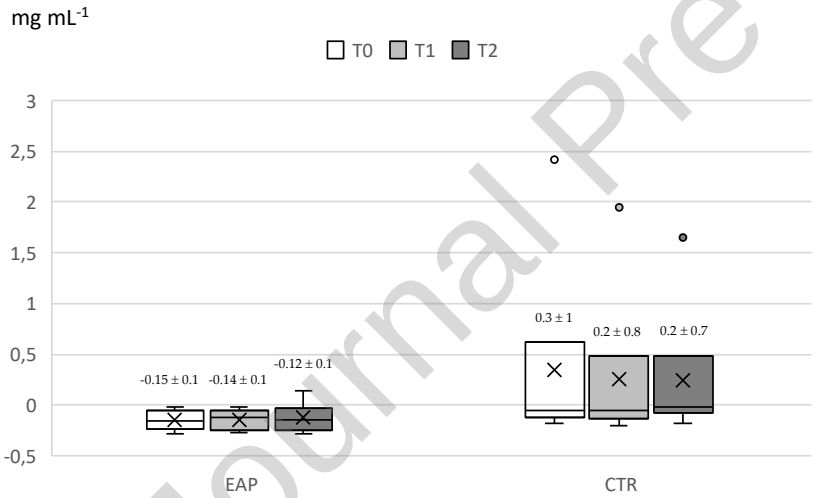

Figure 6. Concentrations of IgM in EAP and CTR groups over time (mean ± sd). T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0.

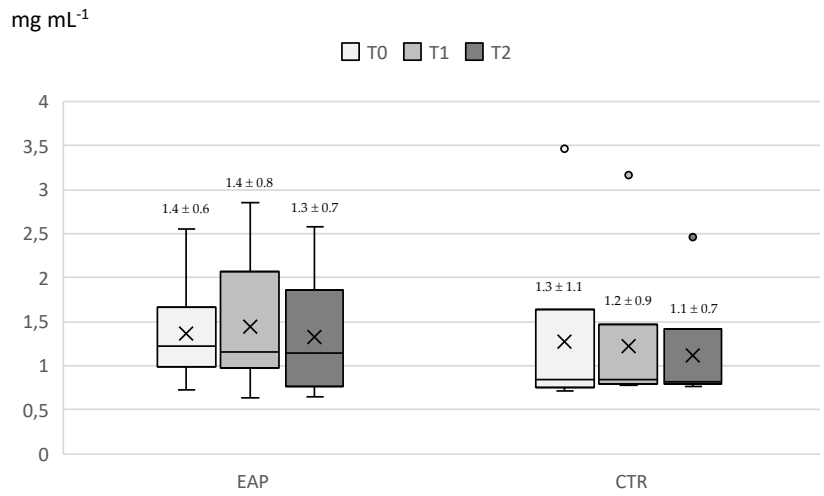

Figure 7. Concentrations of IgA in EAP and CTR groups over time (mean ± sd). T0: time of anesthesia induction, T1: 1 h after T0; T2: 2.5 hours after T0.

Highlights

The early effect of electroacupuncture acts on neutrophils, monocytes and T-cells

Electroacupuncture can modulate the response of the immune system

Electroacupuncture shows promise in reducing perioperative immunosuppression