# UNIVERSITA' DEGLI STUDI DI MILANO

**PhD Course in Veterinary and Animal Science**

**Department of Veterinary Medicine and Animal Sciences**

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**Class XXXV**

# ANAESTHESIA AND PAIN MANAGEMENT IN COMPANION ANIMALS

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# Summary

# 1. Introduction

## 1.1 Physiology of Pain

The International Association for the Study of Pain (IASP) has defined pain as "*an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage*" and recently added that "*the inability to communicate in no way negates the possibility that an individual is experiencing pain and is in need of appropriate pain relieving treatment*"[1]. Pain is a uniquely individual experience in humans and animals and its mechanisms serve as a natural protective function of organisms against noxious stimuli by changing the physiology and behavior to reduce or avoid further damage[2].

Nociception, or the normal processing of noxious stimuli, involves the transduction, transmission and central nervous system modulation of nociceptive signals[3]. During transduction, a noxious stimulus (mechanical, thermal or chemical) is converted into an electrical impulse which is propagated through nerve fibers (mostly myelinated Aδ or unmyelinated C-fibers) or the first-order neurons leading from the peripheral nociceptors to the spinal cord. The receptive properties of these sensory fibers are determined by their expression of transducing ion-channel receptors, which have a high threshold of activation to external stimuli. Unmyelinated C-fibers are activated by intense mechanical, chemical and thermal stimuli contributing to the "slow burn" sensation of pain. The Aδ fibers transmit impulses more quickly and contribute to the rapid onset of the acute pain response, resulting in rapid and protective withdrawal from the noxious stimulus. Modulation take places mainly in the dorsal horn of the spinal cord where the first-order neurons synapse with second-order neurons[3]. Nociceptive input can be amplified or attenuated at this site by a number of neurotransmitters released from close neurons and descending pathways. Second-order neurons project from here to third-order neurons in supraspinal structures in the ascending pathway of the spinal cord. The third-order neurons link to the cerebral cortex where further processing results in perception[3]. The conscious, cognitive elaboration of nociception results in pain, that is the endpoint of a complex information-processing network and can only occur in a conscious animal[4,5]. However, there is also the involvement of autonomic pathways and deeper centers of the brain involved with emotion and memory; hence pain results in a complex and multi-dimensional experience[3]. Pain perception is the affective component, which is the experience associated with either actual or potential tissue damage[6] (Figure 1).

All tissue injury, including that from surgery, may cause pain. Pain-induced stress responses, mediated by the endocrine system, are one of the negative consequences of pain. Increased cortisol, catecholamines and inflammatory mediators cause physiologic changes, such as tachycardia, vasoconstriction, decreased gastrointestinal motility and delayed healing. In addition, this condition may cause changes in the central nervous system. In animals, the inadequate pain prevention and management can lead to a prolonged pain state and to a magnification of pain perception[7].

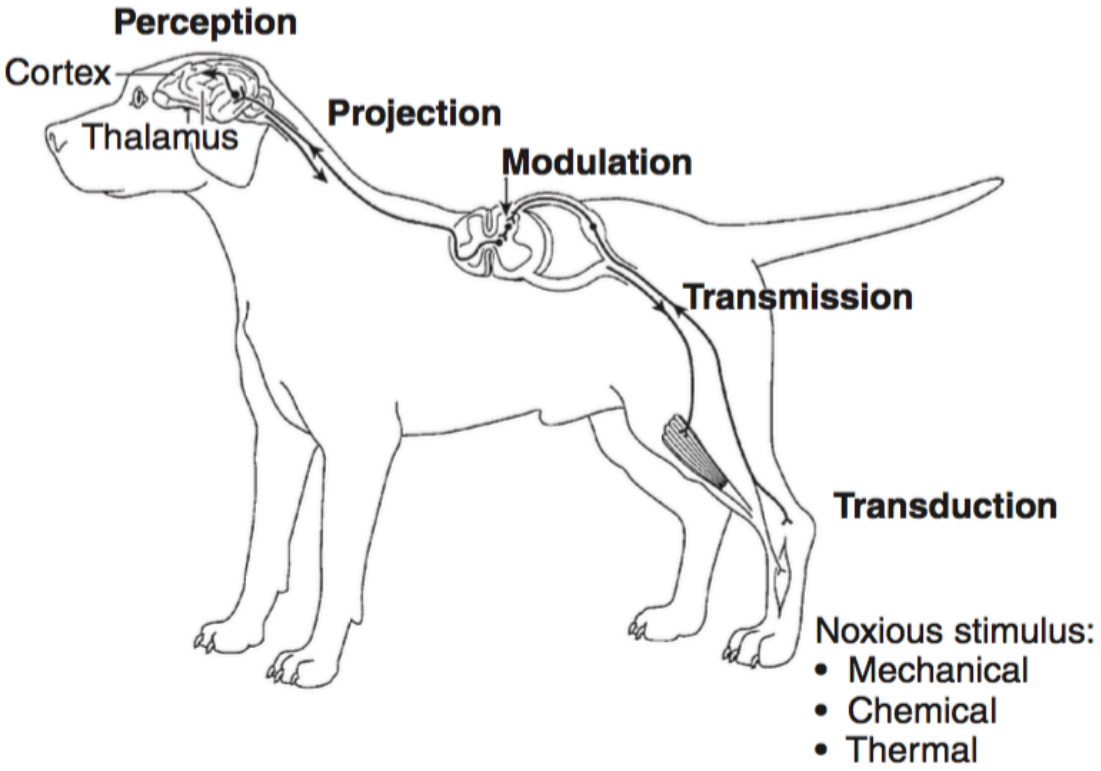


**Figure 1.** Pathways involved in nociception. Noxious stimuli are transduced (transduction) into electrical signals that are transmitted (transmission) to the spinal cord, where they are modulated (modulation) before being relayed (projection) to the brain for final processing and awareness (perception) (*From: Gaynor et al. 2015, Handbook of Veterinary Pain Management*)

Although traditionally pain has been classified as acute or chronic based on duration, a more contemporary approach considers it as adaptive or maladaptive[8]. Adaptive pain includes inflammatory pain: inflammation is the major component of many pain states (including acute pain following trauma or surgery) and some chronic pain states such as osteoarthritis. Inflammatory mediators sensitize neural pathways, increasing the individual perception of pain[7]. If adaptive pain is not properly managed, physical changes occur in the central nervous system, leading to

maladaptive pain. Actually, the thalamus typically serves as a station sending nerve impulses from the periphery to the cortex but may become a spontaneous pain generator if adaptive pain becomes maladaptive. These pain-induced changes in the central nervous system cause it to become more sensitive and these neuro-physiologic processes promote a switch from adaptive to maladaptive pain, which is more serious and difficult to control[7]. Maladaptive pain is characterized by hyperalgesia (excessive response to noxious stimulus), allodynia (painful response to non-noxious stimulus, such as touch or pressure) and pain protracted beyond the expected time of inflammation and healing. Under these conditions, genomic and phenotypic changes can occur and create neuropathic pain, whereby pain can be considered a disease of the central nervous system[6].

## 1.2 Adaptive Pain

Adaptive pain, or physiological pain, occurs after most types of noxious stimulus and is usually protective. It is usually short-acting, relatively easy to treat and plays an adaptive role as part of the body's normal defensive mechanisms. This type of pain includes inflammatory pain, often categorized with acute pain as "nociceptive". Inflammatory pain results gradually from activation of the immune system in response to injury or infection[6]. The inflammatory process is mediated by the local release of different chemicals, including bradykinin, prostaglandins, leukotrienes, serotonin, histamine, substance P, thromboxanes, platelet-activating factor, adenosine and free radicals. Cytokines, such as interleukins and *tumor necrosis factor*, and neurotropins, especially *nerve growth factor*, are also generated during inflammation[5] (Figure 2).

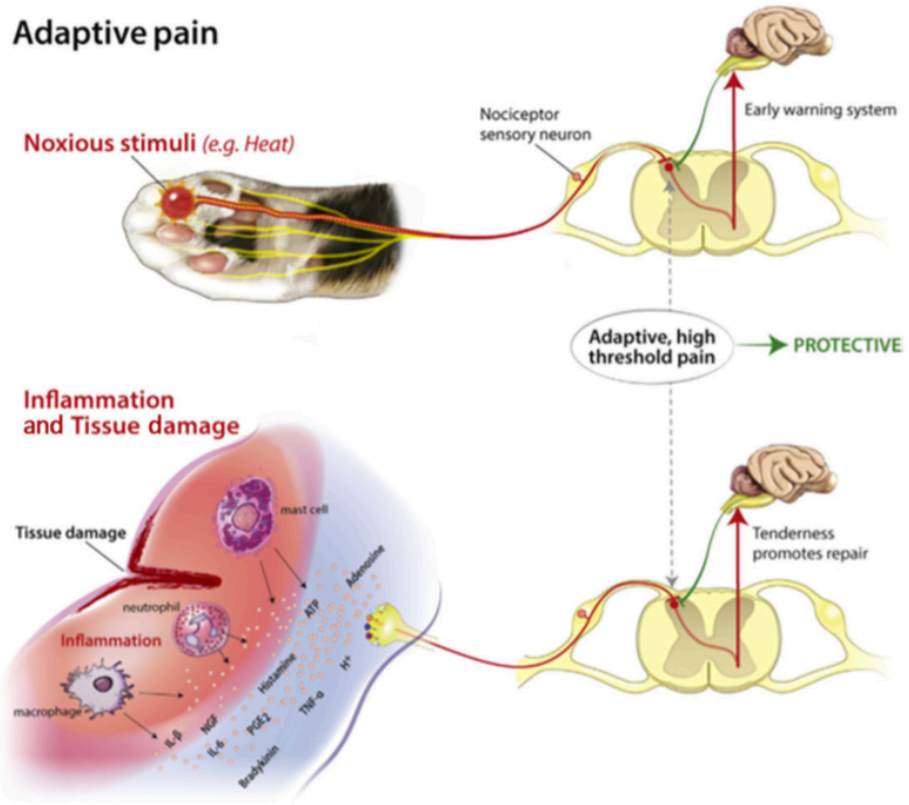


**Figure 2.** Schematic illustration of adaptive pain. A noxious stimulus (red starburst) activated high-threshold primary afferent neurons (red/yellow lines). The nociceptive message is transmitted to second order neurons in the dorsal horn of the spinal cord and then to the brain via ascending pathways in the spinal cord (red arrow). Descending inhibitory controls (green line) from higher brain modulate the nociceptive message in the spinal cord before conscious perception in the brain cortex. In inflammatory pain, local tissue damage results in release of inflammatory mediators which sensitize sensory nerves, resulting in generation of nociceptive signals; they are transmitted by afferent neurons (red line) through the spinal cord and then to the brain (red arrow). Descending inhibitory controls (green line) may modulate the nociceptive message at the spinal cord (*From: Adrian et al. 2017*)[13].

## 1.3 Maladaptive Pain

Maladaptive pain occurs when adaptive pain is amplified and sustained by molecular, cellular and microanatomic changes, collectively termed peripheral and central hypersensitization[6]. If after an acute injury, hyperalgesia and allodynia do not resolve or if a chronic disease process is driving pain, than it can be considered maladaptive[9]. Normally, a steady state is maintained in which there is a close correlation between injury and pain. Long-lasting or very intense nociceptive input or the removal of a portion of the normal input can distort the nociceptive system and the close correlation between injury and pain can be lost[5]. A progression from adaptive to maladaptive pain might be considered as different stages of pain, proposing that multiple neurophysiological mechanisms are involved, depending on the nature and time course of the originating stimulus. If a noxious stimulus is very intense or prolonged, leading to tissue inflammation and damage, it might be influenced by response properties of various components of the nociceptive system changing. These changes note that the central nervous system has moved to a more excitable state as a result of the noxious input generated by tissue injury and inflammation. Inflammation could expand the pool of receptors in nociceptive terminals and increase the nerve responsiveness to stimuli, including the "silent nociceptors"[10]. During their course to the dorsal horn of the spinal cord it is possible that "cross-excitation" occur between neurons, and this could be another mechanism through which peripheral sensitization may occur[11]. Patients experience spontaneous pain and sensation changes evoked by continuous stimulation of the injured and surrounding area (Figure 3). This change is a leftward shift of the stimulus-response curve and it is known as hyperalgesia. Hyperalgesia in the area of injury is known as primary hyperalgesia, while in the areas of normal tissue surrounding the injury site, as secondary hyperalgesia[5]. The increase in dorsal horn excitability exaggerates inputs from nociceptors and elicits responses from Aβ-fibers that normally would not respond. The activity of nociceptive-specific and nonspecific (wide dynamic range) neurons in the dorsal horn of the spinal cord is stimulated by afferent C-fibers and results in temporal summation and cumulative depolarization (known as windup) of synaptic inputs in dorsal horn neurons[5].

In the case of nerve injury, the electrical properties and central connectivity of neurons can change, bringing disorganization on normal sensory processing, and sometimes inducing maladaptive chronic neuropathic pain. This type of pain is attributable to changes that damage the axon or soma of sensory neurons or disrupts the myelin sheath that surrounds axons (dysmyelination and demyelination)[12]. Such changes include alterations in expression of neurotransmitters,

neuromodulators, receptors, ion-channels and structural proteins; some of these changes are involved in the reparative process, but others contribute to neuropathic pain[12].

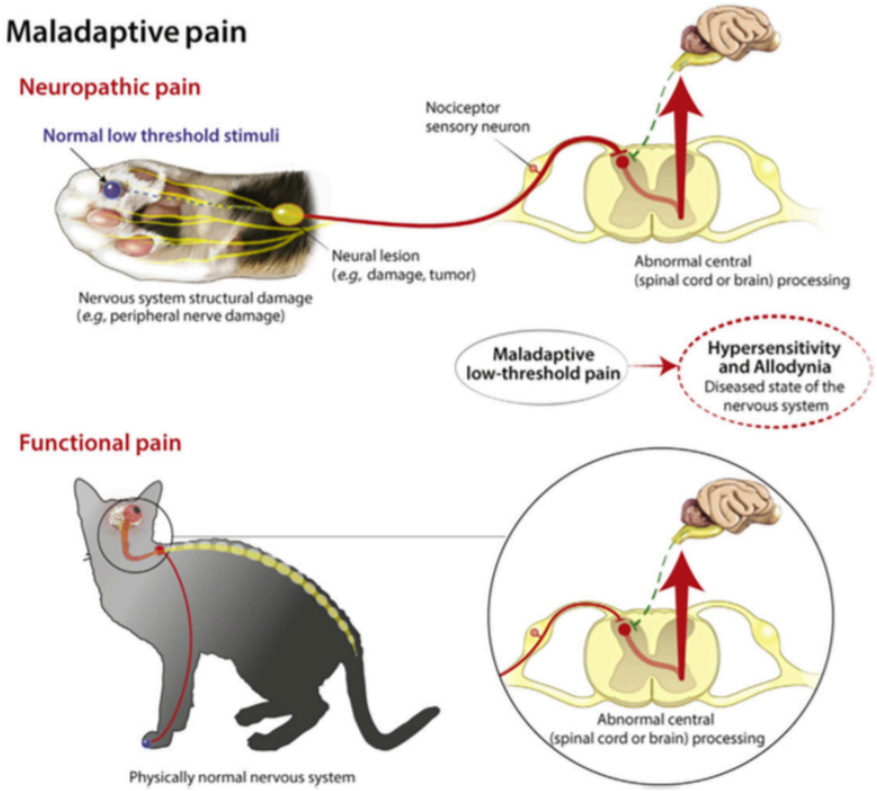


**Figure 3.** Schematic illustration of maladaptive pain. In maladaptive pain, physical damage to nervous system tissues results in abnormal activation of sensory neurons which become activated in response to sub-threshold stimuli. The subsequent changes (nervous system plasticity) occur at the level of the dorsal root ganglion and dorsal horn of the spinal cord, resulting in amplification and facilitation of the nociceptive signals. The descending inhibitory controls (dashed green line) less effective. Hyperalgesia and allodynia occur as a result of these changes. In functional pain, the functioning of nervous system is abnormal. This abnormal central processing results from repeated input to the system, causing nervous system plasticity. Under these conditions, a nociceptive stimulus activates a normal nociceptor (red line) but abnormal central processing in the spinal cord or brain results in the stimulus being interpreted as painful. Hyperalgesia, allodynia and spontaneous pain may occur (*From: Adrian et al. 2017*)[13].

## 1.4 Clinical Pain

Clinical pain results from an altered pain transmission system, due to either adaptive and maladaptive changes; it must be highlighted that most clinical pain conditions reflect a mixture of different types of pain (e.g. inflammatory and pathological types of pain occur simultaneously in pain related to osteoarthritis)[13]. The nociceptive sensory system is an inherently plastic system and when tissue injury or inflammation occurs, the sensitivity of an injured area is enhanced so that both noxious and, sometimes normally innocuous stimuli, are provided as painful. Peripheral sensitization is the result of changes in the environment bathing nociceptive terminals secondary to tissue injury and inflammation. Inflammatory mediators and neurotransmitters are released by damaged cells which either directly activate nociceptors or sensitive nerve terminals. This results in long-lasting changes in the functional properties of peripheral nociceptors. Sensitized and activated nerves also play a role in local inflammation through a phenomenon called "neurogenic inflammation"; collectively, all these changes result in what is called "peripheral sensitization"[14]. Trauma and inflammation can also upregulate nociceptive transmission. Sustained noxious stimuli to the spinal cord and higher centers result in progressive changes in the pain mechanisms and endogenous analgesic system and consequent facilitation and amplification of these signals. The term "central sensitization" describes changes in the spinal cord and at supraspinal levels, such as decreased activity of descending inhibitory noxious controls including endogenous analgesic systems[14]. Central sensitization can occur as a result of surgery[15] and in long-standing painful conditions where there is prolonged input of noxious signals into the central nervous system (e.g. dogs and cats with osteoarthritis or degenerative joint disease[16-17], or dogs with maladaptive neuropathic pain[18]). Central sensitization and maladaptive chronic pain are ubiquitous in companion animals, most commonly as the results of osteoarthritis, whose reported prevalence appears to be close to 40% in dogs and more than 50% in cats[19-20]. Osteoarthritis is a slowly progressive degenerative joint disease characterized by whole-joint structural changes including articular cartilage, synovium, subchondral bone and periarticular components, which can lead to pain and loss of joint function[20]. Central sensitization and maladaptive chronic pain may also be associated to dental, spinal, cancer or neuropathic pain and other chronic conditions[19].

## 1.5 Pain Management

Pain is a complex disease: it is an unpleasant experience involving sensory and emotional components and is unique to each individual[14]. In the last decades, pain management has become one of the most important issues in veterinary medicine. In companion animal practice, it is evolving from a "damage control" to a "proactive" strategy, including the prevention and the rapid detection of pain, combined with early multimodal intervention[14,19]. Preventive analgesia is a clinical approach that refers to the administration of analgesics pre-, intra- and postoperatively[14]. This type of management can also be applied to maladaptive chronic pain, treating earlier in the disease process in order to prevent the adverse effects of ongoing noxious stimuli[19]. It considers factors in all perioperative moments or disease processes that can contribute to peripheral and central sensitization and involves any drugs and analgesic techniques for pain relief[21]. Multimodal analgesia is an integrative approach that consists in the use of pharmacological and non-pharmacological therapies. The concomitant administration of drugs and non-pharmacological treatments that act at different sites of the nociceptive pathway provide the best approach to pain management[19]. Drugs frequently used for preventive analgesia include opioids, non-steroidal anti-inflammatory drugs (NSAIDs), local anesthetics, alpha$_2$-adrenoceptor agonists, N-methyl-D-aspartate (NMDA) antagonists and gabapentinoids. Because they target different pain mechanisms, lower doses of each drug can be administered, minimizing the occurrence of adverse effects. The choice of drug(s) used to treat pain will depend on the underlying cause of pain, its severity and duration. Furthermore, the pharmacokinetic profiles of drugs are likely to be different among adults, pediatrics, seniors and animals with comorbidities, which may alter dosage regimens. Pain control using non-pharmacological therapies is far more advanced in human than in veterinary medicine and consists in a wide variety of procedures, from physical modalities to advanced interventional techniques[22]. However, this approach should be added to the pain management strategy whenever practicable. All measures to reduce stress, fear and anxiety, and to provide positive mental and physical stimulation are also encouraged[19].

### 1.5.1 Pharmacological Management of Pain

Effective pharmacological pain management generally involves a multimodal strategy using several classes of drugs. This approach addresses targeting multiple sites in pain pathways, potentially allowing lower doses of each medication and minimizing the potential for side effects associated with any single drug. The choice of medications should be based on type and level of pain and

individual patient needs. Preventive analgesia, provided prior to pain onset, is more effective than analgesia provided once pain has occurred, contributing to a dose sparing effect[6].

**Opioids** are the most effective drug class for managing acute adaptive pain and can play a role in managing chronic maladaptive pain. They bind to opioid receptors (μ, κ, δ, nociceptin and their subtypes) in the central and peripheral nervous systems, inhibiting release of excitatory neurotransmitters from afferent fibers in the spinal cord and the synaptic transmission of nociceptive stimuli; at a postsynaptic level, enhanced $K^+$ efflux causes neuronal hyperpolarization of spinal cord projection neurons and inhibits ascending nociceptive pathways[23]. Opioids are widely used in the perioperative setting as part of multimodal and preventive analgesia as well as for their anesthetic sparing effects; they are also widely administered in the emergency and critical care setting[14]. Although they are commonly used in the treatment of chronic maladaptive pain in humans[24], there is no published data regarding the clinical efficacy and safety of the long-term use of opioids in veterinary species. Opioid tolerance and opioid-induced hyperalgesia are reported in humans and laboratory animals[25] however, they are not been documented in small animal practice.

**NSAIDs** block the activity of cyclooxygenase (COX) enzymes and the consequent production of prostaglandins or, as in the case of piprant class of drugs (PGE2 receptor antagonists), block the interaction of prostaglandins with their receptors. COX-1 produces prostaglandins involved in physiological processes including gastroprotection, vascular homeostasis, renal blood flow and perfusion and blood clotting; COX-2 is primarily released after tissue injury to produce inflammatory prostaglandins[26]. Individual NSAIDs inhibit COX-1 and COX-2 to different degrees[26]. By inhibiting COX activity, NSAIDs exert antipyretic, anti-inflammatory and analgesic effects, but may also result in adverse effects, by inhibiting physiological functions[14,26]. In small animals practice, COX-inhibiting NSAIDs are effective analgesics in the perioperative period, as well as in other acute and chronic maladaptive pain states such as osteoarthritis, cancer and other chronic inflammatory conditions. They are given as a sole medication or in combination with adjuvant drugs depending on the nature and the severity of pain[26]. When used for chronic maladaptive pain conditions (e.g. osteoarthritis), they are often titrated to the lowest effective dose, but this should be combined with careful patient reassessment[27,28]. The NSAIDs side effects are most commonly related to gastrointestinal tract (vomiting, diarrhea and decreased appetite); these effects are usually self-limiting although ulceration and perforation can occur following inappropriate administration[29]. Other less frequent adverse effects include decreased platelet aggregation and renal and hepatic toxicity[14,30]. NSAIDs are contraindicated in dogs and cats with uncontrolled gastrointestinal, renal or hepatic disease,

coagulation disorders, hypovolemia, dehydration or hypotension[14]. Glucocorticoids are analgesic in inflammatory states due to their strong anti-inflammatory action and they may modulate nociceptive processing in the dorsal horn[31]. However, they are commonly associated with moderate-to-severe adverse effects and so must be used cautiously and with consideration of the type of pain present[9,14].

**Local anesthetics** block inward $Na^+$ currents through voltage-gated $Na^+$ channels and consequently inhibit membrane depolarization, nerve excitation and conduction. They are weak bases and therefore equilibrate within the body according to their pKa; this is important for local anesthetics because it is the non-ionized form of the drug that cross the neuronal cell membrane to access the voltage gated $Na^+$ channel and that binds to the $Na^+$ channel receptor to block $Na^+$ entry into neurons. Therefore, local anesthetics with a low pKa, similar to physiological pH (e.g. lidocaine), have a more rapid onset because a greater proportion of the drug will be non-ionized at physiological pH[14]. This is the only class of drugs that promote complete analgesia[6]. The evidence in human and veterinary medicine reveal the predictable analgesic and anesthetic drug-sparing effect of local anesthetics; in addition, they are reported to have antimicrobial and immunomodulatory properties and can diminish postoperative maladaptive pain states[6,32]. In small animal practice, local anesthetic techniques can reduce the dose of other anesthetic drugs required for maintenance of general anesthesia and contribute to a multimodal analgesic management. Use of local anesthetics in specific nerve blocks can inhibit the relay of nociceptive information from the site of injury to the spinal cord and can also provide preventive analgesia and prevent or reduce the development of central sensitization[14]. In dogs, lidocaine can be administered intravenously by constant rate infusion to provide analgesia and reduce concentration of inhalant agents required to maintain general anesthesia[33,34]. Systemically, the mechanisms of analgesia are considered to be multiple. The plasma concentration of lidocaine following systemic administration is too low to block $Na^+$ channels directly. Therefore, mechanisms to block the production of cytokines and inhibition of NMDA receptors are considered to be more important[14].

**Alpha$_2$-adrenoceptor agonists** produce sedation, analgesia and muscle relaxation. This class of drugs binds to different alpha$_2$-adrenoceptor subtype receptors in the dorsal horn of the spinal cord (spinal analgesia) and cerebral cortex and *locus coeruleus* (sedation and supraspinal analgesia). Noradrenaline is the endogenous ligand for these receptors and is present on noradrenergic and non-noradrenergic neurons. These drugs inhibit the release of excitatory neurotransmitters through complex mechanisms causing membrane hyperpolarization in a similar way to opioid analgesic

drugs. They also bind to their receptors in the vascular endothelium causing peripheral vasoconstriction with increases in systemic and pulmonary vascular resistance while decreasing cardiac output in a dose-dependent manner[35]. In dogs, concurrent use of alpha$_2$-adrenoceptor agonists and opioids may improve analgesia due to a synergistic effect with consequent decrease in further opioids requirement[14]. Furthermore, intravenous infusions of medetomidine and dexmedetomidine are commonly used to provide sedation and continuous analgesia during the perioperative period[36].

**Ketamine** is an antagonist of NMDA receptors; their activation is one of the primary contributors to the initiation and maintenance of central sensitization. By reversibly antagonizing NMDA receptors, ketamine modulates central sensitization and exerts anti-hyperalgesic effects, as part of a multimodal perioperative pain management in dogs and cats undergoing major, invasive surgery and in traumatized patients[14]. In humans, ketamine may also have immunomodulatory effects and directly suppress proinflammatory cytokine production[37].

In small animals practice, there are several medications that can be incorporated into a pain management protocol that do not fall into the major traditional classes of analgesics (Table 1). These drugs are not considered “standard” analgesic agents and are most often used in conjunction with opioids, NSAIDs, local anesthetics and alpha$_2$-adrenoceptor agonists. These drugs may especially play a role in the treatment of chronic maladaptive pain however, more scientific and clinical trial on their use are gathered in dogs and cats.

| DRUG | INDICATIONS | PREDOMINANT MECHANISM |
|---|---|---|
| **Grapiprant** | • Chronic Osteoarthritic Pain | EP4 receptor antagonist |
| **Tramadol** | • Perioperative Adaptive Pain<br>• Cancer Pain<br>• Chronic Osteoarthritic Pain | Adrenergic, serotonin and opioid receptors |
| **Gabapentin** | • Perioperative Adaptive Pain<br>• Neuropathic Pain | $Ca^{++}$ channels, GABA receptors, suppressing glutamate and substance P. |
| **Pregabalin** | • Neuropathic Pain | GABA receptors |
| **Amantadine** | • Chronic Osteoarthritic Pain | NMDA receptors antagonist. |
| **Amitriptyline** | • Refractory Chronic Pain<br>• Neuropathic Pain | Reuptake of catecholamines blockage, NMDA receptors antagonist. |
| **Biphosphonates (Pamidronate)** | • Cancer Pain (malignant osteolytic pain) | Interference with geranylation of small GTPase proteins involved in cell signaling |
| **Cannabidiol (CBD)** | • Chronic Osteoarthritic and Neuropathic Pain | $CB_1$ and $CB_2$ receptors, opioidergic, NMDA and GABA receptors |
| **Anti-nerve growth factor (NGF) monoclonal antibodies** | • Chronic Osteoarthritic Pain | Interaction with tropomyosin kinase A receptor |
| **Paracetamol** | • Perioperative Adaptive Pain<br>• Contraindicated in cats | Interaction with a sub-form of COX- 1 in the central nervous system |

**Table 1.** Adjuvant drugs in canine and feline pain management

### 1.5.2 Nonpharmacological Management of Pain

Although pharmacological agents are often necessary to assist with managing pain and discomfort in companion animals, nonpharmacological modalities are critically important in their management. The use of these methods is becoming popular as veterinarians and owners seek alternative therapies for pain control with minimal side effects. Furthermore, it has become clear that multimodal approaches to pain management are superior to “single-modal” therapy. Using more

than one drug combined with nonpharmacological modalities can provide better analgesia than if these techniques were used alone[38].

**Acupuncture** is a nonpharmacological treatment option for numerous conditions in humans[39]. The contribution of acupuncture to pain control includes muscle relaxation, restoration of blood flow, decrease in joint compression and improvement in oxygen and nutrient distribution to the affected site; additionally, acupuncture reduces local signs of pain and tissue inflammation[40]. Pain modulating effects are mediated principally at the segmental level, with needle stimulation of afferent nerves resulting in action potentials entering the dorsal horn of the spinal cord. Descending inhibitory systems may also play a role in the analgesia provided by acupuncture[41]. Central regulatory effects mediated by the limbic system, which plays a central role in the affective and cognitive dimensions of pain, may be responsible for general calming effects as well as an improved sense of well-being reported in humans[41]. In the veterinary literature, acupuncture has been reported to be helpful as an adjunct treatment for perioperative pain following ovariohysterectomy in dogs and cats and for managing intervertebral disc disease, but it was not found to be beneficial for the treatment of pain associated with osteoarthritis in dogs[19,42,43]. Further work is needed to fully define the role of acupuncture in pain control[19].

**Cold therapy** is a nonpharmacological analgesic tool medically useful, globally available and not limited by regulation; it involves topical application of ice or frozen substrate via buckets and bags or use of cold compression devices and circulation sleeves. It has a long history as an analgesic modality for acute adaptive pain management in humans. Applying cold therapy to skin decreases temperature up to a depth of 2-4 cm, resulting in decreased activation of tissue nociceptors and slowed conduction velocity along peripheral axons[44]. Cold therapy also decreases edema via vasoconstriction, delivery of inflammatory mediators to injured tissues and reduces neuronal activity in sensory nerves. A study in dogs has demonstrated that cold compression therapy applied with the first 72 hours following tibial plateau leveling osteotomy (TPLO) surgery resulted in decreased pain, decreased lameness and increased joint range of motion[38]. In cases of chronic maladaptive pain with an inflammatory component or muscle spasm, cold therapy might also be valuable[14]. As with any drug or medical procedure, it has dose-related, time-related and disease-related effects that vary on a patient basis. Therefore, it should be used after careful consideration of its potential value to each individual[14].

**Regenerative medicine** is focused on strategies to grow, repair or replace injured or diseased cells, organs or tissues. Mesenchymal stem cells (MSC) are used in regenerative medicine and are

unspecialized adult cells with immunomodulatory and anti-inflammatory effects that have the ability to migrate to sites of tissue injury. These cells can be isolated from various tissues such as bone marrow or adipose tissue sourced from the patient itself (autologous) or from a donor of the same species (allogenic) or different species (xenogeneic) and can be administered by intravenous, intra-articular or other routes[45]. In dogs with osteoarthritis, MSC therapy is promising and current studies show decreased lameness, pain and an increase of range of motion[46]. This type of therapy resulted in complete remission or substantial clinical improvement in cats affected by severe refractory gingivostomatitis[47]. Hyaluronic acid is a natural component of joint fluid and cartilage that can be injected into osteoarthritic joints or given orally. Platelet rich plasma contains growth factors and proteins with anti-inflammatory properties. It involves the collection and processing of the patients' blood with subsequent injection into affected joints. Both hyaluronic acid and platelet rich plasma improve joint pain and mobility in people[48]. Evidence is still limited in veterinary medicine but seems to indicate positive effects on pain and function when intra-articular administration is used in dogs affected by osteoarthritis[46,49,50].

**Advanced interventional techniques** are minimally invasive procedures, often conducted under the guidance of an imaging modality. They are largely used in human medicine for management of severe maladaptive chronic pain and as part of palliative medicine and hospice care. Most of these procedures hold a great potential for the relief of pain that is unresponsive to currently available therapies. Some of these techniques are already widely used also in veterinary medicine, while others are relatively nascent or unavailable[22].

Epidural injection is one of the most common interventional pain management procedures performed in humans. Cervical, thoracic, lumbar or caudal epidural injections in humans are used for treatment of different maladaptive, chronic, nonmalignant pain syndromes. Epidural steroid (e.g. triamcinolone, methylprednisolone, dexamethasone) injection for radiculopathy/radiculitis due to spinal or foraminal stenosis is most common, but other indications include vertebral fractures, phantom limb pain and postherpetic neuralgia[51]. Local anesthetics and other drugs are often combined with the steroids[52]. In humans, epidural injection can be performed under local anesthesia with or without sedation and it is ideally guided by fluoroscopy, computed tomography or ultrasonography[51]. Epidural injection for chronic and neuropathic pain unresponsive to conservative therapy is not as well established in veterinary medicine, primarily because of the paucity of data on the object. A retrospective study by Janssens et al. (2004) reported that 80% of

dogs affected by lumbosacral degenerative stenosis showed some improvement, after epidural injection of methylprednisolone acetate every two weeks for a total of three injections[53].

Peripheral nerve, ganglion and plexus blocks, from the trigeminal nerve block for trigeminal neuralgia to the celiac plexus block for pancreatitis and pancreatic cancer to medial branch blocks for low back pain, are performed in humans for diagnostic, therapeutic and/or prognostic purposes[22,54]. Peripheral nerve, ganglion and plexus blocks can be performed using a "blind technique", through palpation of the landmarks, electrolocation or imaging modalities, such as ultrasonography. The perineural injection of local anesthetics is widely used and several adjuvants have been added to the local anesthetic in an attempt to prolong the duration of the block, a concept known as "multimodal perineural analgesia"[55]. These approaches are gaining interest also in veterinary medicine[22,56]. In human medicine, if significant short-term pain relief is achieved, a neurolytic procedure, such as cryoneurolysis or thermal radiofrequency, may be used to provide long-term analgesia[22].

Cryoneurolysis (or cryoablation) uses a special probe to freeze nerves. Pressurized gas (usually $N_2O$ or $CO_2$) travels through the inner tube of the probe and passes into a larger outer tube; the gas expands rapidly into the tip of the probe and heat is extracted. This process generates temperatures as low as -88°C to -79°C at the level of the tip, and an ice ball measuring several millimeters is formed; Wallerian degeneration ensues, but because myelin and endoneurium remain intact, the nerve will eventually regenerate[57]. Chemical neurolysis is a method still used in veterinary medicine but has largely been replaced in human pain medicine by other methods[22]. Today its use is primarily limited to celiac plexus neurolysis for pain from terminal neoplasia. Nociceptive primary afferent neurons pass through several sympathetic ganglia/plexuses on their way to the dorsal horn of the spinal cord; thus, injection of a neurolytic agent into a ganglion or plexus can relieve pain from specific anatomic structures. Destruction of the celiac plexus will decrease pain from the pancreas, the gastrointestinal tract from the esophagus to the transverse colon, the liver, the adrenal glands and the ureters, with a reduction in opioid requirement[58,59]. Ethanol and phenol are most commonly used and complications are uncommon[59].

In human medicine, radiofrequency therapy is applied to nerves to produce long-term (months to a year) analgesia. A radiofrequency cannula coated with insulation, except for a short length at the distal end, is used; this cannula is positioned so that the active tip is adjacent to the target nerve. An electrode connected to a radiofrequency generator is then inserted through the cannula until its tip reaches the active tip of the cannula. The generator produces an alternating current at radio

wave frequency (250 kHz to 1 MHz) that creates an electromagnetic field and consequently heat, in the tissues surrounding the active tip[22,60]. With thermal radiofrequency, a target temperature of 80°C is maintained for 1 to 2 minutes, resulting in localized necrosis of axons, myelin breakdown and hemorrhage. This thermal lesioning induces Wallerian degenerations of neurons, which prevents the transmission of nociceptive stimuli. Functional, but incomplete regeneration can occur, but can take months to years[61]. Thus, thermal radiofrequency can only be used on sensory nerves. The biophysical concepts underlying pulsed radiofrequency are the same of those for thermal radiofrequency. However, with pulsed radiofrequency, the generator produces current in short, high-voltage bursts. The silent period between bursts allows for heat dissipation, resulting in an average tissue temperature of 40°C, which is below the temperature considered lethal for neurons[60,61]. Because pulsed radiofrequency does not cause clinical motor deficits, in human pain medicine it is used on numerous mixed sensory-motor nerves to treat many painful conditions[62,63]. Although a preliminary study evaluating histopathologic changes of sciatic and saphenous nerves treated with thermal and pulsed radiofrequency has been conducted in dogs[64], clinical trials in companion animals with different painful conditions would be needed to determine if and to what extent these interventional modalities provide analgesia, and how long such analgesia might last.

## 1.6 Pain Assessment

Because animals are nonverbal and cannot self-report the presence of pain, the pain recognition and assessment lie with veterinary professionals[6]. It is now accepted that the most accurate method for evaluating pain in animals is not by physiological parameters but by observation of behavior. Pain assessment should be a component of every physical examination and obtaining an accurate patient history from the owner can help determine abnormal behavior patterns that may be pain related[2,6,7,14]. Behavioral expressions of pain are species-specific and influenced by age, breed, demeanor, type and duration of pain, clinical condition and the presence of additional stressors such as anxiety or fear[14]. Debilitating diseases can dramatically reduce the range of behavioral indicators of pain that the animal would normally show (e.g. dogs and cats may be reluctant to move to prevent worsening of pain). Therefore, when assessing an animal for pain, it is helpful to know the normal behaviors as changes in behaviors are important means of pain assessment[6,14]. Acute pain recognition is based on routine assessment of the animal for sign of pain; these signs are better identified through observation and interaction with the patient along with knowledge of the disease/surgical status and history of the animal. The behavioral changes associated with chronic maladaptive pain in dogs and cats may develop gradually and may be subtle, so that they can only be detected by someone familiar with the animal (usually the owner or the caregiver)[14,65]. A study has highlighted the importance of owner and caregiver education in the identification of maladaptive chronic pain in cats; in fact, caregiver education is important because long-standing pain conditions produce gradual behavioral changes that may not be noticed by caregivers or may be ascribed to ageing[66]

Although there is currently no gold standard for assessing pain in small animal practice, the guidelines Task Force strongly recommends utilizing pain-scoring tools for both acute and chronic pain[6]. Those tools have varying degrees of validation and reliability, acute and chronic pain scales are not interchangeable, and also canine and feline scales. The use of pain scales can decrease subjectivity and bias by observers, resulting in more effective pain management, which ultimately leads to an improvement of quality of life of patients[6,14]. Examples of composite pain scales with reported validation for assessment of adaptive acute pain in dogs include the Glasgow Composite Measure Pain scale and its short form (CMPS-SF)[67,68] and the French Association for Animal Anaesthesia and Analgesia pain scoring system (4A-Vet)[69], which are easy to use and include interactive components and behavioral categories. The CMPS-SF is a clinical decision-making tool used in conjunction with clinical judgement. Concurrent sedation is a confounding factor as deeply

sedated dogs tend to score highly irrespective of whether they are painful or not. The effect of sedation on CMPS-SF scores should be considered when assessing patients and deciding on the requirement for additional analgesia[14]. Multi-dimensional composite pain scales for assessing acute postoperative pain in cats include the short form of UNESP-Botucatu multi-dimensional feline pain assessment scale (UFEPS-SF)[70,71] and the feline Glasgow composite pain scale (CMPS-Feline)[72]. These tools require interaction with the patient, which is not always possible (e.g. stray and unsocialized cats); however, many components of these scales can be used to assess also these populations[14]. Facial expressions of pain appear to be exhibited in all mammals including cats, making species-specific scales valuable. The Feline Grimace Scale has been developed for cats and correlates well with multi-dimensional composite pain scales; it is a valid and reliable tool for rapid assessment for different types of pain, also when interaction whit patients is not possible[73].

Numerous pain scoring systems are available for evaluation of chronic maladaptive pain and quality of life in dogs; however, only a few have been validated[74]. Based on current evidence, the Canine Brief Pain Inventory (CBPI)[75] and the Liverpool Osteoarthritis in Dogs (LOAD)[76] are recommended for use in practice. Other tools such as Quantitative Sensory Testing and activity monitors are used in dogs[14]. Quantitative Sensory Testing evaluates the transmission of information related to thermal, mechanical and chemical stimuli from the periphery to the somatosensory cortex. It uses calibrated devices to induce a noxious stimulus against the skin of the animal until a behavioral reaction is observed; the end point is objectively recorded. Quantification of sensory sensitivity allows to compare animals with and without painful conditions, as well as the effects of treatment[14].

In cats, a recent checklist (Feline Musculoskeletal Pain Screening Checklist) has been produced to assist with the identification of cats affected by degenerative joint disease-associated pain, based on a valid scientific approach[66]; this checklist helps identify cats that may have degenerative joint disease-associated pain and can be used as an important caregiver education tool[14]. The Feline Musculoskeletal Pain Index (FMPI) and the Montreal Instrument for Cat Arthritis Testing (MI-CAT) are instruments designed to score the impact of degenerative joint disease-associated pain on the cat and monitor the effectiveness of treatment[77]. When used at intervals over time, they provide consistent data measuring the severity of chronic degenerative joint disease-associated pain in cats.

## 1.7 General Aims

In the last decades, small animal practice has reported an increasing interest in assessment and management of acute adaptive and chronic maladaptive pain and remarkable efforts have been made to identify new strategies for pain recognition and treatment. Management of animal pain has become a significant ethical as well as economic component in the modern veterinary practice. The incorporation of analgesic protocols in the perioperative period has garnered attention and expertise of the practicing veterinarians. Evidence to support specific anaesthetic and analgesic protocols, effective in the treatment of perioperative pain, continues to increase. In this scenario, the first aim of this research project was to explore the potential role of two alternative, simple and low-cost routes of administration of analgesics in the management of adaptive perioperative pain in dogs. In human medicine, the intraperitoneal and the intra-articular administration of analgesic drugs are a simple and effective example of perioperative pain management, as they may provide local analgesia with minimal systemic side effects; furthermore, they reduce early postoperative rescue analgesic requirements and prolong time to first-intervention analgesia[78,79]. The current veterinary literature on intraperitoneal and intra-articular perioperative analgesia has several limitations and provides no consensus regarding their efficacy. Published studies used different drugs, doses and volumes and different pain scoring systems, which render the results difficult to interpret[80,81]. Therefore, the first part of this PhD dissertation aimed to implement data regarding the efficacy of different analgesic drugs, administered via intraperitoneal and intra-articular routes, in perioperative management of acute adaptive surgical pain in dogs.

On the other hand, maladaptive chronic pain differs in fundamental aspects when compared with adaptive acute pain. Certain types of maladaptive chronic pain, like osteoarthritis-related and neuropathic pain, could be extremely difficult to treat. Patients generally need higher dosages of analgesic drugs than patients affected by adaptive acute pain and are more likely to require multimodal analgesia, including drugs that are not traditionally considered analgesics[82]. However, despite aggressive treatment, pain may not be successfully relieved[82]. In recent years, the human and veterinary medical fields have grown an increased interest in improving their understanding and treatment of maladaptive chronic pain. Advances in this type of pain management, both pharmacological and nonpharmacological, are increasing the quality of time that a patient suffering a chronic painful condition has. In small animal medicine, the treatment of this type of pain lacks in a consensus[14]. For this reason, the second aim of this project was to study the clinical efficacy of a new multimodal combination of analgesics for treating maladaptive chronic osteoarthritis-related

pain in dogs and of radiofrequency, a new interventional pain therapy in veterinary medicine, for managing different types of maladaptive chronic pain in canine patients.

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# 2. Comparison of intraperitoneal and incisional lidocaine or ropivacaine irrigation for postoperative analgesia in dogs undergoing major abdominal surgeries

**Brioschi FA**, Ravasio G, Ferrari F, Amari M, Di Cesare F, Valentini Visentin M, Rabbogliatti V; *PLoS One* 2023; 18(4):e0284379. DOI: 10.1371/journal.pone.0284379

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***Brioschi FA**, Rabbogliatti V, Amari M, Di Cesare F, Ferrari F, Romussi S, Ravasio G. Comparison of intraperitoneal lidocaine and ropivacaine for postoperative analgesia in dogs undergoing major abdominal surgeries*

## 2.1 Introduction

Major abdominal surgical procedures are very common in small animal practice[1]. These procedures are performed under general anesthesia and are considered to cause moderate-to-severe postoperative abdominal pain[2,3]. The pain from abdominal surgeries originates from incision, from manipulation of the abdominal viscera and from stretching of the associated ligaments[2]. Correct pain management reduces the recovery time, decreases the risk for surgical complications and results in a faster return to normal activities[4].

Intraperitoneal (IP) and incisional (INC) administration of local anesthetics are simple, safe and low-cost techniques[5] that have been used in human medicine during minimally invasive surgeries[6] and in open abdominal procedures[7,8]. During the last decade, these non-invasive techniques have gained interest also in small animal practice[9-12]. During IP administration, local anesthetics are applied to the surgical site and the viscera before suturing the abdominal wall[11,13], while INC local anesthesia consists in an infiltration or topical administration of local anesthetics on superficial muscles or subcutaneous tissue before or after surgical incision. Local anesthetics produce complete blockage of sensory nerve fibers and prevent the development of central sensitization to pain[14]. These agents have also the advantages of being inexpensive and widely available and do not have the adverse effects of systemically administered opioids (sedation, postoperative nausea and impairment of return of gastrointestinal motility)[15].

Lidocaine is a short-acting local anesthetic, easily adsorbed from the injection site due to its chemical structure[16]. Largely used in people to provide analgesia following abdominal surgery[17,18], IP lidocaine has been anecdotally advocated in dogs for treatment of pain related to diseases such as pancreatitis that are very difficult to manage with systemic analgesics[14]. In a canine study, there was a postoperative pain score trend suggesting efficacy of IP and INC lidocaine in providing analgesia following ovariohysterectomy in the dog[13]. Ropivacaine is an amino-amide longer-lasting local anesthetic with low risk of cardiotoxicity and neurotoxicity[19,20]. Some studies have recently demonstrated that IP ropivacaine has advantages, including prolonged analgesia and low risk of systemic toxicity in dogs[11,21].

The purpose of this study was to compare the postoperative analgesic efficacy of the combination of IP and INC lidocaine *versus* ropivacaine in dogs undergoing major abdominal surgeries. The authors hypothesized that IP and INC lidocaine and ropivacaine would provide effective post-surgical pain relief and that ropivacaine would promote a longer lasting analgesic effect than lidocaine.

## 2.2 Materials and Methods

### 2.2.1 Animals

This study was approved by the Institutional Ethical Committee for Animal Care at the University of Milan (OPBA_46_2020), and all dogs were enrolled after obtaining owner's written informed consent. The study included thirty-three client-owned dogs, older than six months of age and weighing more than five kg, of different breeds and genders, presented to the Veterinary Teaching Hospital of the University of Milan (Lodi, Italy) for elective major abdominal surgeries. The health status of dogs at admission was confirmed by physical examination, complete blood cell count, serum biochemical analysis, electrocardiographic and echocardiographic examinations. Dogs with severe systemic manifestations of disease (American Society of Anesthesiology class > III) and dogs that were administered analgesics within 72 hours prior to surgery were excluded from the study.

### 2.2.2 Study Design

This prospective, randomized, blinded clinical study was completed within a 12-month period. All dogs were fasted for 10 hours and water was withheld for two hours before the beginning of anesthesia. A temperament evaluation was carried out in all dogs using a score ranging from 1 (calm and friendly) to 4 (very excitable and nervous)[22]. Preoperative pain (T0, baseline) was assessed using

the Short Form-Glasgow Composite Measure Pain Scale (SF-GCMPS) scoring from 0 (no pain) to 24 (severe pain)[23]. Additionally, a 10 cm visual analogue scale (VAS) with end points labelled as "no pain" (0) and "worst pain imaginable" (10) was used[24]. All dogs were premedicated with dexmedetomidine (5 μg $kg^{-1}$) (Dexdomitor 0.5 mg $ml^{-1}$; Vetoquinol, Italy) and methadone (0.3 mg $kg^{-1}$) (Semfortan 10 mg $ml^{-1}$; Dechra Veterinary Products, Italy), mixed in the same syringe and injected into the lumbar epaxial muscles. After 15 minutes, a catheter was aseptically placed in a cephalic vein and anesthesia was induced with intravenous propofol (Proposure 10 mg $ml^{-1}$; Merial Italia S.p.A., Italy) titrated to effect to permit the endotracheal intubation. Anesthesia was maintained with isoflurane (Isoflo; Esteve, Italy) in oxygen, dogs were mechanically ventilated using a volume-controlled ventilation mode and respiratory rate was set in order to keep end-tidal carbon dioxide concentration between 35 and 45 mmHg. Lactated Ringer's solution (Ringer Lattato; Fresenius Kabi, Italy) was administered intravenously at the rate of 3-5 ml $kg^{-1}$ $hour^{-1}$ until extubation. Cefazolin 25 mg $kg^{-1}$ (Cefazolina; Teva S.r.l., Italy) was administered intravenously 20 minutes before surgery. During the intraoperative period, heart rate, invasive blood pressure, hemoglobin oxygen saturation, end-tidal carbon dioxide and body temperature were continuously monitored. In the event of a nociceptive response to surgery, defined as a 20% increase in heart rate and/or mean arterial pressure compared with pre-stimulation values, a fentanyl 1 μg $kg^{-1}$ intravenous bolus (Fentadon; Eurovet Animal Health B.V., The Netherlands) was administered[25]. The number of intraoperative fentanyl boluses given to each dog was recorded. Abdominal surgeries were performed via a midline approach by the same experienced clinical surgeon.

Dogs were randomly (Microsoft Office Excel 2013; Microsoft Corp, USA) divided into three groups according to the IP and INC treatments. Dogs in the L group received IP lidocaine 4 mg $kg^{-1}$ (Lidocaina Cloridrato 1%; Salf, Italy). Dogs in the R group received IP ropivacaine 4 mg $kg^{-1}$ (Naropina 1%; AstraZeneca, Italy). Both treatments were of equal volume by diluting drugs with sterile saline to 5 ml $kg^{-1}$. Dogs in the C group received IP sterile saline 5 ml $kg^{-1}$ (sodium chloride 0.9%; Fresenius Kabi, Italy). Solutions for IP instillation were aseptically prepared by an anesthesiologist and were administered prior to complete closure of the linea alba, through an intravenous catheter deprived of the inner stylet and inserted at the cranial portion of the midline incision[11]. After complete closure of the linea alba, just prior to skin closure, dogs in the L group received INC irrigation of lidocaine 2 mg $kg^{-1}$ and dogs in the R group received INC ropivacaine 2 mg $kg^{-1}$. Both treatments were of equal volume by diluting drugs with sterile saline to 0.2 ml $kg^{-1}$. Dogs in the C group received INC sterile

saline 0.2 ml $kg^{-1}$. Time from induction of general anesthesia to extubation (anesthesia time) and surgery time were recorded.

Thirty minutes after extubation, 0.2 mg $kg^{-1}$ meloxicam (Meloxidyl; Ceva, Italy) was subcutaneously administered to dogs. At 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 18 and 24 hours after extubation, sedation and postoperative pain were evaluated by a trained observer who was not aware of treatment allocation. The degree of sedation was assessed with a numerical scoring system ranging from 0 (no sedation) to 3 (profound sedation). Pain assessments were performed using the SF-GCMPS scoring from 0 (no pain) to 24 (severe pain)[23] and VAS scoring from 0 (no pain) to 10 (worst pain imaginable)[24]. Rescue analgesia (methadone 0.2 mg $kg^{-1}$ IM) was administered to dogs with a SF-GCMPS score $\geq$ 5/20 or $\geq$ 6/24 and/or a VAS score > 4. Pain scores obtained from dogs receiving the rescue analgesia were excluded from further statistical analysis. Small amounts of food were offered 5 hours after extubation and at any consecutive time points; elapsed time from extubation to first food intake was recorded. A follow-up period of 30 days was planned to evaluate any side effects.

### 2.2.3 Statistical Analysis

Sample size calculation was performed to identify the number of dogs necessary to detect a difference between treatments in SF-GCMPS, assuming that the C group would have higher scores than the L and R groups (anticipated means, 4.1, 2.3, 2.3 for C, L and R respectively, with 0.5 standard deviation). Based on this calculation, ten dogs per group would provide power of 80% at the α level of 0.05. Mean SF-GCMPS scores and standard deviation were estimated from a pilot study. Statistical analysis was performed using PASW 18.0 (SPSS Inc, Chicago, IL, USA). The normality of data distribution was assessed by a Shapiro-Wilk test at the α = 0.05 level. Data are expressed as mean $\pm$ standard deviation (parametric variables) or as median and range (nonparametric variables). Body weight, age, anesthesia and surgery times were compared among groups using one-way analysis of variance followed by a Tukey's test. A Kruskal-Wallis test was used to compare ASA status, temperament, the number of fentanyl boluses administered intraoperatively, sedation, SF-GCMPS and VAS scores, time to first food intake and the number of methadone doses administered postoperatively among groups. A Friedman test was used to compare differences in SF-GCMPS scores over time within each group. Wilcoxon signed-rank tests with a Bonferroni adjustment were also employed as Friedman post hoc. Values for $p < 0.05$ were considered significant.

## 2.3 Results

Thirty-one out of 33 client-owned dogs met the inclusion criteria and were assigned to the L group (n = 11), to the R group (n = 10) or to the C group (n = 10). Two dogs (one in the R group and one in the C group) were excluded from the study after presurgical evaluation, because of biochemical abnormalities. Table 1 summarizes dogs' information about breed distribution, age, body weight, gender, ASA status, temperament and preoperative SF-GCMPS and VAS scores. There were no statistically significant differences between groups regarding age ($p$ = 0.96), body weight ($p$ = 0.92), ASA status ($p$ = 0.65), temperament ($p$ = 0.85), preoperative SF-GCMPS ($p$ = 0.63) and VAS ($p$ = 0.66) scores, highlighting the homogeneity of groups.

**Table 1**. Breed, age, body weight, gender, ASA status, temperament and preoperative SF-GCMPS and VAS scores of the dogs recruited in L, R and C groups.

| Group | Patient | Breed | Age (months) | Body Weight (kg) | Gender | ASA status | Temperament score | SF-GCMPS score | VAS score |
|---|---|---|---|---|---|---|---|---|---|
| **L** | **1** | Cocker Spaniel | 168 | 13 | Female | 3 | 1 | 6 | 4 |
| **L** | **2** | Mongrel | 132 | 21 | Male | 3 | 2 | 7 | 4 |
| **L** | **3** | Bracco Italiano | 42 | 25 | Male | 2 | 2 | 6 | 4 |
| **L** | **4** | Golden Retriever | 36 | 36 | Male | 3 | 1 | 8 | 5 |
| **L** | **5** | English Bulldog | 96 | 25 | Female | 3 | 1 | 6 | 4 |
| **L** | **6** | Bouledogue Francais | 98 | 13 | Female | 2 | 2 | 8 | 5 |
| **L** | **7** | Labrador Retriever | 144 | 37 | Male | 2 | 2 | 6 | 3 |
| **L** | **8** | English Bulldog | 120 | 25 | Female | 2 | 3 | 4 | 2 |
| **L** | **9** | Mongrel | 54 | 37 | Female | 1 | 2 | 2 | 1 |
| **L** | **10** | Bernese Mountain Dog | 64 | 33 | Male | 3 | 1 | 10 | 7 |
| **L** | **11** | Cocker Spaniel | 60 | 11 | Female | 3 | 1 | 8 | 6 |
| **R** | **1** | Bull Terrier | 60 | 21 | Male | 3 | 1 | 8 | 5 |
| **R** | **2** | American Staffordshire Terrier | 48 | 30 | Male | 3 | 2 | 8 | 6 |
| **R** | **3** | Labrador Retriever | 150 | 36 | Female | 3 | 2 | 4 | 2 |
| **R** | **4** | Pinscher | 128 | 6 | Male | 2 | 2 | 4 | 2 |
| **R** | **5** | Bull Terrier | 72 | 25 | Male | 2 | 1 | 6 | 4 |
| **R** | **6** | Mongrel | 60 | 22 | Female | 3 | 2 | 7 | 4 |
| **R** | **7** | German Sheperd | 72 | 44 | Male | 2 | 3 | 8 | 5 |
| **R** | **8** | Mongrel | 110 | 22 | Male | 3 | 2 | 8 | 5 |
| **R** | **9** | Mongrel | 132 | 43 | Male | 3 | 1 | 12 | 7 |
| **R** | **10** | Mongrel | 73 | 21 | Male | 3 | 2 | 12 | 8 |
| **C** | **1** | Great Dane | 18 | 55 | Female | 1 | 2 | 1 | 0 |
| **C** | **2** | Pug | 48 | 10 | Female | 2 | 2 | 5 | 2 |
| **C** | **3** | Mongrel | 14 | 18 | Male | 3 | 3 | 10 | 6 |
| **C** | **4** | Mongrel | 156 | 22 | Female | 2 | 1 | 6 | 3 |
| **C** | **5** | English Bulldog | 90 | 29 | Female | 3 | 1 | 6 | 4 |
| **C** | **6** | Mongrel | 132 | 25 | Male | 3 | 1 | 8 | 5 |
| **C** | **7** | Fox Terrier | 161 | 7 | Female | 3 | 1 | 9 | 5 |
| **C** | **8** | English Setter | 142 | 19 | Female | 3 | 1 | 14 | 8 |
| **C** | **9** | Golden Retriever | 125 | 37 | Male | 2 | 2 | 8 | 5 |
| **C** | **10** | Labrador Retriever | 76 | 31 | Male | 2 | 3 | 6 | 4 |

Table 2 summarizes information about the type of abdominal surgery each dog underwent and about anesthesia and surgery times. The statistical analysis detected no differences between groups, with respect to anesthesia ($p$ = 0.49) and surgery ($p$ = 0.32) times. The number of fentanyl boluses administered during the intraoperative period did not differ among groups ($p$ = 0.16).

**Table 2**. Types of major abdominal surgery and anesthesia and surgery times of the dogs recruited in L, R and C groups.

| Group | Patient | Type of Major Abdominal Surgery | Anesthesia Time (minutes) | Surgery Time (minutes) |
|---|---|---|---|---|
| **L** | **1** | Enterotomy | 85 | 58 |
| **L** | **2** | Splenectomy | 131 | 92 |
| **L** | **3** | Enterectomy | 154 | 116 |
| **L** | **4** | Enterotomy | 128 | 103 |
| **L** | **5** | Cervical Stump Revision | 156 | 114 |
| **L** | **6** | Enterectomy | 86 | 60 |
| **L** | **7** | Splenectomy | 117 | 87 |
| **L** | **8** | Ovariohysterectomy for Pyometra | 105 | 52 |
| **L** | **9** | Ovarian Remnant Removal | 131 | 100 |
| **L** | **10** | Gastrotomy + Enterotomy | 136 | 113 |
| **L** | **11** | Enterectomy | 174 | 109 |
| **R** | **1** | Gastrotomy | 119 | 75 |
| **R** | **2** | Gastrotomy + Enterotomy | 125 | 90 |
| **R** | **3** | Enterectomy | 130 | 110 |
| **R** | **4** | Splenectomy | 112 | 75 |
| **R** | **5** | Enterectomy | 120 | 80 |
| **R** | **6** | Gastrotomy + Enterotomy | 176 | 140 |
| **R** | **7** | Prostatic Cyst Omentalization | 148 | 126 |
| **R** | **8** | Enterectomy | 195 | 150 |
| **R** | **9** | Intra-abdominal Testicular Neoplasia Removal | 117 | 80 |
| **R** | **10** | Choledochal Stent and Anastomosis | 128 | 102 |
| **C** | **1** | Ovariohysterectomy + Prophylactic Gastropexy | 149 | 123 |
| **C** | **2** | Ovariohysterectomy for Pyometra | 95 | 52 |
| **C** | **3** | Gastrotomy | 131 | 94 |
| **C** | **4** | Splenectomy | 115 | 75 |
| **C** | **5** | Ovariohysterectomy for Pyometra | 152 | 103 |
| **C** | **6** | Prophylactic Gastropexy | 127 | 77 |
| **C** | **7** | Enterotomy | 119 | 87 |
| **C** | **8** | Splenectomy | 83 | 52 |
| **C** | **9** | Splenectomy | 124 | 81 |
| **C** | **10** | Enterectomy | 138 | 115 |

Postoperative sedation scores did not significantly differ between groups; after T3, the sedation score was 0 in all dogs. In group C, postoperative SF-GCMPS scores were significantly higher than in groups L and R at T0.5, T1, T2, T3, T4, T5 and T6 ($p < 0.05$), while at T9 they were significantly higher than in group R ($p$ = 0.033); in R group, postoperative SF-GCMPS were significantly lower than in

groups L and C, at T12, T18 and T24 ($p < 0.05$) (Fig 1). In group C, postoperative VAS scores were significantly higher than in groups L and R at T0.5, T1, T2, T3, T5 and T6, while at T9, T18 and T24 they were significantly higher than in group R ($p < 0.05$); in R group, postoperative VAS scores were significantly lower than in groups L and C at T12 ($p = 0.028$) (Fig 2).

**Figure 1. Box-and-whisker plots of the perioperative Short Form-Glasgow Composite Measure Pain Scale (SF-GCMPS) scores in 31 dogs undergoing major abdominal surgeries.** Dogs received intraperitoneal and incisional lidocaine (Group L), ropivacaine (Group R) or sterile saline (Group C) at the end of surgery. Dogs were evaluated immediately before surgery (baseline) and from 30 minutes (T0.5) up to 24 hours (T24) after extubation. Each box represents the interquartile range, and the median value is the horizontal line within each box. The upper and lower whiskers represent the upper and lower range of values, respectively.

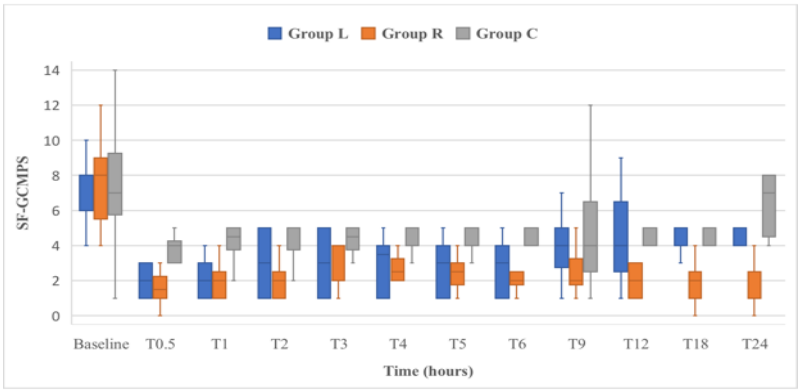
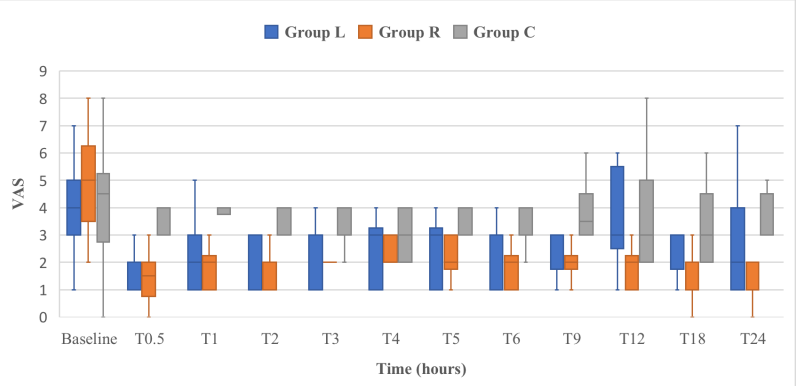



**Figure 2. Box-and-whisker plots of the perioperative visual analogue scale (VAS) scores in 31 dogs undergoing major abdominal surgeries.** Dogs received intraperitoneal and incisional lidocaine (Group L), ropivacaine (Group R) or sterile saline (Group C) at the end of surgery. Dogs were evaluated immediately before surgery (baseline) and from 30 minutes (T0.5) up to 24 hours (T24) after extubation. Each box represents the interquartile range, and the median value is the horizontal line within each box. The upper and lower whiskers represent the upper and lower range of values, respectively.




Postoperative SF-GCMPS and VAS scores significantly decreased in groups L and R when compared to baseline scores *(p* < 0.05*)*. The number of dogs that required postoperative methadone was significantly higher in group C than in group R (*p* = 0.002). In L group, one dog received rescue methadone at T1, one dog at T9, two dogs at T12 and one dog at T24. One dog in R group was administered rescue methadone at T12. In C group, one dog received rescue methadone at T3, two dogs at T9, one dog at T12, one dog at T18 and three dogs at T24. Treatment failure rate was 45,5% (5/11 dogs) at T24 in L group, 10% (1/10 dogs) at T12 in R group, and 80% (8/10 dogs) at T24 in C group. The time to first food intake was significantly lower in group R than in groups L (*p* = 0.01) and C (*p* = 0.002). Medians (min-max range) of the time of first food intake were 9 (6-12), 5 (5-9) and 9 (6-18) in L, R and C groups, respectively. Vomiting was observed in two dogs at T4 in L group and in one dog at T9 in C group. All dogs recovered without complications and no other adverse effects were observed during the 30 day follow-up period.

## 2.4 Discussion

Intraperitoneal anesthesia is an inexpensive, simple, and safe method for controlling intraoperative and postoperative pain during abdominal surgery in human patients[5]. Results of the present study showed that dogs that received IP and INC lidocaine and ropivacaine (IP: 4 mg $kg^{-1}$, diluted to 5 ml $kg^{-1}$; INC: 2 mg $kg^{-1}$, diluted to 0.2 ml $kg^{-1}$) at the end of major abdominal surgeries recorded lower postoperative pain scores if compared to baseline; these two groups of dogs experienced less postoperative pain (lower SF-GCMPS and VAS scores) during the first 6 hours after extubation, compared with findings in dogs of the control group. Administration of IP and INC ropivacaine provided lower postoperative pain scores than lidocaine and saline up to 24 hours after extubation, reduced the need for rescue analgesia and promoted a rapid food intake. In veterinary medicine,

pain after major abdominal surgeries is a multifactorial process that includes visceral and somatic pain, from the incision of the abdominal wall, the distension of the peritoneum, stretching of the associate ligaments and traction of nerves and blood vessels[26]. It can cause decrease in food intake, depression of respiratory function and central sensitization to noxious stimuli that can lead to the development of maladaptive pain[27]. Therefore, to manage postoperative pain after this type of surgery, a multimodal approach is required; in small animal practice this involves the use of opioids, non-steroidal anti-inflammatory drugs and local anesthetics[28]. The main advantages of using local anesthetics is that they produce complete blockage of sensory nerve fibers and that they do not cause the adverse effects of opioids and non-steroidal anti-inflammatory drugs, that may reduce the quality of recovery and delay discharge from hospital[26]. Among different regional analgesic techniques, IP and INC administration of local anesthetics are simple and inexpensive methods that reduce early postoperative analgesic requirements, time to first-intervention analgesia and pain scores after abdominal surgery in humans[7,29]. The topical application of local anesthetics to the incisional site, the viscera and to the peritoneum exhibits an analgesic effect by blocking nociception from the area of tissue damage; the systemic absorption of local anesthetics through the peritoneal surface may also play a role in the analgesic effect by attenuating nociception both in human[7,29,30] and veterinary medicine[31]. Furthermore, local anesthetics have anti-inflammatory actions. A proinflammatory cytokine cascade in the peritoneal cavity, with direct action on the visceral afferents and the vagus as major vehicle, is a feasible contributor to postoperative visceral pain. By using IP and INC local anesthetics, it may be possible to modulate somatic, visceral and peritoneal signaling to the brain, thereby attenuating the metabolic impact of surgery[32]. To the best of our knowledge, no studies evaluating the combined use of IP and INC local anesthesia during major abdominal surgeries and quantification of its postoperative analgesic effect have been described in companion animals. In dogs, numerous studies have evaluated the effectiveness of IP and INC administration of local anesthetics for pain relief after ovariohysterectomy and have provided variable results probably due to differences in site and timing (preoperatively or postoperatively) of administration and differences in local anesthetic doses, concentrations and volumes of injection. Findings of a previous study suggest a possible efficacy of IP and INC lidocaine for treatment of postoperative pain in dogs undergoing ovariohysterectomy; in fact, dogs tended to have lower pain scores at 0.5 hours post-extubation and receive less rescue analgesics than dogs who received IP and INC saline[13]. However, results of another study showed no benefit of IP lidocaine for postoperative pain management after ovariohysterectomy, compared to a group of placebo-treated

dogs[33]. Intraperitoneal 0.5% ropivacaine (3 mg $kg^{-1}$), administered in combination with morphine and carprofen, provided postoperative analgesia for 6 hours after extubation in dogs undergoing ovariohysterectomy[11], while it did not promote lower postoperative SF-GCMPS and VAS scores compared with dogs that received IP saline, if administered at 3 mg $kg^{-1}$ and diluted to a final volume of 1.2 ml $kg^{-1}$ [21]. One of the factors that might contribute to failure of IP local anesthesia for postoperative pain management after abdominal surgeries may be related to inadequate distribution of local anesthetics throughout the visceral and peritoneal surface. In fact, achieving an even distribution of local anesthetics into the tissues of a surgical site can sometimes be technically difficult and often result in "patchy" analgesia[28]. In contrast, higher volumes should provide a uniform spread of local anesthetics throughout the peritoneal cavity and thus may be beneficial to improve pain relief after major abdominal surgeries. The volume of IP solution (5 ml $kg^{-1}$) administered in the present study was larger than those previously reported in veterinary literature[11,13,21,33] and it was determined by the experience of anesthetists and surgeons at the Veterinary Teaching Hospital of the University of Milan, with the goal of providing adequate exposure to the whole visceral and peritoneal surfaces while limiting the risk of leakage from the abdominal cavity during surgery. Furthermore, it is clearly demonstrated that visceral and parietal peritoneum exposure to room air during abdominal surgery promotes local early inflammatory responses[34], probably concurring to the amplification of the nociceptive stimulus. In this study, the large volume (5 ml $kg^{-1}$) in which the local anesthetics were diluted was probably evenly distributed over the entire peritoneal surface, contributing to limit the painful stimuli resulting from general peritoneal cavity inflammation, and not only of that deriving from the area manipulated by the surgeon. Solutions for IP instillation were administered prior to complete closure of the linea alba so that the surgeon could confirm the distribution at the surgical site and that there was no leakage from the abdominal cavity. Local instillation of large volumes of local anesthetic may increase the risk of their systemic absorption and side effects in people[35], but no clinically relevant adverse consequences or signs of toxicity were noted during the postoperative period in dogs included in the present study. Doses administered for lidocaine and ropivacaine were 4 mg $kg^{-1}$ and 2 mg $kg^{-1}$, by IP and INC routes, respectively. Higher doses of lidocaine had been administered in dogs, combining IP (8.8 mg $kg^{-1}$) and INC (2 mg $kg^{-1}$) routes; the doses studied generated plasmatic levels of lidocaine well below toxic and no adverse effects were observed in dogs up to 18 hours after administration[31]. To the authors' knowledge, there are no studies reporting the maximum recommended doses for IP and INC ropivacaine administration in dogs. However, in animal models,

it is reported that ropivacaine has delayed cardiotoxic and neurotoxic side effects and a wider margin of safety compared to bupivacaine at equipotent doses[19]; in dogs undergoing ovariohysterectomy, Carpenter et al. (2004) did not observe any side effects with a combination of 4.4 mg $kg^{-1}$ of IP and 2 ml of INC 0.75% bupivacaine. Furthermore, Lambertini et al. (2018) did not observe any adverse effects with 3 mg $kg^{-1}$ of IP ropivacaine in dogs, a similar dose to that used in the present study. In this study, the efficacy of both IP and INC lidocaine and ropivacaine in reducing postoperative pain in dogs undergoing major abdominal surgeries was represented by lower postoperative SF-GCMPS and VAS scores compared with baseline. Despite no significant variations in SF-GCMPS and VAS scores between baseline and other examined periods, also dogs assigned to control group experienced a progressive decrease in pain scores. These findings, although not statistically significant, allow authors to suppose that even the combination between dexmedetomidine, methadone and meloxicam resulted in some beneficial effects in terms of postoperative pain relief. It is also possible that this multimodal approach may have contributed to the postoperative pain scores reduction observed in L and R groups. This study revealed a significant difference in the need of postoperative rescue methadone between dogs that received IP and INC ropivacaine (1/10) and dogs included in the control group (8/10). Although the number of postoperative rescue analgesia administrations did not significantly differ between groups L (5/11) and R (1/10), 4/5 dogs in L group received postoperative methadone from 9 hours after extubation; furthermore, SF-GCMPS scores did not significantly differ between the two groups from T0.5 to T9 and VAS score from T0.5 to T6, suggesting a comparable analgesic effect of the two treatments for six hours after extubation. Thereafter, in R group, postoperative SF-GCMPS scores were significantly lower than in groups L and C, at T12, T18 and T24, while postoperative VAS scores were significantly lower at T12; these results suggested a longer duration of the postoperative analgesic effect of IP and INC ropivacaine than IP and INC lidocaine. Considering the lower postoperative SF-GCMPS and VAS scores and the number of postoperative rescue methadone administrations, the faster postoperative food intake in dogs in group R was unsurprising. This result confirms the beneficial effect on food intake of reducing postoperative pain and postoperative opioids administration. Our findings support the conclusion of another study, where the use of peripheral nerve block in dogs undergoing tibial plateau levelling osteotomy promoted a lower requirement of postoperative methadone and a consequent greater postoperative food intake compared with a group of dogs treated with systemic analgesia[25].

This study has several limitations, one of which is that dogs had different abdominal pathologies and underwent different abdominal surgeries, with various degrees of tissue inflammation and pain. These factors could have affected the drugs' absorption from IP and INC sites into the bloodstream and, consequently, the drugs' systemic and local analgesic effects. Furthermore, assessment of postoperative abdominal pain in small animals is far from being objective and lacks any gold standard; in our study, in order to limit subjectivity and to increase the reliability of pain evaluation, two different pain scales were used and pain was assessed by the same trained investigator, who was unaware of treatment allocation. Another limitation of the study is that the dilution of lidocaine and ropivacaine with 0.9% sterile saline to 5 mL $kg^{-1}$ may have changed the physicochemical properties of both drugs[36] and consequently altered local anesthetics onset of action, duration and efficacy.

## 2.5 Conclusions

In conclusion, this study compared the postoperative analgesic efficacy of IP and INC irrigation of lidocaine *versus* ropivacaine in dogs undergoing major abdominal surgeries. The authors demonstrated that, as part of a multimodal approach to postoperative pain management for dogs undergoing major abdominal surgeries, IP and INC lidocaine and ropivacaine (IP: 4 mg $kg^{-1}$, diluted to a final volume of 5 mL $kg^{-1}$; INC: 2 mg $kg^{-1}$, diluted to a final volume of 0.2 mL $kg^{-1}$) provide effective and comparable post-surgical pain relief for 6 hours after extubation. In accordance to what has been previously hypothesized, IP and INC ropivacaine provided a longer lasting analgesic effect (up to 24 hours after extubation) than lidocaine and this finding results in a decreased postoperative opioids requirement and in a more rapid food intake, compared with dogs in L and C groups.

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# 3. Clinical effects of preemptive intra-articular lidocaine, dexmedetomidine and lidocaine-dexmedetomidine administration in dogs

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***Brioschi FA**, Gioeni D, Lazzarini E, Jacchetti A, Bronzo V, Carotenuto AM. Effects of intra-articular administration of lidocaine, dexmedetomidine and a lidocaine-dexmedetomidine combination in dogs*

## 3.1 Introduction

Arthroscopic surgery plays a major role in the treatment of joint diseases both in human and veterinary medicine[1,2] and can evoke different levels of pain. Various techniques, including peripheral nerve block and intra-articular (IA) drug administration, have been used for analgesic management in humans[3,4]. Intra-articular analgesic drug administration has the advantages of better preservation of motor function and technical simplicity, while providing good pain relief[5]. In veterinary medicine, the clinical evidence clearly states that perioperative IA injection of analgesics can accomplish pain control with low side-effects in dogs and horses, although available data need to be confirmed by further studies[6].

Lidocaine is a local anesthetic with anti-inflammatory action[7]. In humans, analgesia without toxic effects was obtained during arthroscopic procedures with IA administration of lidocaine[8]. In a canine study, IA lidocaine showed no signs of cardiac or neurologic toxicity, but its perioperative analgesic efficacy was not evaluated[9]. Dexmedetomidine is a highly selective $\alpha_2$-adrenoceptor agonist with sedative, anxiolytic and analgesic properties[10]. It has cardiovascular effects including a decrease in heart rate (HR), a baroreceptor-mediated response to the hypertensive effect caused by the increase in systemic vascular resistance[11]. In humans, IA administration of dexmedetomidine enhanced postoperative pain relief after arthroscopic surgery[12-14]. In dogs undergoing stifle joint surgery, postoperative IA administration of dexmedetomidine, in combination with morphine, provided

longer-lasting postoperative analgesia, compared with either dexmedetomidine or morphine alone[15]. Furthermore, $\alpha_2$-agonists are used in combination with local anesthetics to perform peripheral nerve blocks, providing a useful adjunct, because they prolong sensory and motor blockade, compared to local anesthetics alone[16].

The purpose of this study was to assess the perioperative effects of IA lidocaine, dexmedetomidine and their combination in dogs undergoing arthroscopy. We hypothesized that IA lidocaine, dexmedetomidine and their association would be similar in providing intraoperative analgesia and that IA dexmedetomidine would reduce postoperative analgesic requirements. It was further hypothesized that IA dexmedetomidine, alone or in combination with lidocaine, would have some effects on HR and arterial blood pressure.

## 3.2 Materials and Methods

### 3.2.1 Animals

The study was approved by the AVA Ethical Committee (Protocol number 2019-006; Approval date, 14 April 2019) and for each dog a written informed consent was acquired from the owner. The study included 30 client-owned dogs scheduled for unilateral arthroscopy (stifle, elbow or shoulder arthroscopy) older than 6 months, of any breed, sex and bodyweight. The dogs included were considered healthy (ASA I-II) based on physical examination, complete blood cell count, serum biochemical analysis, electrocardiographic and echocardiographic examinations. The type of IA pathology was noted and recorded. A scoring system ranging from 1 to 4 (1: low, 2: low/moderate, 3: moderate, 4: high) was applied to establish the score of articular inflammation[9]. Dogs with significant capsular swelling related to severe synovitis (score 4) and dogs that were administered analgesics and/or anti-inflammatory medications within 72 h prior to surgery were excluded.

### 3.2.2 Study Design

This prospective, randomized, masked clinical study was completed within a 9-month period. Food was withheld for 8 hours, and water for 2 hours before the beginning of anesthesia. Acepromazine (0.02 mg $kg^{-1}$; Prequillan; Fatro, Italy) and methadone (0.2 mg $kg^{-1}$; Semfortan; Eurovet, Italy) were mixed in the same syringe and injected into the lumbar epaxial muscles. After 20 minutes, a catheter was placed in a cephalic vein, and induction of anesthesia was performed using intravenous (IV) propofol (Proposure; Merial, Italy) titrated to effect. Anesthesia was maintained with isoflurane (Isoflo; Esteve, Italy) in a mixture of medical air and oxygen (fraction of inspired oxygen between 0.6

and 0.7), via a circle breathing system. Dogs were mechanically ventilated (Cato Dräger Medical) using the volume-controlled ventilation mode in order to maintain end-tidal carbon dioxide concentration between 35 and 45 mmHg. The end-tidal isoflurane concentration (FE'Iso) was initially set at 1.3% and adjusted by ± 0.1% every 5 minutes, according to the assessment of the anesthesiologist, who was masked to group allocation, to reach a plane of anesthesia that maintained a ventral eye position, absence of palpebral reflex and relaxed jaw tone. Intravenous fluid therapy was provided with 5 ml $kg^{-1}$ $hour^{-1}$ lactated Ringer's solution (Ringer Lattato, Fresenius Kabi). Cefazolin 30 mg $kg^{-1}$ (Cefazolina; Teva, Italy) was administered IV 20 minutes before surgery. A catheter was aseptically placed in a dorsal pedal artery to measure invasive blood pressure. The invasive blood pressure transducer (Transpac IV Disposable Pressure Transducer; ICU Medical, Italy) was zeroed at atmospheric pressure and placed at the level of the right atrium.

Dogs were randomly (Microsoft Office Excel 2013, Microsoft Corp, USA) assigned to three groups according to the IA treatment. Dogs in the L group received IA lidocaine 1 mg $kg^{-1}$ (Lidocaina Cloridrato; Esteve, Italy). Dogs in the group D received IA dexmedetomidine 2.5 µg $kg^{-1}$ (Dexdomitor; Vetoquinol, Italy). Dogs in the group LD were administered a lidocaine-dexmedetomidine combination (lidocaine 1 mg $kg^{-1}$ and dexmedetomidine 2.5 µg $kg^{-1}$). All treatments were of equal volume by diluting drugs with sterile saline to 0.2 mL $kg^{-1}$ [15]. In the L and LD groups the final lidocaine concentration was 0.5%. All syringes were aseptically prepared by an anaesthesiologist and labelled in a way that did not reveal their content. The IA injection was performed by a surgeon in the operating theatre after a sterile preparation of the skin. To assure a correct positioning of the needle into the joint, the IA solution was slowly injected after withdrawal of synovial fluid. To allow the distribution of IA solution, the joint was flexed and extended at its maximum range for 1 minute. The arthroscopic procedure started 15 min after IA injection[9]. During this time the joint was aseptically prepared for the arthroscopy.

Heart rate, systolic (SAP), mean (MAP) and diastolic (DAP) invasive blood pressure, FE'Iso, body temperature (T), peripheral oxygen saturation and end-tidal carbon dioxide concentration were continuously monitored with a multiparameter monitor (S5 Compact Anaesthesia Monitor, Datex-Ohmeda, Italy) by an anesthesiologist who was masked to IA treatment. Data regarding HR, SAP, MAP, DAP, FE'Iso and T were recorded 5 minutes before IA injection (T0), at IA injection (TIA) and every 5 minutes after IA injection. A lead II electrocardiogram was obtained during general anesthesia in order to diagnose any arrhythmias. In the case of bradycardia, defined as HR lower than 45 beats per min, 0.02 mg $kg^{-1}$ IV atropine (Atropina solfato; ATI, Italy) was administered. In

case of hypotension (MAP below 60 mmHg), an IV bolus of lactated Ringer's solution (10 ml $kg^{-1}$ over 15 minutes) was administered. Unresponsive hypotension was treated with synthetic colloid bolus (5 ml $kg^{-1}$ over 15 minutes; Gelplex; Fresenius Kabi, Italy) and then with an IV dopamine infusion starting at 2 µg $kg^{-1}$ $minute^{-1}$ and increased by 0.5 µg kg $minute^{-1}$ every 5 minutes. Dogs that were administered atropine or any treatment for hypotension were excluded from the study. In the event of a nociceptive response to surgery, defined as a 20% increase in HR and MAP compared with the pre-stimulation values (defined as HR and MAP values recorded immediately before skin incision), rescue IV fentanyl (Fentanest; Dechra, Italy) 1 µg $kg^{-1}$ was administered[17]. The number of intraoperative IV fentanyl boluses administered to each dog was recorded.

Thirty minutes after extubation, 2 mg $kg^{-1}$ carprofen (Rimadyl, Pfizer, Italy) was administered subcutaneously to all dogs. Time from induction of general anesthesia to extubation (general anesthesia time) and time from injection of IA treatment (TIA) to the skin closure (surgery time) were recorded. Postoperative pain was assessed every hour until 6 hours, then at 8, 10 and 12 hours after extubation, using the Short Form-Glasgow Composite Measure Pain Scale (SF-GCMPS)[18] scoring from 0 (no pain) to 24 (severe pain) by a trained investigator unaware of the treatment allocation. Intramuscular rescue analgesia (methadone, 0.2 mg $kg^{-1}$) was administered to dogs with a SF-GCMPS score $\geq$ 6. Dogs receiving rescue analgesia were excluded from further statistical analysis. A follow-up period of 30 days was planned to evaluate any side effects.

### 3.2.3 Statistical Analysis

Sample size calculation was performed using G-Power Software. A minimum of eight dogs per group was required to have a power of 80% with an alpha level of 0.05 and an effect size of 0.68 with regard to intra-operative fentanyl administration (anticipated mean number of fentanyl boluses, 1.5, 0.125, 0.125 for L, D and LD respectively, with standard deviation 0.79). Another sample size calculation was performed to identify the number of dogs necessary to detect a difference between treatments in SF-GCMPS, assuming that the L group would have higher scores than the D and LD groups (anticipated means, 3.6, 2.2, 2.2 for L, D and LD respectively, with 0.8 standard deviation). Based on this calculation, eight dogs per group were necessary. Statistical analysis was performed using IBM SPSS Statistics 26.0. The normality of data distribution was assessed by a Shapiro-Wilk test at the $\alpha = 0.05$ level. Normally distributed data were presented as mean ± standard deviation and compared using one-way analysis of variance test for repeated measures with a post hoc Bonferroni test, in order to assess differences for each group in relation to time. The same approach

was used to compare differences from the baseline within each treatment. Non-normally distributed data were presented as median and 95% confidence interval and compared by Generalized Estimating Equation while categorical variables were compared with Chi-square test. A *p* value of 0.05 was taken as statistical significance.

## 3.3 Results

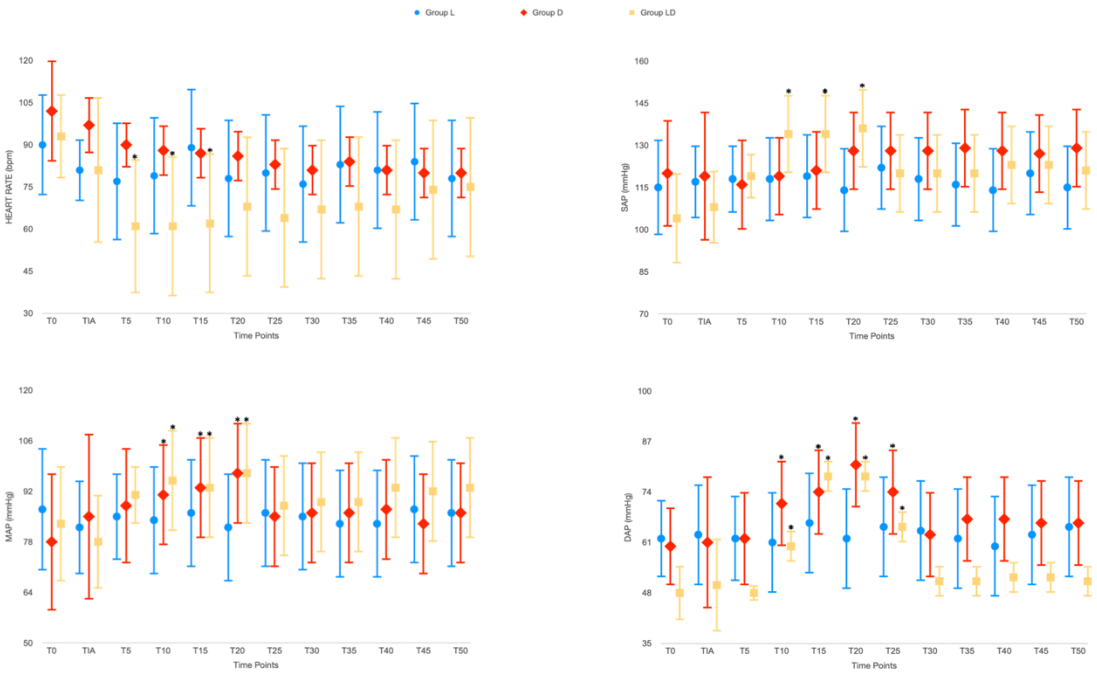
A total of 24 client-owned dogs (eight in each group) completed the study without complications (six dogs were excluded from the study after presurgical evaluation). The data on breed, age, bodyweight, sex, involved joint, IA pathology, score of articular inflammation, general anesthesia and surgery times are summarized in Table 1. No significant differences were observed between groups for age, bodyweight, sex, score of articular inflammation and general anesthesia and surgery times (Table 1).

**Table 1.** Breed, age, bodyweight, sex, involved joint, joint disease, score for articular inflammation (1 = low, 2 = low/moderate, 3 = moderate, 4 = high), number of IV fentanyl (FNT) boluses (1 µg $kg^{-1}$) administered, general anesthesia time (time from induction of general anesthesia to extubation) and surgery time (time from intra-articular [IA] treatment administration to skin closure) for dogs undergoing arthroscopy treated IA with lidocaine (group L, *n* = 8), dexmedetomidine (group D, *n* = 8) or lidocaine-dexmedetomidine combination (group LD, *n* = 8). F, female; M, male.; CaCLR, caudal cruciate ligament rupture; CrCLR, cranial cruciate ligament rupture; ED-FCP, elbow dysplasia-fragmented coronoid process; OA, osteoarthritis; OCD, osteochondritis dissecans.

| Group | Breed | Age (months) | Bodyweight (kg) | Sex | Joint | Disease | Score (1-4) | FNT (Number of boluses) | General anaesthesia time (min) | Surgery time (min) |
|---|---|---|---|---|---|---|---|---|---|---|
| **L** | Labrador Retriever | 84 | 31 | F | Knee | CrCLR | 1 | 0 | 125 | 52 |
| **L** | German Shepherd | 72 | 34 | M | Elbow | OA | 2 | 2 | 135 | 53 |
| **L** | Labrador Retriever | 96 | 28 | F | Elbow | OA | 2 | 2 | 145 | 60 |
| **L** | Labrador Retriever | 7 | 27 | M | Elbow | ED-FCP | 1 | 0 | 130 | 54 |
| **L** | Cane Corso | 7 | 28 | F | Shoulder | OCD | 3 | 1 | 135 | 50 |
| **L** | German Shepherd | 15 | 42 | M | Knee | OCD | 2 | 1 | 100 | 51 |
| **L** | Border Collie | 16 | 19 | F | Shoulder | OCD | 3 | 4 | 140 | 50 |
| **L** | Bouledogue Francois | 12 | 10 | M | Knee | CaCLR | 3 | 2 | 105 | 53 |
| **D** | American Staffordshire Terrier | 26 | 24 | M | Elbow | ED-FCP | 1 | 0 | 130 | 56 |
| **D** | Bernese Mountain Dog | 14 | 33 | F | Shoulder | OCD | 2 | 0 | 105 | 54 |
| **D** | Labrador Retriever | 18 | 31 | M | Elbow | ED-FCP | 2 | 0 | 145 | 58 |
| **D** | American Staffordshire Terrier | 26 | 24 | M | Elbow | ED-FCP | 1 | 0 | 105 | 53 |
| **D** | Labrador Retriever | 18 | 32 | M | Elbow | ED-FCP | 3 | 1 | 125 | 50 |
| **D** | Bernese Mountain Dog | 14 | 33 | F | Shoulder | OCD | 2 | 0 | 96 | 50 |
| **D** | Cane Corso | 60 | 56 | F | Elbow | ED-FCP | 3 | 0 | 131 | 52 |
| **D** | German Pointer | 78 | 25 | M | Shoulder | OA | 2 | 0 | 148 | 50 |
| **LD** | Mixed breed | 36 | 36 | F | Elbow | OA | 3 | 0 | 149 | 57 |
| **LD** | Labrador Retriever | 58 | 28 | F | Knee | CaCLR | 1 | 0 | 140 | 61 |
| **LD** | Labrador Retriever | 9 | 27 | M | Elbow | ED-FCP | 3 | 1 | 110 | 55 |
| **LD** | Mixed breed | 26 | 21 | M | Elbow | ED-FCP | 2 | 0 | 111 | 53 |
| **LD** | Rottweiler | 74 | 61 | M | Elbow | ED-FCP | 1 | 0 | 140 | 50 |
| **LD** | Mixed breed | 26 | 21 | M | Elbow | ED-FCP | 2 | 0 | 135 | 60 |
| **LD** | Golden Retriever | 9 | 27 | M | Elbow | ED-FCP | 1 | 0 | 120 | 51 |
| **LD** | Cane Corso | 9 | 28 | F | Shoulder | OCD | 1 | 0 | 100 | 50 |

There were no significant differences between groups regarding HR, SAP, MAP and DAP at any evaluation times (Fig. 1). In the LD group, SAP was significantly higher at T10, T15 and T20 when compared with T0, with a *p* value of 0.027, 0.021 and 0.022 respectively (Fig. 1). In the LD and D groups, MAP was significantly higher at T10 ($p = 0.024$ and $p = 0.022$ respectively), T15 ($p = 0.09$ and $p = 0.024$ respectively) and T20 ($p = 0.021$ and $p = 0.019$ respectively), compared with T0 (Fig. 1). In the LD and D groups, DAP was significantly higher at T10 ($p = 0.047$ and $p = 0.026$ respectively), T15 ($p = 0.023$ and $p = 0.021$ respectively), T20 ($p = 0.012$ and $p = 0.011$ respectively) and T25 ($p = 0.027$ and $p = 0.019$ respectively), compared with T0; no hypotension was recorded (Fig. 1). In the LD group HR was significantly lower at T5, T10 and T15 when compared with T0, with a *p* value of 0.031, 0.026 and 0.034 respectively (Fig. 1). In the LD group, 5/8 dogs experienced a self-limiting second-degree atrioventricular block in the first 20 minutes after IA lidocaine-dexmedetomidine administration, while in the L and D groups no atrioventricular block were detected ($p = 0.002$). No dogs required atrioventricular block treatment and no other cardiac arrhythmias were recorded.

**Figure 1**. Heart rate (HR; beats per min, bpm), systolic (SAP, mmHg), mean (MAP, mmHg) and diastolic (DAP, mmHg) arterial blood pressure measurements (mean ± standard deviation) at different time points (baseline: T0, 5 min before intra-articular injection; intra-articular injection: TIA; intraoperative time points: 5 min intervals) for dogs undergoing arthroscopy. Dogs were treated with intra-articular (IA) lidocaine (group L, $n = 8$), dexmedetomidine (group D, $n = 8$) or lidocaine-dexmedetomidine combination (group LD, $n = 8$). *Significantly different from T0 within the same treatment ($P \leq 0.05$).




Significantly more fentanyl boluses were required in the L group ($p$ = 0.03) than in the D and LD groups (Table 1). Regardless of groups, fentanyl boluses ($n$ = 14) were administered during skin incision ($n$ = 4/14), joint distension ($n$ = 4/14) and bone/cartilage debridement ($n$ = 6/14). Regarding FE'Iso, no significant differences were found within each group in comparison with T0; mean FE'Iso in the D group was significantly lower ($p$ = 0.001) than in the L and LD groups at TIA and at any consecutive time point (Table 2). No significant differences were found between groups in body temperature at any time point and within groups during the procedure (Table 2). No significant differences were detected in median SF-GCMPS scores between groups at the evaluated postoperative time points ($p$ = 0.121) (Table 3). One dog in the L group received methadone 4 hours after extubation and was removed from data analysis for the remaining postoperative time points. No systemic side effects or cutaneous alterations at the surgical site were observed during the 30-day follow-up period.

**Table 2.** Baseline (T0, 5 min before intra-articular injection, TIA), TIA and intraoperative time points (5 min intervals) end-tidal isoflurane concentration in % (FE'Iso) and body temperature (T, °C) measurements (mean ± standard deviation) for dogs undergoing arthroscopy treated with intra-

articular (IA) lidocaine (group L, *n* = 8), dexmedetomidine (group D, *n* = 8) or lidocaine-dexmedetomidine combination (group LD, *n* = 8). [a] Statistically different from all other treatments at the same time point ($p \leq 0.05$).

| Variable | Group | T0 | TIA | T5 | T10 | T15 | T20 | T25 | T30 | T35 | T40 | T45 | T50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FE'Iso | L | 1.5 ± 0.3 | 1.4 ± 0.3 | 1.4 ± 0.22 | 1.4 ± 0.3 | 1.4 ± 0.3 | 1.3 ± 0.3 | 1.3 ± 0.2 | 1.3 ± 0.2 | 1.3 ± 0.2 | 1.3 ± 0.2 | 1.3 ± 0.2 | 1.3 ± 0.2 |
| | D | 1.4 ± 0.2 | 1.3 ± 0.1 [a] | 1.2 ± 0.1 [a] | 1.2 ± 0.1 [a] | 1.2 ± 0.1 [a] | 1.1 ± 0.1 [a] | 1.1 ± 0.1 [a] | 1.1 ± 0.1 [a] | 1.1 ± 0.1 [a] | 1.1 ± 0.1 [a] | 1.1 ± 0.1 [a] | 1.1 ± 0.1 [a] |
| | LD | 1.5 ± 0.2 | 1.4 ± 0.2 | 1.3 ± 0.1 | 1.3 ± 0.1 | 1.3 ± 0.1 | 1.3 ± 0.2 | 1.2 ± 0.1 | 1.3 ± 0.1 | 1.3 ± 0.1 | 1.3 ± 0.1 | 1.2 ± 0.1 | 1.2 ± 0.2 |
| T | L | 38.1 ± 0.3 | 37.1 ± 0.4 | 37.0 ± 0.3 | 36.9 ± 0.3 | 37.0 ± 0.3 | 36.8 ± 0.3 | 36.8 ± 0.3 | 36.7 ± 0.3 | 36.7 ± 0.4 | 36.7 ± 0.4 | 36.6 ± 0.4 | 36.5 ± 0.5 |
| | D | 38.3 ± 0.3 | 37.3 ± 0.4 | 37.1 ± 0.4 | 37.1 ± 0.6 | 37.0 ± 0.5 | 37.0 ± 0.5 | 37.0 ± 0.5 | 36.9 ± 0.5 | 36.9 ± 0.5 | 36.9 ± 0.5 | 36.8 ± 0.4 | 36.7 ± 0.4 |
| | LD | 37.7 ± 0.5 | 36.8 ± 0.6 | 36.7 ± 0.7 | 36.6 ± 0.7 | 36.5 ± 0.7 | 36.4 ± 0.7 | 36.4 ± 0.7 | 36.3 ± 0.3 | 36.3 ± 0.7 | 36.3 ± 0.7 | 36.2 ± 0.7 | 36.2 ± 0.7 |

**Table 3.** Median (95% confidence intervals) post-operative Short Form-Glasgow Composite Measure Pain Scale (SF-GCPS) pain scores for dogs undergoing arthroscopy treated with intra-articular (IA) lidocaine (group L, *n* = 8), dexmedetomidine (group D, *n* = 8) or lidocaine-dexmedetomidine combination (group LD, *n* = 8). One dog in L group was administered methadone at 4 hours after extubation and was excluded from data analysis for the remaining postoperative time points.

| Group | Time (h) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 8 | 10 | 12 |
| L | 2 (1-5) | 2.5 (1-5) | 2.5 (1-5) | 2.5 (1-8) | 2 (1-5) | 3 (2-5) | 3 (2-5) | 2 (1-5) | 2 (1-5) |
| D | 1 (0-2) | 1.5 (0-2) | 2 (1-4) | 2.5 (1-5) | 2.5 (1-5) | 3 (1-5) | 3 (1-5) | 3 (1-4) | 2.5 (1-4) |
| LD | 1 (1-2) | 1 (1-2) | 2 (1-4) | 3.5 (2-4) | 3.5 (2-5) | 3 (2-4) | 3 (1-5) | 1.5 (1-3) | 1.5 (1-3) |

## 3.4 Discussion

The findings of this study suggested a possible systemic absorption of IA dexmedetomidine. Furthermore, IA lidocaine-dexmedetomidine was associated with a greater incidence of atrioventricular blocks. In contrast to what had been hypothesized, IA dexmedetomidine, alone or combined with lidocaine, provided a good level of analgesia during the arthroscopic surgery, reducing intraoperative fentanyl requirement more than lidocaine alone. All IA analgesic protocols produced an adequate 12 hours postoperative pain relief.

It is reported that IA drug administration can cause an eventual absorption of the injected drugs from the joint into the bloodstream and this event can lead to systemic effects in dogs[9] and horses[19]. Our results suggested a possible systemic uptake of dexmedetomidine from the IA injection site since the effects on systemic blood pressure in the D and LD groups were characterized by a significant increase with respect to baseline in the first 25 minutes after IA injection. This transient increase suggested a possible dexmedetomidine systemic effect. Dexmedetomidine causes an initial increase in blood pressure that results from peripheral vasoconstriction caused by activation of post-synaptic $\alpha_2$-receptors in peripheral vascular smooth muscle[11]. Moreover, a significant decrease in HR and higher frequency of atrioventricular blocks was observed after IA lidocaine-dexmedetomidine injection. Although a retarding action of dexmedetomidine on absorption of locally administered lidocaine has been proposed[20], the effect of lidocaine on the absorption of dexmedetomidine has not been studied. However, results of a previous study demonstrated the potential for lidocaine to accelerate the absorption of epinephrine in humans, due to its vasodilating action[21]. The results of the present study suggested that lidocaine might have accelerated and increased the systemic uptake of dexmedetomidine, leading to a transient decrease in HR and to a higher frequency of appearance of atrioventricular blocks.

To the authors' knowledge, postoperative IA analgesic efficacy of dexmedetomidine has been demonstrated in dogs[15], while this is the first study showing its analgesic efficacy during the intraoperative period. The intra-articular analgesic action of dexmedetomidine seems to result from direct local action in humans[12], dogs[15] and horses[22], although systemic absorption cannot be excluded. Additionally, expression of $\alpha_2$-adrenergic receptors in chondrocytes suggests possible effects of this drug on cellular signalling pathways[22,23]. Another possible mechanism is the dexmedetomidine modulation activity of transient receptor potential vanilloid 1 (TRPV1), demonstrated in mice[24]. TRPV1 is expressed in osteoarthritic joints, playing a role in the development of inflammatory and chronic pain[25]. Furthermore, $\alpha_2$-agonists are reported to provide local anaesthetic effects by inhibiting the conduction of nerve signals and may stimulate the release of enkephalin-like substances at peripheral sites[26].

The intraoperative use of analgesic drugs as part of a balanced anaesthetic protocol aims to reduce the requirement for volatile anaesthetics in dogs[27]. In the present study, treatment with IA dexmedetomidine was associated with a significant decrease in FE'Iso compared to the L and LD groups at TIA and any consecutive time point. This result could not be ascribed to a specific IA protocol since the significant difference was already present at the time of IA injection. The clinical

variables used in this study to assess the anaesthetic depth were subjective and may have led to biased FE'Iso values. An objective scoring system to evaluate anaesthetic depth should have been used to make comparison between groups more reliable.

No significant differences in the SF-GCMPS scores were detected between groups at any postoperative time points, although one dog in the L group received methadone 4 hours after extubation. This result may indicate that IA lidocaine and dexmedetomidine, alone or combined, were able to manage pain in the 12-hour post-operative period. The preoperative IA drugs administration as well as the use of a multimodal analgesic protocol may have contributed to the similar analgesic effect during the postoperative period in these three groups. It is likely that carprofen could have masked the detection of significant differences among groups.

This study has several limitations, one of which is the absence of a control group. Furthermore, dogs had different articular pathologies with various degrees of tissue inflammation and osteoarthritis. These factors could have affected the drugs' absorption from the joint into the bloodstream and, consequently, the drugs' systemic and local effects. Further studies are advocated to evaluate the effects of IA lidocaine, dexmedetomidine or their combination on the synovial structures in terms of chondrotoxicity, and the presence and function of $\alpha_2$-adrenergic receptors in canine joints. Di Salvo et al. (2016)[28] demonstrated that the exposure of canine chondrocytes to 0.5% lidocaine produced no significant reduction in cell viability *in vitro*; furthermore, a significant improvement in cell viability was noted if lidocaine 0.5% was administered in combination with epinephrine. It is possible to propose that the effects of 0.5% lidocaine on chondrocytes viability could be enhanced by dexmedetomidine, by exerting a protective effect similar to that caused by epinephrine. To the authors' knowledge, there are no studies investigating $\alpha_2$-adrenoceptor agonists cytotoxic activity on canine chondrocytes however, the dexmedetomidine dose used in the present study did not affect cellular viability in equine chondrocytes[22].

## 3.5 Conclusions

As part of multimodal approach to pain treatment for dogs undergoing arthroscopy, IA dexmedetomidine, alone or in combination with lidocaine, provided better intraoperative analgesia compared with IA lidocaine; treatments resulted in similar postoperative analgesic effects. Effects on HR and arterial blood pressure suggested systemic absorption of IA dexmedetomidine. Although cardiovascular variables remained within physiologically acceptable limits, the addition of lidocaine to IA dexmedetomidine increased the incidence of atrioventricular blocks.

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# 4. Oral transmucosal cannabidiol oil formulation as part of a multimodal analgesic regimen: effects on pain relief and quality of life improvement in dogs affected by spontaneous osteoarthritis

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***Brioschi FA**, Rabbogliatti V, Gioeni D, Di Cesare F, Valentini Visentin M, Ravasio G. Effect of oral-transmucosal cannabidiol on pain and quality of life in dogs affected by osteoarthritis*

## 4.1 Introduction

Osteoarthritis (OA) is a progressive and degenerative condition that affects dog populations and causes pain and crepitus in joints, decreased mobility and reluctance to exercise[1]. It is one of the main causes of chronic pain in dogs, owing to both active inflammation and to a maladaptive component caused by central sensitization to pain[2]. Management of osteoarthritic pain includes treatment with anti-inflammatory drugs; non-steroidal (NSAIDs) or corticosteroids. The potential side effects of these drugs may preclude long-term use, particularly in geriatric patients with comorbidities, such as kidney and gastrointestinal diseases[3,4]. Furthermore, clinical experience[5] and a review of experimental studies[6,7], clearly state that anti-inflammatory drugs do not provide complete pain relief in dogs with OA. Adjunctive medications with analgesic properties (e.g. gabapentin and amitriptyline) are used in combination with anti-inflammatory therapy in human patients[8], and a similar approach has been suggested in dogs[5]. Gabapentin is an anticonvulsant drug that exerts its analgesic effects via blockade of voltage-dependent calcium channels[9]. Due to this mechanism of action, it can be used in dogs affected by OA for pain management with minimal side effects, though owners should be warned about possible sedation when beginning administration[10]. Amitriptyline is a tricyclic antidepressant drug that inhibits the reuptake of serotonin and norepinephrine in the central nervous system and is therefore expected to reinforce the descending

inhibitory nociceptive modulation[9]. To the authors knowledge, there are no clinical trials or experimental studies evaluating the use of amitriptyline for OA-related pain in dogs, but an insight for their use can be gathered in human literature[11]. Anti-inflammatory drugs, gabapentin and amitriptyline are available options for long-term treatment in osteoarthritic dogs that experienced states of chronic unmanaged pain. However, there is still a lack of knowledge regarding their efficacy whether administered alone or in combination[2]. Moreover, since the lack of consensus in canine OA-related pain management, there is a constant search to find alternative therapies, and new treatments are often suggested and embraced despite the lack of proved clinical effectiveness[12].

Over the last three decades, a new biochemical and physiological receptor system, the endocannabinoid system, has been described[13]. The endocannabinoid receptor system, composed of two cannabinoid receptors (CB1 and CB2) and their ligands, plays a role in pain modulation and inflammation attenuation[14]. Cannabinoid receptors are widely distributed throughout the central and peripheral nervous system[13] and are also present in the human synovium[15]. Cannabidiol (CBD) is a non-psychotropic cannabinoid that exerts immunomodulatory, antihyperalgesic, antinociceptive and anti-inflammatory effects, acting as a non-competitive allosteric antagonist of CB receptors[16]. Given these pharmacological properties, CBD represents an attractive therapeutic option in dogs with OA[17]. Unfortunately, its bioavailability has been reported to be extremely low when given orally to dogs, presumably due to high first-pass effect through the liver[18].

Oral transmucosal (OTM) route is gaining importance in veterinary medicine, because of the advantages it offers over oral, intramuscular and intravenous administration for systemic drug delivery[19-21]. These major advantages are its easy practicability, lack of pain during administration, high blood mucosal supply and avoidance of the hepatic first-pass effect or gastrointestinal degradation[22,23].

The purpose of the study was to evaluate the efficacy of a CBD oil formulation, included within a multimodal pharmacological regimen, in alleviating pain in dogs affected by spontaneous OA, following OTM administration. Secondary objectives included the identification of any adverse clinical effect associated with 12-week multimodal pharmacological therapy, and in particular with CBD oil administered through OTM route. The authors hypothesized that CBD oil, administered to the buccal mucosa of dogs, would enhance the effectiveness of a selected multimodal analgesic protocol for the treatment of OA-related pain without causing greater side effects.

## 4.2 Materials and Methods

### 4.2.1 Animals

This study was approved by the Institutional Ethical Committee for Animal Care at the University of Milan (OPBA_15_2020) and all dogs were enrolled for CBD oil administration after obtaining owner's written informed consent. The study included twenty-four client-owned dogs, of different breed, age, body weight and gender, presented to the Veterinary Teaching Hospital of the University of Milan (Lodi, Italy) for evaluation and treatment of pain related to OA. Inclusion criteria were: radiographic evidence of OA (i.e. periarticular osteophytes, irregular or narrowed joint space and subchondral bone sclerosis), OA-associated signs of joint dysfunction (i.e. lameness, difficulty lying down, standing up, going up or down stairs, reluctance to jump or difficulty jumping) and painful joint(s) on palpation. Radiographic findings and OA localization were noted and recorded by a boarded radiologist. Patient screening at baseline (T0) included a physical examination, blood cell count and serum biochemical analysis. Exclusion criteria included demonstrated neurologic, neoplastic, renal or uncontrolled endocrine disease and history of coagulopathy. Dogs that received anti-inflammatory medications and/or other analgesic therapies or that underwent orthopedic procedures within four weeks prior the initial evaluation were excluded from the study.

### 4.2.2 Study Design

Dogs were enrolled over a period of 12 months and were involved in a 12-week multimodal therapeutic program for OA-related pain treatment. Upon enrollment, all subjects were randomly assigned to two groups (CBD and C), using a commercial software program (Microsoft Office Excel 2013; Microsoft Corp, USA). Regardless of the group considered, all dogs were orally administered an anti-inflammatory drug (i.e. firocoxib or prednisone), gabapentin and amitriptyline. In CBD group, patients received also a CBD oil at the dose of 2 mg $kg^{-1}$ every 12 hours, which was added to the multimodal pharmacological protocol and was administered by OTM route. In C group, the administration of CBD was not included. Firocoxib was the first-choice anti-inflammatory treatment. In case of reported adverse effects following NSAIDs assumption, prednisone administration was decided as an alternative to firocoxib. Specifically, the dose of anti-inflammatory medications was lowered during the observational study period as follows: treatment was given orally for the first week at a standard dose (5 mg $kg^{-1}$ every 24 hours for firocoxib or 0.5 mg $kg^{-1}$ every 12 hours for prednisone), then the daily dose was reduced by 50% during the second week and decreased again by 50% during the remaining study period. In case of poor response to dosage lowering, defined as an increase of $\geq 1$ in Pain Severity Score (PSS) and/or $\geq 2$ in Pain Interference Score (PIS)[24], the anti-

inflammatory daily dose was restored to the previous higher dosage. Dogs also received oral gabapentin (10 mg $kg^{-1}$ every 12 hours during the first week, 5 mg $kg^{-1}$ every 12 hours during the remaining study period) and oral amitriptyline (1 mg $kg^{-1}$ every 24 hours for the entire study period). The CBD oil used in this study was a galenic formulation that can be prepared and sold only in authorized pharmacies. The CBD oil contained 40, 100, or 200 mg of CBD $mL^{-1}$, according to the patient weight, with only trace amounts of the other cannabinoids (< 0.01 mg $mL^{-1}$). The remaining ingredient was Medium Chain Triglycerides (MCT) oil. Access to food was withheld for one hours before CBD administration and was reinstated one hour post treatment. Water was given *ad libitum*. Oral transmucosal administration of the CBD oil was performed by the owner using a syringe without needle inserted into the buccal pouch. To assess the dog's pain and quality of life, owners were contacted through email and asked to complete the Canine Brief Pain Inventory (CBPI), a validated numeric rating scale-based questionnaire, which contained 11 questions on the dog's lameness, mood and willingness to move, play and jump[25,26]. Four questions required the owners to grade the severity of their dog's pain over the previous days. The 4 pain severity questions were scored on a discrete numerical scale of 0 (no pain) to 10 (extreme pain); the responses for these questions were averaged to generate the PSS[25]. Six questions evaluated the pain interference with dog's general activity, enjoyment of life and locomotive function. The 6 pain interference questions were scored on a discrete numerical scale of 0 (does not interfere) to 10 (completely interferes); the responses for these questions were averaged to generate the PIS[25]. In addition, a final question was included at the end of the questionnaire to obtain the owner's overall assessment of the dog's quality of life (Quality of Life Index, QoL)[26]. Question 11 (QoL) was graded on a discrete 0 to 4 numerical scale, with 0 representing a poor quality of life and 4 an excellent quality of life. The owners received an Italian version of the CBPI questionnaire, translated and reviewed by three authors who were expert in chronic pain management and fluent in the original and target languages. All of the owners were asked to evaluate their dogs based on CBPI scoring system before treatment initiation (T0) and at one (T1), two (T2), four (T3) and twelve weeks (T4) thereafter. Mean CBPI results for each time point were compared between CBD and C groups, and mean CBPI results for T1, T2, T3 and T4 were compared with T0 within each group. Individual treatment success, defined as a reduction of $\geq 1$ in PSS and $\geq 2$ in PIS[24], was also calculated. Furthermore, owners were asked to record the occurrence of any mild to moderate or severe adverse event; mild ptyalism and temporary somnolence were considered mild to moderate adverse effects (slightly interfering with the dog's usual habit), while serious ptyalism, gastrointestinal disorders, lethargy and changes in

behavior or distress were considered severe (significantly interfering with the dog's usual habit). Blood cell count and serum biochemical analysis were performed at the end of the twelve-week evaluation period.

### 4.2.3 Statistical Analysis

An *a priori* sample size calculation was performed to determine the number of dogs needed for this study, with 80% power, an alpha level of 0.05 and a 95% confidence interval, using prior data suggesting a Canine Brief Pain Inventory (CBPI) total score change of 3 out of 20 points from baseline as an indicator of successful treatment, with a standard deviation of 4 out of 20 points[27]. Calculation assessed that 7 dogs for each group would be necessary to find differences in outcomes of interest. Statistical analysis was performed using PASW 18.0 (SPSS Inc, Chicago, USA). The assumption of data normality was examined by a Shapiro-Wilk test with an $\alpha = 0.05$ level. Results are presented as mean ± standard deviation (SD) or as number of patients (%) where appropriate. For continuous variables that were normally distributed, comparisons between CBD and C groups were performed with independent Student's t-test. The same approach was used to assess differences for each group in relation to time. For categorical variables, Fisher exact test was used to compare differences between the treatment groups. Statistical significance was set at $p < 0.05$.

## 4.3 Results

Twenty-one out of 24 client-owned dogs met the inclusion criteria, were enrolled in the study and assigned to the CBD group (n = 9) or to the C group (n = 12). Reasons for withdrawal for the other three dogs included presence of neurologic abnormalities during baseline evaluation (n = 1 dog in the C group) and owner's inability to return CBPI questionnaire (n = 2 dogs in the CBD group). Table 1 summarizes dogs' information about radiographic findings and OA localization of recruited dogs. Table 2 summarizes dogs' information about breed, age, weight, gender and dosages of the analgesics included in the multimodal protocol. The statistical analysis detected no differences between group CBD and C with respect to age ($p = 0.07$), weight ($p = 0.06$) and gender ($p = 1.00$), highlighting the homogeneity of groups.

**Table 1**. Radiographic findings and osteoarthritis (OA) involved joint of the dogs recruited in CBD (n = 9) and C (n = 12) groups.

| | Group | Radiographic findings | OA localization |
|---|---|---|---|
| **1** | **CBD** | Moderate left stifle OA with intracapsular swelling | Left stifle |
| **2** | **CBD** | Moderate bilateral elbow OA, mild bilateral coxofemoral OA | Left elbow, right elbow, bilateral hip |
| **3** | **CBD** | Severe right elbow OA | Right elbow |
| **4** | **CBD** | Severe medial coronoid remodelling (with fragmentation on the right) and bilateral elbow OA | Left elbow, right elbow |
| **5** | **CBD** | Severe right medial coronoid remodeling, and bilateral elbow OA | Left elbow, right elbow |
| **6** | **CBD** | Moderate left medial coronoid remodeling, severe left elbow OA | Left elbow |
| **7** | **CBD** | Severe right stifle OA with moderate intracapsular swelling, bilateral moderate coxofemoral OA | Right stifle, bilateral hip |
| **8** | **CBD** | Bilateral severe stifle OA due to cranial cruciate ligament disease | Left stifle, right stifle |
| **9** | **CBD** | Moderate-to-severe bilateral coxofemoral OA | Bilateral hip |
| **1** | **C** | Moderate right coxofemoral OA, severe left coxofemoral OA | Bilateral hip |
| **2** | **C** | Severe right shoulder OA, moderate right elbow OA | Right shoulder, right elbow |
| **3** | **C** | Severe bilateral elbow OA, moderate bilateral coxofemoral OA | Left elbow, right elbow, bilateral hip |
| **4** | **C** | Moderate right shoulder OA | Right shoulder |
| **5** | **C** | Severe bilateral elbow OA, moderate bilateral coxofemoral OA | Left elbow, right elbow, bilateral hip |
| **6** | **C** | Bilateral severe coxofemoral OA | Bilateral hip |
| **7** | **C** | Severe right elbow OA | Right elbow |
| **8** | **C** | Severe bilateral coxofemoral OA | Bilateral hip |
| **9** | **C** | Severe right elbow OA, mild left stifle OA | Right elbow, left stifle |
| **10** | **C** | Moderate bilateral coxofemoral OA | Bilateral hip |
| **11** | **C** | Moderate right shoulder OA, severe bilateral elbow OA | Left elbow, right elbow, right shoulder |
| **12** | **C** | Severe bilateral coxofemoral OA | Bilateral hip |

**Table 2**. Breed, age, weight, gender and analgesic therapies administered to the dogs recruited in CBD (n = 9) and C (n = 12) groups. SID, once daily; BID, twice daily.

| | Group | Breed | Age (months) | Weight (kg) | Gender | NSAIDs | Glucocorticoids | Gabapentin | Amitriptyline | CBD |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CBD | Mongrel | 156 | 23 | Female | Firocoxib (5-1.25 mg $kg^{-1}$ SID) | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | 2 mg $kg^{-1}$ BID |
| 2 | CBD | Épagneul Breton | 144 | 18 | Female | None | Prednisone (0.5-0.12 mg $kg^{-1}$ BID) | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | 2 mg $kg^{-1}$ BID |
| 3 | CBD | English Bulldog | 96 | 25 | Male | Firocoxib (5-2.5 mg $kg^{-1}$ SID) | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | 2 mg $kg^{-1}$ BID |
| 4 | CBD | Cane Corso | 125 | 45 | Female | Firocoxib (5-2.5 mg $kg^{-1}$ SID) | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | 2 mg $kg^{-1}$ BID |
| 5 | CBD | Labrador Retriever | 110 | 45 | Male | Firocoxib (5-1.25 mg $kg^{-1}$ SID) | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | 2 mg $kg^{-1}$ BID |
| 6 | CBD | Dogue de Bordeaux | 84 | 60 | Male | Firocoxib (5-1.25 mg $kg^{-1}$ SID) | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | 2 mg $kg^{-1}$ BID |
| 7 | CBD | Border Collie | 156 | 20 | Male | None | Prednisone (0.5-0.12 mg $kg^{-1}$ BID) | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | 2 mg $kg^{-1}$ BID |
| 8 | CBD | Boxer | 108 | 33 | Male | Firocoxib (5-1.25 mg $kg^{-1}$ SID) | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | 2 mg $kg^{-1}$ BID |
| 9 | CBD | Boxer | 108 | 40 | Female | Firocoxib (5-1.25 mg $kg^{-1}$ SID) | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | 2 mg $kg^{-1}$ BID |
| 1 | C | Australian Sheperd | 156 | 24 | Male | Firocoxib (5-1.25 mg $kg^{-1}$ SID) | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |
| 2 | C | Labrador Retriever | 152 | 41 | Male | Firocoxib (5-1.25 mg $kg^{-1}$ SID | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |
| 3 | C | Golden Retriever | 173 | 29 | Male | Firocoxib (5-2.5 mg $kg^{-1}$ SID | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |
| 4 | C | Cocker Spaniel | 167 | 13 | Female | Firocoxib (5-2.5 mg $kg^{-1}$ SID | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |
| 5 | C | Labrador Retriever | 161 | 30 | Female | Firocoxib (5-1.25 mg $kg^{-1}$ SID | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |
| 6 | C | German Sheperd | 115 | 25 | Female | Firocoxib (5-1.25 mg $kg^{-1}$ SID) | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |
| 7 | C | Labrador Retriever | 153 | 34 | Male | None | Prednisone (0.5-0.12 mg $kg^{-1}$ BID) | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |
| 8 | C | German Sheperd | 108 | 25 | Female | None | Prednisone (0.5-0.12 mg $kg^{-1}$ BID) | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |
| 9 | C | Mongrel | 180 | 10 | Male | Firocoxib (5-2.5 mg $kg^{-1}$ SID | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |
| 10 | C | Mongrel | 127 | 22 | Male | None | Prednisone (0.5-0.12 mg $kg^{-1}$ BID) | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |
| 11 | C | English Bulldog | 108 | 27 | Female | Firocoxib (5-2.5 mg $kg^{-1}$ SID) | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |
| 12 | C | Mongrel | 182 | 18 | Male | Firocoxib (5-1.25 mg $kg^{-1}$ SID) | None | 10-5 mg $kg^{-1}$ BID | 1 mg $kg^{-1}$ SID | None |

Baseline scores for PSS (5 ± 2 in CBD group and 6 ± 2 in C group, $p = 0.29$), PIS (6 ± 2 in CBD group and 7 ± 2 in C group, $p = 0.24$) and QoL (3 ± 1 in CBD group and 2 ± 1 in C group, $p = 0.12$) were similar between groups. Pain Severity Score was significantly lower in CBD than in C group at one (T1), two (T2) and four weeks (T3) after treatment initiation: 3 ± 2 versus 7 ± 2 ($p = 0.0002$), 3 ± 1 versus 5 ± 2 ($p = 0.0043$) and 3 ± 2 versus 5 ± 2 ($p = 0.016$), respectively. Pain Interference Score was significantly lower in CBD than in C group at one (T1), two (T2) and twelve weeks (T4) after treatment initiation: 2 ± 1 versus 7 ± 2 ($p = 0.0002$), 3 ± 1 versus 6 ± 2 ($p = 0.0007$) and 2 ± 1 versus 6 ± 2 ($p = 0.004$), respectively. Quality of Life Index was significantly higher in CBD than in C group at T1: 4 ± 1 versus 2 ± 1 ($p = 0.003$), respectively. The PSS, PIS and QoL scores of the dogs recruited in CBD and C group are reported in Table 3.

Within CBD group, the comparison of mean PSS, PIS and QoL scores between T0 and each successive time point showed a decrease in PSS between baseline and T2 ($p = 0.01$) and between baseline and T3 ($p = 0.03$). Pain Interference score (PIS) was significantly lower in CBD group at T1 ($p = 0.001$), T2 ($p = 0.0007$), T3 ($p = 0.04$), and T4 ($p = 0.004$) compared to baseline. In CBD group, QoL increased at T1 ($p = 0.008$), T2 ($p = 0.04$), and T4 ($p = 0.01$) compared with baseline. Despite no significant variations in PSS, PIS and QoL scores between baseline and other examined periods, dogs assigned to group C experienced a decrease in pain scores and an improvement in QoL.

Treatment was successful in reducing PSS in 6 out of 9 (67%) dogs of group CBD at T1, T2 and T3 and in 5 out of 9 dogs at T4 (56%). In group C, considering PSS, treatment was classified as successful in 1 out of 12 (8%) dog at T1, 2 out of 12 (17%) dogs at T2 and T4 and 3 out of 12 (25%) dogs at T3. When considering PIS, treatment in group CBD was successful in 6 out of 9 (67%) dogs at T1 and T2, 5 out of 9 (56%) dogs at T3 and 4 out of 9 (44%) dogs at T4. In group C, considering PIS, treatment was classified as successful only in one dog (8%) at T2, T3 and T4.

**Table 3**. Pain Severity Score (PSS), Pain Interference Score (PIS) and Quality of Life Index (QoL) (adopted by Brown et al. 2008) of the dogs enrolled in CBD (n = 9) and C (n = 12) groups. $p<0.05$ between groups at the same time point (*). $p<0.05$ intra CBD group compared to baseline (T0): PSS T0 versus PSS T2 (a); PSS T0 versus PSS T3 (b); PIS T0 versus PIS T1 (c), PIS T0 versus PIS T2 (d); PIS T0 versus PIS T3 (e); PIS T0 versus PIS T4 (f); QoL T0 versus QoL T1 (g); QoL T0 versus QoL T2 (h), QoL T0 versus QoL T4 (i). No statistical differences intra C group compared to baseline (T0).

| Time point | | T0 | | | T1 | | | T2 | | | T3 | | | T4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Score | | PSS | PIS | QoL | PSS | PIS | QoL | PSS | PIS | QoL | PSS | PIS | QoL | PSS | PIS | QoL |
| Sig. | | a,b | c,d,e,f | g,h,i | * | *, c | *, g | *, a | *, d | h | *,b | e | | | *, f | i |
| 1 | CBD | 4 | 5 | 3 | 3 | 2 | 3 | 2 | 4 | 3 | 2 | 4 | 3 | 2 | 3 | 4 |
| 2 | CBD | 2 | 2 | 3 | 1 | 1 | 4 | 1 | 1 | 4 | 1 | 1 | 4 | 2 | 1 | 3 |
| 3 | CBD | 5 | 5 | 3 | 1 | 3 | 4 | 3 | 4 | 3 | 6 | 6 | 3 | 6 | 3 | 3 |
| 4 | CBD | 9 | 9 | 1 | 4 | 5 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 1 | 4 |
| 5 | CBD | 5 | 6 | 2 | 2 | 1 | 4 | 3 | 2 | 4 | 3 | 3 | 4 | 5 | 4 | 4 |
| 6 | CBD | 9 | 9 | 2 | 5 | 3 | 3 | 5 | 2 | 2 | 2 | 6 | 2 | 3 | 2 | 2 |
| 7 | CBD | 5 | 6 | 3 | 5 | 4 | 4 | 4 | 3 | 3 | 3 | 4 | 4 | 3 | 4 | 4 |
| 8 | CBD | 3 | 7 | 3 | 1 | 1 | 4 | 3 | 4 | 3 | 5 | 6 | 3 | 5 | 2 | 3 |
| 9 | CBD | 6 | 8 | 3 | 2 | 2 | 3 | 3 | 4 | 3 | 4 | 5 | 3 | 4 | 2 | 4 |
| mean | | 5.33 | 6.33 | 2.55 | 2.66 | 2.44 | 3.55 | 3 | 3 | 3.11 | 3.22 | 4.33 | 3.22 | 3.66 | 2.44 | 3.44 |
| SD | | 2.4 | 2.2 | 0.7 | 1.6 | 1.4 | 0.5 | 1.2 | 1.1 | 0.6 | 1.5 | 1.6 | 0.6 | 1.4 | 1.1 | 0.7 |
| 1 | C | 4 | 8 | 3 | 7 | 7 | 3 | 4 | 7 | 4 | 4 | 4 | 4 | 3 | 6 | 4 |
| 2 | C | 8 | 8 | 1 | 8 | 8 | 1 | 8 | 9 | 1 | 8 | 8 | 2 | 7 | 8 | 2 |
| 3 | C | 9 | 9 | 2 | 9 | 8 | 1 | 8 | 8 | 2 | 7 | 7 | 2 | 7 | 9 | 3 |
| 4 | C | 7 | 8 | 2 | 8 | 7 | 2 | 7 | 7 | 3 | 7 | 6 | 3 | 6 | 7 | 1 |
| 5 | C | 4 | 8 | 2 | 5 | 8 | 2 | 4 | 8 | 2 | 4 | 6 | 2 | 3 | 8 | 2 |
| 6 | C | 6 | 6 | 3 | 9 | 5 | 3 | 4 | 5 | 3 | 6 | 4 | 3 | 6 | 5 | 3 |
| 7 | C | 5 | 6 | 3 | 5 | 5 | 3 | 3 | 2 | 4 | 3 | 4 | 4 | 2 | 2 | 4 |
| 8 | C | 2 | 3 | 4 | 3 | 3 | 4 | 3 | 3 | 4 | 2 | 1 | 4 | 2 | 3 | 4 |
| 9 | C | 8 | 9 | 1 | 6 | 9 | 1 | 7 | 8 | 2 | 6 | 7 | 1 | 7 | 8 | 3 |
| 10 | C | 7 | 9 | 2 | 7 | 8 | 1 | 6 | 8 | 2 | 7 | 7 | 2 | 7 | 9 | 3 |
| 11 | C | 7 | 8 | 2 | 7 | 7 | 2 | 7 | 7 | 2 | 7 | 6 | 3 | 6 | 6 | 3 |
| 12 | C | 3 | 5 | 2 | 5 | 5 | 2 | 3 | 5 | 2 | 3 | 3 | 2 | 3 | 5 | 2 |
| mean | | 5.83 | 7.25 | 2.25 | 6.58 | 6.66 | 2.08 | 5.3 | 6.41 | 2.58 | 5.33 | 5.25 | 2.66 | 4.92 | 6.33 | 2.83 |
| SD | | 2.2 | 1.9 | 0.8 | 1.8 | 1.7 | 0.9 | 2.0 | 2.2 | 0.9 | 2.0 | 2.1 | 1.0 | 2.1 | 2.3 | 0.9 |

Within group CBD, 7 out of 9 (78%) dogs received firocoxib and 2 out of 9 (22%) received prednisone. Within group C, 9 out of 12 (75%) dogs received firocoxib and 3 out of 12 (25%) received prednisone. No statistical differences between CBD and C groups were observed for firocoxib ($p$ = 0.47) and prednisone ($p$ = 0.47) administration. In addition, in 2 out of 7 (29%) dogs (CBD group) and 4 out of 9 (44%) dogs (C group) OA-related symptoms worsened shortly after firocoxib therapy was reduced to the lowest dose. However, increasing firocoxib to 50% of the standard dose resulted in reversal of this worsening.

In all dogs oral transmucosal CBD administration was well tolerated, with mild or absent gastrointestinal side effects. In two dogs in CBD group (2 out of 9, 22%) minimal ptyalism was observed, while in one dog in CBD group and in two dogs in C group (3 out of 21, 14%) somnolence and mild ataxia were reported. No relevant changes in the measured blood cell count and serum

biochemical analysis were noted in either the CBD or C groups at the end of the twelve-week evaluation period (data not shown).

## 4.4 Discussion

To the authors' knowledge, the present study is the first to evaluate the clinical effects of the OTM administration of CBD oil in dogs. A significant reduction in perceiving pain and a significant increase in quality of life was achieved in dogs affected by spontaneous OA receiving OTM CBD oil (2 mg $kg^{-1}$ every 12 hours) in addition to a multimodal analgesic regimen, compared with findings in dogs of the control group.

Because of the complex neurobiology of chronic pain, it is reasonable to believe that multimodal pharmacologic therapy is advantageous for the treatment of OA[28], although this approach has received poor attention in the veterinary literature[29]. Furthermore, the use of a multimodal therapeutic approach may reduce doses of analgesics and therefore their adverse effects[30]. The present study included a wide range of analgesic drugs, strengthening the importance of a multimodal treatment in dogs with osteoarthritic chronic pain. Osteoarthritis can cause hyperalgesia and evolve into neuropathic pain[31], therefore the use of analgesic adjuvants, such as amitriptyline and gabapentin, appears advisable. Despite the lack of high-quality evidence to support their use, in the Authors' experience gabapentin and amitriptyline have provided the most interesting results in pain relief in addition to NSAIDs therapy in dogs with OA. At present, NSAIDs and glucocorticoids are the most widely used drugs for OA treatment in animals[32]. The effects of these two groups of pharmaceuticals are similar as they both have anti-inflammatory effects, have direct effects on cartilage metabolism and may stimulate synthesis of interleukin-1[33,34]. The number of dogs that received NSAIDs or glucocorticoids in this study was similar between groups. Thanks to the similarity between the two treatment groups and to the proved affinity between the effects on OA of NSAIDs and glucocorticoids, it is possible to make a comparison between CBD and C groups.

In the present study, the CBPI questionnaire was used to detect changes in pain scores and to identify differences in terms of pain relief and quality of life improvement in response to treatment. This scoring system was specifically designed to quantify the intensity of pain and its impact on daily activities in dogs in their environment and it has been validated as an owner tool to assess OA-related pain[24,25]. The questionnaire is divided into a PSS, that assesses the magnitude of pain of an animal, a PIS, that assesses the degree by which pain affects daily activities and a global assessment

of the quality of life[25,26]. In the present study, the increase in comfort and activity for dogs included in CBD group was represented by lower PSS and PIS mean values, as well as higher QoL mean values, compared with group C, at each time point. Although these values did not always differ significantly, the improvement in PSS, PIS and QoL scores was consistent. In fact, changes from baseline values were found to be significantly different in group CBD at T2 and T3 for PSS, and at every time point for PIS. In CBD group, QoL increased at T1, T2, and T4 compared with baseline. Despite no significant variations in PSS, PIS and QoL scores between baseline and other examined periods, dogs assigned to group C experienced a progressive decrease in pain scores and an improvement in QoL. These findings, although not statistically significant, allow authors to suppose that even the combination between an anti-inflammatory, gabapentin and amitriptyline resulted in some beneficial effects in terms of pain relief and quality of life improvement. It is also possible that significant results within C group could be observed with a larger sample size. Recent evaluation of the ability of the CBPI to detect a significant improvement in osteoarthritic dogs treated with carprofen found that a decrease in PSS $\geq$ 1 and a decrease in PIS $\geq$ 2 resulted in the highest statistical power to predict whether a treatment would lead to a response in an individual dog[24]. When considering individual results in the present study, treatment success was obtained in more dogs in CBD group, compared with C group. Our results suggest that the changes detected might be due to a positive response to CBD OTM treatment, also in long-term use. In fact, a significative improvement in the CBPI scores was shown also at T4, after 12-week treatment with CBD. The use of oral CBD oil for osteoarthritic pain management in dogs has been previously studied[17]. This study demonstrated a significant decrease in PSS and PIS and a significant increase in dogs' activity at week 2 and 4, when compared to baseline, but long-term efficacy was not evaluated[17]. Pharmacologically, CBD has a complex signalling mechanism. It can both activate and silence cannabinoid receptors as well as modulate cannabinoid receptor pathways, influencing nociceptive signalling and reducing long-term inflammation progression[35]. Including CBD in a multimodal drug treatment is a strategy that the authors have used in order to manage more effectively OA-related pain in dogs. The results showed that this approach can be effective, suggesting that CBD may enhance concurrent analgesic drugs effects, probably by exerting a positive modulation at glycine and vanilloid TRPV1 receptors which play a central role in the development of OA[35]. Moreover, it is well known that cannabinoid system could be exposed to degradation by cyclooxygenase type 2 (COX-2), and that this important degradative pathway might convert cannabinoids into pro-inflammatory and pro-nociceptive mediators, such as prostamides, prostaglandins and prostacyclin glycerol esters[36]. Consequently,

NSAIDs that inhibit COX-2 could attenuate cannabinoids breakdown prolonging its effects, and selectively prevent the formation of pro-inflammatory and pro-nociceptive mediators[37]. Furthermore, it has been demonstrated that COX-2 play a role in central sensitisation and that COX-2 inhibitors can prevent this process[38]. Authors strongly advise the use of NSAIDs that inhibit COX-2 (unless specifically contraindicated) as part of a multimodal treatment for osteoarthritic pain in dogs, especially if CBD is co-administered, since this pharmacological interaction could lead to a progressive reduction in pain perceived by the animal. The benefits of this long-term therapy could include better control of pain, greater improvements in mobility and the potential slowing down of the osteoarthritic process through improved joint usage, even if the continuous administration of anti-inflammatories might lead to an increased incidence of adverse events. As a result, recent human guidelines suggest the administration of the lowest effective dose of NSAID to minimize side effects, and only for the time required[39]. To date, there are no studies concerning the long-term use of anti-inflammatory drugs in dogs, while a study conducted in human medicine by Luyten and colleagues (2007)[40] showed that there were no significant differences between patients exposed to either long-term or intermittent NSAIDs treatment, except for the intake of rescue analgesia, which was less frequent in the long-term treatment group. Another study, by Gunew and colleagues (2008)[41], reported that oral meloxicam was safe for long-term treatment of OA in cats, including those of advanced age. In the present study, firocoxib or prednisone were gradually decreased over time in order to reach the lowest effective dose, as a possible solution for the challenge of long-term osteoarthritic pain treatment in dogs. Concurrent analgesic therapies may have helped to reduce anti-inflammatory effective dosage, especially in dogs that received CBD in addition to the multimodal pharmacological protocol; in fact, the subjects in CBD group experienced a better response to firocoxib reduction to the lowest dose in comparison to dogs assigned to C group. Thus, according to the authors, including CBD and concurrent analgesic therapies (i.e. gabapentin and amitriptyline) within a multimodal analgesic protocol seems to be a promising strategy in dogs affected by OA, in order to minimize adverse effects occurrence associated to long-term anti-inflammatory drugs consumption.

A recent study in dogs has shown that delivery of CBD through an oil base appears to be the preferential method for absorption, while oil beadlets and transdermal do not appear as effective as infused oils[42]. In fact, the oil-based vehicle seems to be the first choice due to the lipophilic nature of CBD[43]. Unfortunately, CBD bioavailability has been reported to be low (ranged from 13 to 19%) when given orally to both dogs and humans, presumably due to high first-pass effect from the

liver[18,44] together with its demonstrated poor gastrointestinal permeability[45]. As the drug has low aqueous solubility and undergoes first-pass metabolism, alternative delivery routes are needed to achieve successful therapeutic effects by bypassing the first-pass effect. To confirm this statement, unpublished authors' data pointed out inadequate pain relief following oral CBD administration as a part of multimodal therapeutic protocol in dogs affected by spontaneous OA. In the aforementioned patients, the administration of CBD oil via OTM route, instead of oral route, resulted in satisfactory pain relief and in quality of life improvement. OTM route allows to avoid the first-pass metabolic effect and gastrointestinal degradation observed for the orally administered drugs. The rich blood supply of the oral mucosa allows drugs administered by this route to reach systemic therapeutic concentrations[22-23]. Moreover, OTM route represents an attractive alternative to other drug delivery routes, being a non-invasive, pain-free technique, which requires minimal restraint and does not cause distress in patients[46]. The easy practicability for the owner is another major advantage, requiring minimal technical skills compared to other routes of administration[23]. In humans, the development of an oromucosal spray that contains a roughly 1:1 ratio of THC and CBD has provided a non-invasive method of administration, that has proven to show clinically significant improvements for the symptomatic relief of chronic uncontrolled pain in advanced cancer patients[47]. However, OTM route can be more variable than IV or IM administration due to the possibility of swallowing the delivered dose, loss of the drug outside of the mouth, expelled medication by coughing or spitting and vomiting or ptyalism reducing or diluting the quantity of drug for absorption[48]. The oil formulation of CBD administered in the present study was flavourless, and this aspect may have prevented the incidence of marked ptyalism and vomiting. Mild and transient ptyalism was observed in two out of 9 dogs receiving CBD, while vomiting was absent, suggesting a suitable drug formulation palatability. Moreover, the oil formulation contained 40, 100, or 200 mg of CBD $mL^{-1}$, based on the patient weight, in order to minimize the administered volume. In fact, smaller OTM volumes result clinically more effective, having less chance of inducing swallowing, ptyalism and/or loss of drugs outside the mouth[49]. Further studies including a pharmacokinetic investigation of oral transmucosal administration of CBD, alone or in combination with other pharmacologic therapies, are required in order to assess the bioavailability of this drug administered by this type of route in dogs. Somnolence and mild ataxia were observed in one dog in CBD group and in two dogs in C group, but these adverse effects were transient and resolved immediately after gabapentin dosage reduction to 5 mg $kg^{-1}$ every 12 hours. Overall, there were no

moderate/severe clinical adverse effects and there was reliable pain relief and quality of life improvement.

The present study has several limitations. The effect on blood cell count and serum chemistry analysis of the 12-week treatment period was not statistically evaluated. No relevant change was noted in either the CBD or C groups, in accordance with the findings of a previous study showing no clinically significant alterations in blood cell count and serum chemistry during 12-week CBD-rich hemp products administration in healthy dogs[50]. However, a clinical population of osteoarthritic dogs that received oral CBD oil treatment exhibited a significant increase over time in alkaline phosphatase (ALP), from baseline to week 4[17]. Therefore, it could be prudent to monitor liver enzyme values (especially ALP) in dogs receiving CBD oil for long periods, until controlled long-term safety studies will be available. Moreover, the owner compliance to the treatment evaluation may have partially affected the results of the comparison between groups. However, although the CBPI has a subjective component, studies in dogs have indicated that owners are able to assess their pet response to analgesic therapy and that veterinarian chronic pain assessments are not as sensitive as owner assessments[51]. Although in the present study the English version of CBPI questionnaire was translated and reviewed by three authors who were expert in chronic pain management and fluent in the original and target languages, the Italian version of the CBPI questionnaire has not been previously validated and further validation studies are needed. Another important limitation is that a placebo oil was not administered in addition to the multimodal pharmacological protocol assigned to group C. This may have caused a placebo effect for the owners administering a specially formulated oil-medication to dogs in CBD group. However, the authors attempted to limit the potential for bias by blinding the owners of the existence of another treatment group to ensure they considered each of the assigned multimodal protocol as potentially effective.

## 4.5 Conclusions

Overall, according to the CBPI scores assigned by the owner, a satisfactory pain and quality of life management was achieved in dogs receiving OTM CBD oil (2 mg $kg^{-1}$ every 12 hours) in addition to a multimodal pharmacological approach for treatment of OA-related pain. Combined with an anti-inflammatory drug, gabapentin and amitriptyline, CBD appears to enhance osteoarthritic pain relief and quality of life improvement. Furthermore, its co-administration results useful in reducing the other administered drugs' dosage, minimizing the severity and incidence of associated side effects.

The high CBD patient tolerability, the easy practicability and the paucity of adverse effects of OTM route of administration may represent potential benefits for long-term therapy.

## 4.6 References


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# 5. Perineural and intra-articular radiofrequency in dogs affected by maladaptive chronic pain: a case series

## 5.1 Introduction

In dogs, maladaptive chronic pain associated with osteoarthritis (OA) or characterized by a neuropathic component may have a negative impact on quality of life, especially if traditional pharmacological treatments become ineffective[1]. Some dogs with advanced OA and others affected by neuropathic pain may not achieve adequate pain relief even when multimodal analgesic regimens are prescribed[2,3]; they may also develop severe adverse effects, as a consequence of prolonged pharmacological treatments[4]. In this scenario, a non-pharmacological modality that provides prolonged analgesia without systemic adverse effects would be a valuable therapeutic tool.

Thermal radiofrequency (TRF) and pulsed radiofrequency (PRF) of nerves are non-pharmacological and minimally invasive techniques that cause long-term relief of maladaptive chronic pain in people[5,6]. A cannula coated with insulation, except for its distal end, is positioned with the tip contacting the nerve to be treated. An electrode connected to a radiofrequency generator is inserted through the cannula until the end reaches the tip. Alternating current produced by the generator at radio wave frequency creates an electromagnetic field in the tissues surrounding the tip/electrode, generating heat[2]. During TRF, a target temperature of approximately 80°C is maintained, resulting in localized Wallerian degeneration of nerves; Wallerian degeneration prevents action potential transmission and conduction of nociceptive impulses, thereby relieving pain[7]. TRF is not used on motor nerves because Wallerian degeneration would cause motor deficits[5,8]. During PRF, the generator produces current in short, high-voltage "pulses" and the silent period between pulses allows heat to dissipate, resulting in average tissue temperatures of 38-42°C[9,10]. The exact mechanism by which PRF produces analgesia is unknown, despite clinical use in humans, but is thought to involve neuromodulation[9]. Because PRF should not damage nerves, it is used on motor nerves in humans, providing analgesia similar to TRF[7]. Furthermore, intra-articular application of PRF has been reported in different studies as a safe and efficacious technique for pain reduction and mobility improvement in humans patients affected by degenerative OA[11,12].

In a recent study, Wallerian degeneration in TRF-treated canine saphenous nerves appears sufficient to impair transmission of noxious stimuli[2]; PRF of the sciatic nerves did not cause degeneration and motor deficits in treated dogs[2] however, clinical trials are needed, to confirm that both techniques produce analgesia and an improvement of quality of life in dogs affected by maladaptive chronic pain. The present study aimed to evaluate the efficacy of perineural TRF and perineural or intra-articular PRF, in alleviating maladaptive chronic pain in dogs. The secondary objective included the identification of any adverse effects associated with treatments. The authors hypothesized that TRF and PRF, administered to dogs affected by maladaptive chronic pain, would be effective in reducing pain and improving quality of life, without causing severe side effects.

## 5.2 Materials and Methods

### 5.2.1 Animals

All dogs were enrolled after obtaining owner's written informed consent. The case series included client-owned dogs, of different breed, age, body weight and gender, presented to the Veterinary Teaching Hospital of the University of Milan (Lodi, Italy) for evaluation and treatment of maladaptive chronic pain related to OA or neuropathic pain. Inclusion criteria for dogs affected by maladaptive chronic pain related to OA were: radiographic evidence of OA (i.e. periarticular osteophytes, irregular or narrowed joint space and subchondral bone sclerosis), OA-associated signs of joint dysfunction (i.e. lameness, difficulty lying down, standing up, going up or down stairs, reluctance to jump or difficulty jumping) and painful joint(s) on palpation. Radiographic findings and OA localization were noted and recorded by a boarded radiologist. Inclusion criteria for dogs affected by neuropathic pain were: history consistent with nerve injury, pain not necessarily confined to an area of sensory deficit and presence of burning, pulsing or stabbing spontaneous pain. Only animals with pain, lameness and/or activity impairment despite administration of non-steroidal (NSAIDs) and/or steroidal anti-inflammatory drugs were included in this case series. Patient screening at baseline (T0) included a physical examination, blood cell count and serum biochemical analysis. Exclusion criteria included demonstrated neurologic, neoplastic, renal or uncontrolled endocrine disease and history of coagulopathy. Dogs that underwent surgical procedures within four weeks prior the initial evaluation were excluded from the study.

### 5.2.2 Study Design

Dogs were enrolled over a period of 12 months and were involved in a 20-week program for maladaptive chronic pain management. The day of the radiofrequency procedure, food, but non water, was withheld for 8 hours. Radiofrequency was performed under procedural sedation with dexmedetomidine (5 µg $kg^{-1}$; Dexdomitor 0.5 mg $ml^{-1}$; Vetoquinol, Italy) and methadone (0.2 mg $kg^{-1}$; Semfortan 10 mg $ml^{-1}$; Dechra Veterinary Products, Italy), mixed in the same syringe and injected into the lumbar epaxial muscles, and propofol (Proposure 10 mg $ml^{-1}$; Merial Italia S.p.A., Italy), titrated intravenously to effect. Throughout the procedure, oxygen was supplied via facemask and heart rate and rhythm, pulse oximetry and noninvasive blood pressure were continuously monitored. The animal was placed in the appropriate recumbency and the region(s) of interest was/were clipped and aseptically prepared with 4% chlorhexidine gluconate (LH Dermoscrub, CFS, Italy). One side of the thorax was clipped for application of a grounding pad (RF Disposable Grounding Pads, URF-3AP, Diros Technology Inc, Canada) and electrode gel was applied between the skin and pad. Dogs affected by maladaptive chronic pain related to OA were treated with intra-articular PRF of affected joint(s). A 18 gauge, 54 mm radiofrequency cannula with a 5 mm active tip and the inner electrode (RF straight cannula, DHC-018/54/5, Diros Technology Inc, Canada) was aseptically inserted in the treatment joint(s). To assure the correct positioning of the active tip into the joint, the synovial fluid was withdrawn. The cannula was connected to a radiofrequency generator (RF Generator, URF-3AP, Diros Technology Inc, Canada) and the active tip temperature was increased to 42°C, then maintained for 6 minutes. Impedance ranged 300-380 Ω, verifying appropriate active tip placement into the joint and the integrity of the RF system. At the end of the procedure intra-articular dexamethasone (0.2 mg $kg^{-1}$; Dexadreson 2 mg $ml^{-1}$; MSD Animal Health, Italy) was given. Dogs affected by neuropathic pain were given perineural PRF or TRF, according to the pure sensitive or mixed function of the nerve to be treated. Ultrasound-guided of target nerves was performed using a high-frequency, 6-15 MHz linear array transducer and portable ultrasound machine (Sonosite, Fujifilm Sonosite Inc, USA) by the same anesthesiologist. A 18 gauge, 54 mm radiofrequency cannula with a 5 mm active tip and the inner electrode was inserted until the active tip was visualized near the target nerve. The cannula was connected to the radiofrequency generator and the active tip temperature was increased to 42°C (PRF) or 70°C (TRF), then maintained for 6 minutes. Impedance ranged 380-850 Ω, verifying appropriate active tip placement into the soft tissue and the integrity of the RF system. At the end of the procedure perineural dexamethasone (0.2 mg $kg^{-1}$; Dexadreson 2 mg $ml^{-1}$; MSD Animal Health, Italy) was given. After

recovery from sedation, dogs were discharged from the hospital. The owners were instructed to maintain the dog's normal activity and to suspend the prescribed pain medications.

To assess the dog's pain and quality of life, owners were asked to complete the Canine Brief Pain Inventory (CBPI), a validated numeric rating scale-based questionnaire, which contained 11 questions on the dog's lameness, mood and willingness to move, play and jump[13,14]. Four questions required the owners to grade the severity of their dog's pain over the previous days. The 4 pain severity questions were scored on a discrete numerical scale of 0 (no pain) to 10 (extreme pain); the responses for these questions were averaged to generate the Pain Severity Score (PSS)[13]. Six questions evaluated the pain interference with dog's general activity, enjoyment of life and locomotive function. The 6 pain interference questions were scored on a discrete numerical scale of 0 (does not interfere) to 10 (completely interferes); the responses for these questions were averaged to generate the Pain Interference Score (PIS)[13]. In addition, a final question was included at the end of the questionnaire to obtain the owner's overall assessment of the dog's quality of life (Quality of Life Index, QoL)[14]. Question 11 (QoL) was graded on a discrete 0 to 4 numerical scale, with 0 representing a poor quality of life and 4 an excellent quality of life. The owners received a validated Italian version of the CBPI questionnaire[15]. All of the owners were asked to evaluate their dogs based on CBPI scoring system before radiofrequency treatment (T0) and at one (T1), two (T2), three (T3) four (T4) and then every four weeks until five months (T5, T6, T7, T8). Mean CBPI results for T1, T2, T3, T4, T5, T6, T7 and T8 were compared with T0. In case of an increase of $\geq 1$ in PSS and $\geq 2$ in Pain Interference Score PIS[16], an anti-inflammatory drug (firocoxib or prednisone) was prescribed. A repeat radiofrequency treatment was indicated when the PSS was $\geq 8$. Owners were also asked to record the occurrence of any adverse event. Additionally, dogs were monthly evaluated by the same veterinarian and by the same fifth-year veterinary student, using a 10 cm visual analogue scale (VAS), with end points labelled as "no pain" (0) and "worst pain imaginable" (10)[17].

### 5.2.3 Statistical Analysis

Statistical analysis was performed using PASW 18.0 (SPSS Inc, Chicago, USA). The assumption of data normality was examined by a Shapiro-Wilk test with an $\alpha = 0.05$ level. Results are presented as mean ± standard deviation (SD) or as number of patients (%) where appropriate. For continuous variables that were normally distributed, comparisons in relation to time were performed with independent Student's t-test. Statistical significance was set at $p < 0.05$.

## 5.3 Results

Six out of 7 client-owned dogs met the inclusion criteria and were enrolled in this case series. Reason for withdrawal for one dog included presence of neoplastic abnormality during baseline evaluation. Table 1 summarizes dogs' information about breed, age, body weight, gender and body condition score (BCS). The dogs included in the present case series had a mean age of 96.3 $\pm$ 47.3 months and the mean body weight was 18.8 $\pm$ 13 kg.

**Table 1**. Breed, age, body weight, gender and body condition score (BCS) of dogs included in the present case series.

| Dog | Breed | Age (months) | Body weight (kg) | Gender | BCS |
|---|---|---|---|---|---|
| **1** | Mixed Breed | 153 | 31.2 | Male | 7/9 |
| **2** | Golden Retriever | 109 | 31.4 | Male | 5/9 |
| **3** | Border Collie | 127 | 20.8 | Male | 6/9 |
| **4** | Pinscher | 15 | 5 | Female | 5/9 |
| **5** | Cane Corso | 81 | 47.2 | Male | 6/9 |
| **6** | Maltese | 93 | 5.8 | Male | 5/9 |

Three out of 6 dogs (50%) were diagnosed with maladaptive chronic pain related to OA, 2 out of 6 dogs (33%) were diagnosed with neuropathic pain and one dog (17%) was affected by maladaptive chronic pain related to OA and neuropathic pain, arising from two different anatomical regions. Table 2 summarizes dogs' information about type of maladaptive chronic pain, involved anatomical region and type of radiofrequency treatment administered.

**Table 2**. Type of maladaptive chronic pain, involved anatomical region and type of radiofrequency treatment administered to dogs included in the present case series.

| Dog | Type of pain | Involved anatomical region | Type of radiofrequency treatment (kg) | Repeated radiofrequency treatments (YES/NO) |
|---|---|---|---|---|
| 1 | Maladaptive chronic pain related to OA<br>Neuropathic pain | Right elbow<br>S1-S2-S3 nerves | Intra-articular PRF<br>Perineural TRF | NO |
| 2 | Maladaptive chronic pain related to OA | Bilateral coxo-femoral joint<br>Left elbow | Intra-articular PRF<br>Intra-articular PRF | NO |
| 3 | Maladaptive chronic pain related to OA | Bilateral elbow | Intra-articular PRF | NO |
| 4 | Neuropathic pain | Peroneal and tibial nerves | Perineural TRF | YES (TRF repeated treatment at T6) |
| 5 | Maladaptive chronic pain related to OA | Right elbow | Intra-articular PRF | NO |
| 6 | Neuropathic pain | Right dorsal ulnar and superficial radial nerves | Perineural TRF | NO |

The comparison of mean PSS, PIS and QoL scores between T0 and each successive time point showed a decrease in PSS between baseline and T6 ($p$ = 0.038), between baseline and T7 ($p$ = 0.041) and between baseline and T8 ($p$ = 0.018). Pain Interference score and QoL showed no significant variations between baseline and other examined periods. The comparison of mean veterinarian's VAS scores between T0 and each successive time point showed a decrease between baseline and T4 ($p$ = 0.025), between baseline and T7 ($p$ = 0.014) and between baseline and T8 ($p$ = 0.0007). The comparison of mean fifth-year veterinary student's VAS scores between T0 and each successive time point showed a decrease between baseline and T8 ($p$ = 0.009). The PSS, PIS, QoL and VAS scores of the dogs recruited are reported in Table 3 and Table 4.

**Table 3**. Pain Severity Score (PSS), Pain Interference Score (PIS) and Quality of Life Index (QoL) (adopted by Brown et al. 2008)[13] of the dogs included in the present case series

| Dog | T0 | | | T1 | | | T2 | | | T3 | | | T4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PSS | PIS | QoL | PSS | PIS | QoL | PSS | PIS | QoL | PSS | PIS | QoL | PSS | PIS | QoL |
| 1 | 4.5 | 4.8 | 1 | 2 | 2.8 | 1 | 2.3 | 3 | 1 | 1.8 | 2.7 | 1 | 2.5 | 2.7 | 1 |
| 2 | 1.3 | 0.7 | 2 | 1.3 | 0.7 | 2 | 1.3 | 0.3 | 2 | 1.3 | 0.3 | 2 | 1.3 | 0.3 | 2 |
| 3 | 4 | 3.7 | 4 | 3.3 | 2.5 | 4 | 3.8 | 3 | 4 | 4.3 | 3.7 | 4 | 4.3 | 3.8 | 4 |
| 4 | 6.3 | 1.3 | 1 | 4 | 3 | 1 | 2 | 0.8 | 2 | 1.8 | 0.5 | 2 | 1.5 | 0.5 | 2 |
| 5 | 4.5 | 0.5 | 2 | 6.3 | 4.8 | 0 | 6.3 | 4.7 | 0 | 6 | 4.2 | 1 | 6.8 | 5.2 | 0 |
| 6 | 9 | 0.8 | 1 | 7 | 7.2 | 0 | 7 | 6.8 | 1 | 7 | 4.7 | 1 | 7 | 4.7 | 1 |
| **Mean** | **4.9** | **2** | **1.8** | **4** | **3.5** | **1.3** | **3.8** | **3.1** | **1.7** | **3.7** | **2.7** | **1.8** | **3.9** | **2.9** | **1.7** |
| **SD** | **2.6** | **1.8** | **1.2** | **2.3** | **2.2** | **1.5** | **2.4** | **2.4** | **1.4** | **2.4** | **1.9** | **1.2** | **2.6** | **2.1** | **1.4** |

| Dog | T5 | | | T6 | | | T7 | | | T8 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PSS | PIS | QoL | PSS | PIS | QoL | PSS | PIS | QoL | PSS | PIS | QoL |
| 1 | 1.5 | 2.2 | 1 | 1.3 | 2 | 1 | 2.3 | 2.8 | 1 | 3 | 2.7 | 1 |
| 2 | 2.5 | 1.3 | 2 | 1 | 0.3 | 2 | 1 | 0.3 | 2 | 1 | 0.3 | 2 |
| 3 | 4.3 | 3.8 | 4 | 2 | 3.8 | 4 | 3 | 3 | 4 | 4 | 3.7 | 4 |
| 4 | 1.3 | 0.5 | 2 | 8 | 3 | 1 | 0.8 | 0 | 3 | 0.5 | 0.3 | 3 |
| 5 | 6 | 4.8 | 1 | 3 | 4.7 | 1 | 3 | 5.6 | 0 | 5 | 4.2 | 2 |
| 6 | 4 | 3.5 | 2 | 1.3 | 2.3 | 1 | 1.2 | 2 | 2 | 1.2 | 2 | 2 |
| **Mean** | **3.3** | **2.7** | **2** | **3.1** | **2.7** | **1.7** | **1.9** | **2.3** | **2** | **2.5** | **2.2** | **2.3** |
| **SD** | **1.8** | **1.6** | **1.1** | **2.9** | **1.5** | **1.2** | **1** | **2** | **1.4** | **1.8** | **1.7** | **1** |

**Table 4**. Veterinarian's (VET-VAS) and fifth-year veterinary student (STU-VAS) VAS scores of the dogs included in the present case series

| Dog | T0 | | T4 | | T5 | |
|---|---|---|---|---|---|---|
| | VET-VAS | STU-VAS | VET-VAS | STU-VAS | VET-VAS | STU-VAS |
| 1 | 8 | 7 | 5 | 4 | 5 | 5 |
| 2 | 6 | 6 | 4 | 4 | 5 | 5 |
| 3 | 6 | 4 | 4 | 5 | 5 | 5 |
| 4 | 8 | 8 | 5 | 7 | 6 | 7 |
| 5 | 7 | 6 | 2 | 4 | 4 | 4 |
| 6 | 5 | 5 | 4 | 5 | 4 | 4 |
| **Mean** | **6.7** | **6** | **4** | **4.8** | **4.8** | **5** |
| **SD** | **1.2** | **1.4** | **1.1** | **1.2** | **0.8** | **1.1** |
| **Dog** | **T6** | | **T7** | | **T8** | |
| | VET-VAS | STU-VAS | VET-VAS | STU-VAS | VET-VAS | STU-VAS |
| 1 | 4 | 5 | 4 | 5 | 3 | 2 |
| 2 | 4 | 5 | 4 | 4 | 4 | 2 |
| 3 | 4 | 5 | 4 | 5 | 4 | 3 |
| 4 | 4 | 6 | 5 | 4 | 3 | 3 |
| 5 | 5 | 4 | 3 | 5 | 2 | 3 |
| 6 | 5 | 5 | 4 | 4 | 3 | 2 |
| **Mean** | **4.3** | **5** | **4** | **4.5** | **3.2** | **2.5** |
| **SD** | **0.5** | **0.6** | **0.6** | **0.5** | **0.8** | **0.5** |

In 2 out of 6 (33%) dogs pain worsened during the study period; however, administering firocoxib to 50% of the standard dose for two weeks resulted in reversal of this worsening. In all dogs, radiofrequency treatment was well tolerated. In one dog (dog 6) a local cutaneous reaction in the area of TRF treatment was observed: it spontaneously resolved in a two-week period.

## 5.4 Discussion

To the authors' knowledge, the present case series is the first to evaluate the clinical effects of the radiofrequency for maladaptive chronic pain related to OA and neuropathic pain management in dogs. A significant reduction in perceiving pain was achieved in dogs receiving perineural TRF and perineural or intra-articular PRF treatment.

Radiofrequency is a nonpharmacological and interventional technique, largely used in human medicine for the treatment of different subtypes of maladaptive chronic pain[11-12]. Thermal radiofrequency has been widely used, but with the advent of PRF, the risks and complications associated with high-temperature neurolytic injury, have been substantially eliminated without

sacrificing the analgesic efficacy of the procedure. Recent data support the application of this treatment for the management of different disorders associated with the perception of a chronic-type pain component in humans[18,19]. Its clinical use in veterinary medicine, for the treatment of chronic-type forms of pain in dogs, such as pain of osteoarthritic origin or neuropathic pain, has never been evaluated. In a recent veterinary study, Wallerian degeneration in TRF-treated canine saphenous nerves appears sufficient to impair transmission of noxious stimuli[2]; PRF of the sciatic nerves did not cause degeneration and motor deficits in treated dogs[2]. Perineural TRF and perineural or intra-articular PRF were associated with a reduction in CBPI and VAS scores in the present case series. The peak of analgesic efficacy of radiofrequency treatment was obtained between T7 and T8. In humans, pain relief can be observed as early as a few hours or days after treatment, but in most cases it is difficult to obtain an immediate post-treatment efficacy because of the discomfort associated with the procedure, that may interfere with the overall evaluation[18]. In humans, the peak of the analgesic effect after intra-articular PRF treatment can be achieved very rapidly as early as one week post or show up around six weeks post-treatment, depending on the anatomic characteristics of the treated joint[20]. For example, statistically significant improvement, as assessed by patients' VAS scores, was demonstrated at four weeks post treatment in subjects with advanced OA of the knee joint[21], thus much earlier than what was observed in dogs treated with intra-articular PRF in this case series. On the other hand, in other studies conducted in humans, it would appear that the analgesic peak after PRF application at the level of the pudendal nerve for the treatment of chronic forms of pain in the pelvic area succeeds in significantly and durably reducing pain, starting between six and twenty-four months post-treatment, in at least 60% of treated patients[22].

From what emerged from the present case series, it would seem that the best results, in terms of the magnitude of the analgesic effect, were obtained after intra-articular PRF treatment of the elbow joint. In the dog, from an anatomical point of view, the elbow is a simpler and smaller joint than, for example, the coxo-femoral joint[23], in which the approach for performing the radiofrequency procedure is less easy. This would also seem to be reflected in human medicine where an immediate and lasting analgesic effect is reported in smaller joints (e.g. atlanto-axial joint), while in larger joints (e.g. knee joint) gradual pain relief over time has been observed[20]. The explanation could be related to the more "open" geometry of some joints, as shoulder and knee, and the consequent placement of the radiofrequency cannula not close to the bone. When the active part of an electrode is located in soft tissue, the current intensity, and thus the electric field,

is rapidly diluted as the distance from the electrode increases. Within a joint, however, some of the current is deflected by the bony surfaces and tends to remain within the joint space. Thus, an immediate analgesic effect can be observed only if the electric fields throughout the joint are of sufficient strength, and then in those smaller joints where there is no attenuation of the electric field, but also a delayed and gradual analgesic effect probably related to the secondary effects that the electric field exerts on cells of the immune system by going on to modulate cytokine production[20]. Indeed, studies in humans have shown that the concentration of certain cytokines (TNF-α, IL-4 and IL-8) within synovial fluid changes in OA subjects as the disease progresses. This suggests, from a future perspective, their potential use in the clinical setting as predictive biomarkers of OA even before the appearance of characteristic radiographic signs[24].

In 3 out of 6 dogs pain worsened during the study period; two dogs received firocoxib to 50% of the standard dose for two weeks resulting in reversal of worsening, while one dog received a second TRF treatment. Together with systemic therapies, the intra-articular and perineural injection of dexamethasone may have contributed to the long-term analgesic effect observed in the reported cases. Intra-articular and perineural administration of glucocorticoids has been used to treat maladaptive chronic pain conditions in dogs refractory to traditional medical management, showing a long-lasting pain relief[25,26]. In the present case series, the choice to administer dexamethasone was based on the fact that in human medicine, an initial worsening of the patient's clinical condition following treatment with radiofrequency is reported in some cases. In humans, to counteract this potential post-treatment side effect, an anti-inflammatory drug can be administered at the intra-articular or perineural level[27], and for this reason it was decided to adopt this approach in the present case series as well. In dogs suffering from OA, the beneficial effects associated with a single administration of intra-articular triamcinolone hexacetonide, a synthetic corticosteroid, may occur after 90 days, as reported in a recent study[28]. This could be an important aspect to consider also in the present case series. The overall improvement in patients, with reduction in PSS and VAS scores, occurred between T7 and T8 and it could also be minimally due to intra-articular dexamethasone administration in conjunction with radiofrequency treatment. However, further studies are needed to specifically evaluate the long-term effects of intra-articular and perineural dexamethasone to better understand whether such administration could significantly affect the assessment of the analgesic efficacy of radiofrequency treatment over time.

This study has several limitations, one of which is that dogs suffered different pathologic conditions, with various degrees of tissue inflammation and pain. The absence of a control group (sham

inoculated) may hinder the assessment of the radiofrequency treatment efficacy. In addition, the owners, the veterinarian and the fifth-year student were not blinded to treatment and the technique was evaluated in a small nonrandomized group of dogs. Further clinical trials would be needed to define to what extent perineural TRF and intra-articular or perineural PRF provide analgesia in dogs affected by maladaptive chronic pain, and how long such analgesia might last.

## 5.5 Conclusions

Although this case series included a small number of patients, it seems that perineural TRF and intra-articular or perineural PRF are effective procedures that do not result in the development of serious adverse effects in dogs suffering of maladaptive chronic pain related to OA or with a neuropathic component. For this reason, it can be used in a multimodal protocol for the management of chronic painful conditions, although further studies are required to assess the overall success rate.

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# 6. General discussions and conclusions

In the last decades, pain management has become central to small animal practice. Alleviating pain is not only a professional obligation but also a key contributor to successful case outcomes. Although acute and maladaptive chronic pain management is now an established component of therapy, the development of new drugs, analgesic techniques and non-pharmacological modalities makes this a still evolving facet of small animals clinical practice.

In this scenario, the results of this PhD project have contributed to increase knowledge on the perioperative analgesic efficacy of intraperitoneal and intra-articular administration of analgesic drugs and on the long-term effects of a multimodal pharmacological treatment for management of osteoarthritic chronic pain in dogs. Finally, it also introduced the potential role, in treating canine maladaptive chronic pain, of radiofrequency, a non-pharmacological and interventional technique, largely used in humans, but completely new in veterinary medicine.

Intraperitoneal and intra-articular administration of analgesic drugs are two alternative, simple and low-cost techniques for perioperative pain management in small animal practice. Based on the results obtained in our study, intraperitoneal administration of local anesthetics provides effective post-operative pain relief in dogs undergoing major abdominal surgeries. Intraperitoneal ropivacaine, a longer lasting local anesthetic, provided an analgesic effect lasting up to 24 hours after the end of surgery. Its duration was longer than that of intraperitoneal lidocaine and this finding results in a decreased postoperative opioids requirement and in a more rapid food intake in recruited dogs. In veterinary literature, no studies evaluating the use of intraperitoneal local anesthesia during major abdominal surgeries and quantification of its postoperative analgesic effect have been described in companion animals. In dogs, numerous studies have evaluated the effectiveness of intraperitoneal administration of local anesthetics for pain relief after ovariohysterectomy and have provided variable results probably due to differences in site and timing (preoperatively or postoperatively) of administration and differences in local anesthetic doses, concentrations and volumes of injection. In the present study, the use of clinical patients suffering from various abdominal diseases rather than healthy patients undergoing elective procedure is a true reflection of the nature of cases that are seen in daily clinical practice. Subsequently, data and information from this study can find its immediate application in clinics hence improving patients welfare and pain management as well as reducing the overall cost of treatment.

Intra-articular administration of local analgesics is a simple and low cost technique and in human medicine is an effective example of pain management for joint surgery. In veterinary medicine, the intra-articular use of analgesics remains controversial and concerns have been raised about its benefit profile. In the study presented in this research project, preemptive intra-articular dexmedetomidine, alone or in combination with lidocaine, provided effective intraoperative analgesia in dogs undergoing arthroscopy; treatments also resulted in similar postoperative analgesic effects. The topical application of analgesic drugs to the painful area (joint surface) exhibits an analgesic effect by blocking nociception from the area of tissue damage; the systemic absorption of analgesic drugs through the surface may also play a role in the analgesic effect by attenuating nociception. This simple and effective technique could lead not only to an improvement of the multimodal analgesic approach to different types of joint surgeries in dogs, but also to reduce opioid-related side effects. Opioid-free anesthesia is a recent topic in human medicine, while the debate is at a very early stage in veterinary medicine. Future study are advocated to evaluate the analgesic efficacy of this technique in a opioid-free anesthetic scenario.

Chronic pain differs from acute perioperative pain and can result in changes in nociceptive transmission at multiple levels, that facilitate and amplify pain and can be drivers of painful stimuli separately from any peripheral input; this type of pain is often described as “maladaptive” or “pathological” pain. Treatment approach to maladaptive chronic pain in small animal practice should be focused on interrupting nociceptive input from the periphery and on reversing pathological changes and the systemic negative effects long-lasting pain has had. So, a multimodal approach is likely to be the most effective, especially in terms of treatment expectations and prognosis. The mainstays of treatment of maladaptive chronic pain are anti-inflammatory drugs; however, evidence is increasing for other pharmacological and non-pharmacological therapies. Alternative therapies, new pharmacological options should be considered, especially to those that may decrease the central sensitization to pain. Our studies have proposed two possible therapeutic alternatives to maladaptive chronic pain in dogs: a multimodal pharmacological management involving CBD, a new therapeutic option in dogs affected by osteoarthritis, and the use of radiofrequency, an interventional and non-pharmacological technique, completely new in veterinary medicine. According to the results of our study, a satisfactory pain and quality of life management was achieved in dogs receiving oral transmucosal CBD oil in addition to a multimodal pharmacological approach for treatment of osteoarthritis-related pain in dogs. Combined with an anti-inflammatory drug, gabapentin and amitriptyline, CBD appears to enhance osteoarthritic pain

relief and quality of life improvement. Furthermore, its co-administration results useful in reducing the other administered drugs' dosage, minimizing the severity and incidence of associated side effects. The high CBD patient tolerability, the easy practicability and the paucity of adverse effects of oral transmucosal route of administration may represent potential benefits for long-term therapy. The second proposed analgesic technique is radiofrequency, whose long-term efficacy has been evaluated in a small group of dogs affected by maladaptive chronic pain, osteoarthritis-related or characterized by a neuropathic component. Although this study is an ongoing project and in the present form includes a small number of patients, in accordance with the obtained preliminary results, it seems that radiofrequency is an effective procedure that do not result in the development of serious adverse effects in dogs suffering of maladaptive chronic pain. For this reason, it could be included in a multimodal protocol for the management of chronic painful conditions, although further studies are required to assess the overall success rate. Radiofrequency is an interventional technique, completely new in veterinary medicine. This procedure has a great potential for the relief of that pain that is poor responsive or unresponsive to current available therapies, not only in dogs but also in other companion animals, like cats and horses, in which chronic painful conditions severely impact on quality of life of patients affected.

In conclusion, this dissertation includes studies of different nature, involving different analgesic techniques for management of different types of pain in dogs, but the common thread is represented by the attempt to find new treatment options, in order to promote a proactive, preemptive and multimodal pain management in companion animals. The rationale behind this attempt is that using new pharmacological and non-pharmacological techniques, targeting multiple sites in pain pathways, minimize the potential for side effects associated with any single technique and provide a more effective pain management. Pain alleviation, and therefore animals welfare, is an ethical and medical duty and all the new techniques to reduce pain, stress and anxiety should be encouraged.

# 7. Scientific production unrelated to the project

During the years involved in the competition of the present PhD project, I also spent my clinical training as a researcher in the clinical practice of the Veterinary Teaching Hospital of the University of Milan. As a result, I actively participated in studies on other topics related to anesthesia in small, large and zoo animals, that resulted in publications on international, peer-reviewed journals. They are presented in this section:

- Bagardi M, Locatelli C, Brambilla P, Ghilardi S, Rabbogliatti V, Amari M, Casiraghi S, Ravasio G, Galimberti L, **Brioschi FA**. Comparison of two multimodal intramuscular anesthetic protocols in uncooperative feline patients: effects on sedation and echocardiographic measurements. Accepted for publication on: *J Feline Med Surg,* January 2023
- Amari M, **Brioschi FA**, Rabbogliatti V, Di Cesare F, Pecile A, Giordano A, Moretti P, Magnone W, Bonato F, Ravasio G. Comparison of two injectable anaesthetic protocols in Egyptian fruit bats (Rousettus aegyptiacus) undergoing gonadectomy. *Sci Rep* 2022; 12(1):15962 DOI: 10.1038/s41598-022-20408-z
- Rabbogliatti V, Amari M, **Brioschi FA**, Di Cesare F, Zani DD, De Zani D, Di Giancamillo M, Cagnardi P, Ravasio G. Use of dexmedetomidine repeated subcutaneous administration for balanced anaesthesia in horses. *BMC Vet Res* 2022; 18(1):269 DOI: 10.1186/s12917-022-03350-0
- Stranieri A, Lauzi S, Giordano A, Galimberti L, Ratti G, Decaro N, **Brioschi FA**, Lelli D, Gabba S, Amarachi NL, Lorusso E, Moreno A, Trogu T, Paltrinieri S. Absence of SARS-CoV-2 RNA and anti-SARS-CoV-2 antibodies in stray cats. *Transbound Emerg Dis* 2022; 69(4):2089-2095 DOI: 10.1111/tbed.14200
- Lazzarini E, Gioeni D, Del Prete G, **Brioschi FA**, Agostinetto G, Carotenuto AM. Sedative effects of intramuscular dexmedetomidine and ketamine at sub-anesthetic dose alone or in combination with methadone in healthy dogs. *Top Companion Anim Med* 2021; 45:100579 DOI: 10.1016/j.tcam.2021.100579
- Rabbogliatti V, De Zani D, Zani DD, Di Cesare F, **Brioschi FA**, Gioeni D, Crivellari B, Ravasio G. Comparison of four peribulbar anaesthetic techniques: a preliminary study in equine cadavers. *Vet Anaesth Analg* 2021; 48(3):442-450 DOI: 10.1016/j.vaa.2020.10.009

- Gioeni D, **Brioschi FA**, Di Cesare F, Rabbogliatti V, Amari M, Zanzani S, Cagnardi P, Ravasio G. Oral transmucosal or intramuscular administration of dexmedetomidine-methadone combination in dogs: sedative and physiological effects. *Animals (Basel)* 2020; 10(11):2057 DOI: 10.3390/ani10112057
- Ravasio G, **Brioschi FA**, Rabbogliatti V, Gioeni D, Di Cesare F, Corletto F, Oltolina M, Carnevale L. Case report: ultrasound sciatic and saphenous nerve blocks for tibial malunion surgical correction in a pediatric african leopard (Panthera pardus). *Front Vet Sci* 2020; 7:538883 DOI: 10.3389/fvets.2020.538883
- Lazzarini E, Martinelli E, **Brioschi FA**, Gioeni D, Corneliani RT, Carotenuto AM. Intramuscular alfaxalone and methadone with or without ketamine in healthy cats: effects on sedation and echocardiographic measurements. *Vet Anaesth Analg* 2020; 47(5):621-630 DOI: 10.1016/j.vaa.2020.02.010

# 8. Presented abstracts

- **Brioschi FA**, Groppetti D, Rabbogliatti V, Mastellaro S, Pecile A, Ferrari F. *Acute trauma in an unexpected late pregnant bitch: is progesteronemia useful in predicting the date of delivery?* International Symposium on Canine and Feline Reproduction 2022. June 30-July 2, 2022; Milan, Italy
- **Brioschi FA**, Rabbogliatti V, Amari M, Di Cesare F, Ferrari F, Romussi S, Ravasio G. *Postoperative analgesic efficacy of intraperitoneal ropivacaine in dogs undergoing major abdominal surgeries: a preliminary investigation.* SISVET 2022
- **Brioschi FA**, Rabbogliatti V, Amari M, Di Cesare F, Ferrari F, Romussi S, Ravasio G. *Comparison of intraperitoneal lidocaine and ropivacaine for postoperative analgesia in dogs undergoing major abdominal surgeries*. Association of Veterinary Anaesthetists Spring Meeting 2022. May 18-20, 2022; Nafplio, Greece
- Amari M, **Brioschi FA**, Rabbogliatti V, Di Cesare F, Pecile A, Groppetti D, Ferrari F, Oltolina M, Magnone W, Saini M, Ravasio G. *Mechanical ventilation in two male common hippopotami (Hippopotamus amphibius) undergoing surgical castration.* Association of Veterinary Anaesthetists Spring Meeting 2022. May 18-20, 2022; Nafplio, Greece
- Rabbogliatti V, Amari M, **Brioschi FA**, Di Cesare F, Zani DD, De Zani D, Ravasio G. *Clinical comparison between subcutaneous and intramuscular repeated injection of dexmedetomidine in anaesthetized horses: preliminary investigation.* Association of Veterinary Anaesthetists Spring Meeting 2022. May 18-20, 2022; Nafplio, Greece
- Di Cesare F, Ravasio G, Rabbogliatti V, **Brioschi FA**, Amari M, Negro V, Villa R, Cagnardi P. *Pharacokinetics of dexmedetomidine intravenous continuous rate infusion and repeated subcutaneous administration in isoflurane anaesthetized horses.* SISVET 2021
- **Brioschi FA**, Rabbogliatti V, Gioeni D, Di Cesare F, Valentini Visentin M, Ravasio G. *Effect of oral-transmucosal cannabidiol on pain and quality of life in dogs affected by osteoarthritis*. Association of Veterinary Anaesthetists Spring Meeting 2020: March 11-13, 2020; Dublin, Ireland
- Rabbogliatti V, **Brioschi FA**, Di Cesare F, Gioeni D, Amari M, Spediacci C, Ravasio G. *Clinical comparison of continuous rate infusion and subcutaneous administration of dexmedetomidine in isoflurane anaesthetized horses.* Association of Veterinary Anaesthetists Spring Meeting 2020: March 11-13, 2020; Dublin, Ireland