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Clinical Studies

Comparison of a Novel Periarticular Injection Technique with Intra-articular Injection of Platelet-rich Plasma for the Treatment of Osteoarthritis Joint Pain in Dogs

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ABSTRACT

Canine osteoarthritis (OA) is characterized by degenerative changes in the joints resulting in pain and lameness. The study objective was to compare treatment of OA with a novel peri-articular injection (aqua-acupuncture/aqua-AP) technique using platelet-rich plasma (PRP), with standard intra-articular (IA) injection. Sixteen dogs diagnosed with OA in appendicular skeletal joints were enrolled and assigned to Test (n=8) or Control (n=8) treatment groups. Each subject received an injection at Week 0 and again at Week 4. Treatment outcomes assessed included goniometry (flexion and extension), infrared thermal imaging (IRTI), lameness grading scores and Canine Brief Pain Inventory (CBPI) questionnaire. The flexion and extension assessments had no statistically significant changes at 4 or 8-week assessment. Significant score reductions (improvement) were observed in both groups (p<0.001) for pain severity (2, 4, 8 weeks) and activity interference (2, 4, 8 weeks) for CBPI data with no significant difference between study groups concluded (p>0.05). Infrared thermal imaging demonstrated statistically significant (p<0.001), decreased temperature in the inflamed joints for both injection techniques, however, aqua-AP treatment was statistically significant at both 4 and 8 weeks while IA technique didn't reach significance until 8 weeks post-treatment. Lameness scores had significant improvements at 4 and 8 weeks with no significant difference between groups. The findings suggest that PurePRP® treatment delivered with either the proposed aqua-AP or the standard IA injection may improve pain and thermal outcomes (decrease joint inflammation) with more rapid thermal improvement for aqua-AP. There were no clear effects on goniometry measurements. Further investigations with larger scale trials are warranted.

Keywords: aqua-acupuncture, canine osteoarthritis, intra-articular, periarticular, infrared thermal imaging, platelet-rich plasma, PurePRP®, regenerative biologic

ABBREVIATIONS: APIS: Average Pain Interference Score; APSS: Average Pain Severity Score; aqua-AP: aqua-acupuncture; CBPI: Canine Brief Pain Inventory; CCRP: Certified Canine Rehabilitation Practitioner; DJD: degenerative joint disease; IA: intra-articular; IRTI: infrared thermal imaging; NSAID: non-steroidal anti-inflammatory drug; OA: osteoarthritis; PDGF: platelet derived growth factor; PRP: platelet-rich plasma; ROM: range of motion; TCVM: traditional Chinese veterinary medicine

Osteoarthritis (OA) is a degenerative joint disease (DJD) that results in the loss of articular cartilage and has the hallmark of pain. The prevalence of OA in dogs in the United States is estimated to be as high as 38% in dogs over 1 year of age.[1-2] The disease is progressive and there is no known cure. Often, OA goes undiagnosed in clinical practice until the physical signs are advanced. The goal is to recognize the disease and attempt to mitigate its progression. Veterinarians have the role of educating pet owners to develop healthy proactive lifestyles which include a species appropriate diet, regular exercise, and use of chondroprotective joint supplements.[2-4]

There are multiple clinical signs that pet owners may notice in dogs affected with OA which include: refusal to jump/inability to get up on furniture, reluctance to use stairs, difficulty rising from a recumbent position, lethargy and painful swollen joints.[2] During veterinary assessment, there are several useful objective measurements for diagnosing and assessing OA. These include goniometry to measure

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joint flexion and extension, infrared thermal imaging (IRTI) to visually assess inflamed tissues, orthopedic lameness scores and various health risk assessment questionnaires.

A traditional conservative management treatment is based primarily on reducing inflammation and pain. Non-steroidal anti-inflammatory drugs (NSAID) can have beneficial effects for arthritic patients (e.g. Rimadyl, Metacam, Onsior, Deramaxx) but may cause gastrointestinal (GI) upsets and in some canine patients, liver and/or kidney dysfunction.[3,5,6] A more recent medication, Galliprant (non-cox inhibiting NSAID), targets pain and inflammation while reducing the impact on GI, kidney, and liver homeostasis; however although adverse-effects are reduced compared to cox-inhibiting NSAIDs, vomiting, diarrhea, decreased appetite and lethargy are reported in some dogs.[7] Steroids can markedly reduce swelling and inflammation in arthritic joints but long-term use contributes to additional joint damage and tissue breakdown.[3,5] Inflammation and pain left unchecked, however, can lead to central sensitization, establishing acute pain as a chronic condition leading to worsening tissue damage and pain for OA patients.[8] Additional treatments, therefore, with less adverse effects are sought to mitigate these effects.

Regenerative medicine therapy is a developing innovative field that seeks to restore function to injured tissues using the body's own resources.[9-11] Veterinarians have been using regenerative therapies for OA treatment such as: platelet-rich plasma (PRP), bone marrow aspirate concentrate (BMAC), and adipose-derived stem cells (ADSC) with clinical success.[6,12-14] Orthobiologic therapies have gained popularity within the past fifteen years as they aid in the repair of local tissues while avoiding the unwanted side effects of conventional OA medications.[15,16] PRP and other whole blood derivatives have shown benefit for treating soft tissue injuries as well as joint disorders such as OA.[17] In a canine gait analysis model, intra-articular (IA) injection of PRP used to treat dogs with DJD secondary to cranial cruciate rupture resulted in improved gait parameters when compared to untreated controls.[18] In humans, Everts et al. was the first team to report on the effects of PRP intra-articular injection on postoperative pain in humans following shoulder surgery. In their randomized controlled trial on analgesic effects, a significant reduction in visual analog scale scores was reported along with reduction in the use of opioid-based pain medication resulting in a more successful post-surgical rehabilitation.[3,19]

Platelet-rich plasma, as an autologous preparation from whole blood, concentrates a large number of platelets in a small volume of plasma.[17] The processing is uncomplicated and may be accomplished at the point of care with minimal need for expensive equipment. As an autologous biologic, it is safe when administered to the same animal it was obtained from. The platelets in a PRP preparation function as exocytotic cells and play a primary role in healing by activating the clotting process and releasing a variety of bioactive proteins (e.g. cytokines, growth factors) that modify the inflammatory process following degranulation (Table 1).[17,20,21] The secretion of these proteins initiate a complex cellular signaling pathway at the site of injection which affects local tissues and underlies many effects of PRP such as pain relief, soft-tissue and bone healing and regulation of inflammatory processes.[6,19,22]

Table 1: Bioactive proteins produced by platelet activation at the site of PRP injection.[17,21]

Bioactive Proteins	Actions
PDGF	Angiogenesis, mitogenesis (osteoblasts, mesenchymal cells), macrophage activation, chemotaxis (fibroblasts, glial, smooth muscle cells, neutrophils), regulates collagen synthesis and secretion
VEGF	Angiogenesis, vasculogenesis, mitogenesis (endothelial cells)
TGF-β	Bone regeneration and modeling, long term healing, regulation of inflammatory processes, paracrine/ autocrine cell signaling, promotes proliferation of undifferentiated mesenchymal stem cells, regulates collagen production, promotes growth epithelial and vascular endothelium, inhibits macrophage and lymphocyte proliferation
EGF	Regulation of cell growth, proliferation, differentiation
Fibrin, fibronectin, vitronectin	Adhesive proteins/cell surface proteins that mediate binding between cells or cells and extracellular matrix

PDGF = platelet derived growth factor; VEGF = vascular endothelial growth factor; TGF-β = transforming growth factor-β; EGF = epidermal growth factor

Acupuncture is a safe nonpharmacologic intervention used for OA pain relief with minimal side effects.[23] It is very effective for analgesia on a local, segmental, and suprasegmental level. The therapeutic modality originated as part of the medical system in ancient China over 3000 years ago and involves placing sterile needles in acupuncture points.[3,24] These acupoints are located on Meridians which are channels located predominately in the connective tissue fascia network of the body.[25] The mechanism of action for acupuncture is complex, involving local mechanical effects (needle placement), and the modulation of peripheral and central nervous system pain signaling pathways through afferent nerve fibers (A-beta, A-delta, and C fibers) and signaling molecules (opioid peptides, glutamate, 5-hydroxytryptamine, cholecystokinin).[3,26-30]

The local effects of needle stimulation of the connective tissue are of particular interest. The fascial system provides an anatomical continuum penetrating and connecting all districts of the body, from epidermis to bone.[31] It contains nociceptors which can amplify pain through paracrine signaling or react to pain modulation from acupoint stimulation. Essentially when the inserted acupuncture needle impacts connective tissue in fascia, it causes the needle grasp phenomenon (*De Qi* sensation). This results in a mechanical signal that propagates along neighboring fascial structures, intersecting muscles, synovium and bone periosteum downstream, resulting in a flow of paracrine-signaling molecules and creating

multiple adaptive changes including an analgesic and anti-inflammatory response.[8,31,32] The fascial network effects provide an explanation of AP mechanisms that extend beyond the nerve signal mediating effects of the peripheral and central nervous systems.[32]

There are different methods of stimulating acupuncture points that involve needle placement into specific palpable anatomic locations. Aqua-acupuncture (aqua-AP), which injects a substance at an acupoint (i.e. pharmacopuncture), is one of the methods used for stimulating acupuncture points. It combines both the effect of acupoint stimulation on the nervous system, and the action(s) of the substance injected at the acupuncture site.[33] The fascial location of acupoints results in neuromodulation of local tissues starting a cascade that includes stimulation of mechanoreceptors/ nociceptors, microtrauma (increased blood flow), stretched fibroblasts (release growth factors), paracrine signaling and molecules that alter gene expression.[25,30] These actions are similar to PRP local effects, suggesting these two therapeutic modalities would be complimentary and possibly synergistic.

The use of pharmacologic options remains the mainstay of veterinary OA treatment. The purpose of this study was to investigate a reliable non-pharmacologic intervention that could still provide effective pain modulation without the adverse events associated with pharmacologic analgesics. Based on author experiential evidence, the use of a periarticular injection technique which combines 2 proven methods of non-pharmacologic analgesia, acupuncture (aqua-AP) and an orthobiologic (PRP), benefits canine OA pain management programs. Although IA application of PRP has been shown to be efficacious, the present research project proposed investigating whether delivery of PRP by aqua-AP would improve pain control; and at the same time have the advantages of decreasing patient stress with a simpler procedure requiring less sedation/anesthesia, reduce iatrogenic joint trauma, and provide a safer procedure for geriatric dogs (i.e. less sedation).

The objective of this partially randomized, controlled clinical study in dogs with naturally occurring OA, was to compare the effectiveness of a novel periarticular injection technique (aqua-AP) for administration of PurePRP® with the standard administration technique of IA injection. The hypothesis was that the OA-outcome data (goniometry, lameness score, IRTI, Canine Brief Pain Inventory score) generated from the novel injection technique would be better than the outcome data associated with the standard IA injection technique for administration of the orthobiologic, PurePRP®.

MATERIALS AND METHODS

Study Design

The patient population of this study was recruited from client owned dogs referred to Dr. Gerardi's clinical practice, Synergy Integrative Specialty Veterinary Clinic, located in New Bern, North Carolina, USA. Canine patients selected for potential enrollment were those that had OA in appendicular skeletal joints (hip, stifle, hock, shoulder and/or elbow joints), and whose owners sought non-surgical, minimally invasive OA pain management, along with less pharmaceutical use. The facility had on staff a Certified Veterinary Acupuncturist (CVA) to provide standard acupuncture technique and a Certified Canine Rehabilitation Practitioner (CCRP) for physical joint evaluation, objective goniometry measurements and to provide IA injections. Clients that were interested in having their dogs participate in the clinical study signed a consent form describing the study guidelines and risks along with agreement to return for needed evaluation dates at 0, 4 and 8 weeks.

To qualify for enrollment, a potential study candidate had to meet the following criteria: (1) 6-14 years old, (2) body weight of 10-100 pounds, (3) any breed or gender, (4) owners agreed to return at study weeks 4 and 8 by prescheduled recheck appointments for study data collection, (5) referred for previous diagnosis of chronic DJD with OA lesions, (6) no more than two OA affected joints (validated on radiographic scoring: mild, moderate to severe), (7) persistent lameness due to OA during the last 3-4 months despite medical therapy, (8) owners open to a novel, non-drug therapy to treat OA pain.

Exclusion criteria included receiving any of the following treatments during the study or up to 1 month prior to study start: 1) corticosteroids, 2) new supplements/ immune modifying supplements, 3) slow acting disease modifying agents (e.g. Adequan®, Polyglycan®), 4) prosthetics or implants associated with OA (e.g. hip replacement), 5) IA injection, 6) joint instability present (e.g. cruciate ligament rupture). Dogs would also be excluded for a stem cell or bone marrow transplant, or related procedure, within the 9 months prior to study start.

Qualified subjects could remain on their current NSAIDs (no changes to supplements throughout the study). Subjects were required to have baseline hematology (CBC), biochemistry panel, and digital radiographs with 2 views (dorsoventral/lateral) of up to 2 (OA) joints within 4 months or 120 days prior to study enrollment. Each candidate was to continue their normal exercise or physical rehabilitation programs along with continuing their current diet and herbal medicine therapies at prescribed doses per their traditional Chinese veterinary medicine (TCVM) Pattern diagnosis.

A partial randomization was used to assign enrolled dogs into either the Test or Control groups. Subjects in the Test Group received the proposed periarticular aqua-AP delivery of PurePRP®[a], whereas those in the Control Group received the standard PurePRP® delivery technique (IA joint injection). A partial randomization group assignment was chosen as several patients had comorbidities leaving some owners concerned with heavy sedation or general anesthesia needed for the IA injections. For subjects whose owners were comfortable with either injection method, the group assignments were made randomly by a flip of a coin.

After assignment to study groups, each subject received an orthopedic evaluation, along with lameness scoring, followed by a physiological examination using an IRIT camera[b] (prior to manual manipulation). Objective measurements of goniometry were performed next, to avoid thermal tissue changes by manual manipulation.

Study examinations, assessments and treatments were performed in the same manner for all dogs at 0, 4 and 8 weeks. Subjects in either group were given appropriate sedation as determined by the attending practitioner along with a standard joint clip and surgical aseptic cleanse preparation prior to any aqua-AP or IA injection of the PurePRP®[a] product. Dogs in both groups were treated with the first PurePRP® injection at study start (Day 0), followed by a second injection at Day 30 (Week 4). This was followed by a recheck assessment at Day 60 (Week 8, study termination).

Platelet-rich Plasma Treatment Procedures

➢ Selection of PRP Kit

Differences in autologous whole blood processing techniques result in varied cellular composition of the final PRP product, and kits are classified based on postprocessing components.[21] In order for a cellular PRP to be effective, it must have a consistent preparation and proper acceptable cellular composition for the treated species to avoid detrimental inflammatory or cytotoxic consequences in the treated tissue.[6,12,13,34] Multiple commercial PRP separation systems have been developed with varying concentrations of platelets, red blood cells (RBC), white blood cells (WBC), neutrophil, monocyte and lymphocyte numbers. The end goal of tissue healing, pain relief and tissue remodeling may vary dependent of the PRP kit used and the species it is used in.[10,19,35-37]

A prospective study on the validation of 5 commercially available PRP kits sold to veterinarians was conducted.[6,14] All blood samples were processed according to the manufacturer's protocols with mean baseline of whole blood concentrations of platelet, RBC, WBC, neutrophil, monocyte, and lymphocyte numbers determined for each PRP system. In general, it is believed that red blood cells and neutrophils should be reduced as they have an inflammatory effect, while the effect of mononuclear cells remains largely unknown.[6,19,36,38] The PurePRP® commercial product was found to consistently produce the highest platelet yield and lowest RBC and neutrophil count by Carr's group.[6,14]

The PurePRP® system[a], designed as a closed aseptically prepared product, was selected for use in the present study. It offered consistent performance with optimal cell numbers for use in dogs as demonstrated by Carr et al., and supports evidence-based treatment methodology in the emerging field of regenerative medicine.[6,14,36]

➢ Preparation of the Platelet-rich Plasma (PurePRP® Kit)

The autologous PurePRP® product was prepared from study dog whole blood collection consistent with the manufacture's processing guidelines and anticoagulant dilution factors.[6,14,36] In general, preparation and administration of PRP consisted of 1) fasting dogs for 8-12 hours prior to sedation/anesthesia, 2) aseptic whole blood draw with primed syringe (30 ml) containing 5 ml anticoagulant, 3) centrifuge (2-spin technique) utilizing a closed sterile preparation system (concentration cylinder), 4) first-spin concentrated RBC's, centrifuge for 1 minute at 3600 rpm, 5) second-spin concentrated platelets, centrifuge for 5 minutes at 3800 rpm, 6) PRP preparation extracted, drawn into 3 ml sterile syringe yielding final product (neutrophil poor, RBC poor, PRP fraction), 7) final PurePRP® ready, divided into volume appropriate aliquots for injection (Figures 1 and 2).[6,14]

Intra-articular Delivery Technique

Dogs were given general anesthesia to completely immobilize them in order to perform a pain-free smooth IA joint injection. The investigator chose a combination of dexmedetomidine[c] (0.5 mg/ml) dosed at 125-500 mcg/m^2 intramuscularly (IM) and butorphanol[d] (10 mg/ml) dosed at 0.1-0.3 mg/kg (intravenous or intramuscular). A standard aseptic surgical preparation of the site was performed with the clinician wearing sterile gloves. The arthritic joint was entered with an appropriate size needle (i.e. 22-gauge 1–1½ inches), then attached to a syringe (6.0 ml), and correct placement was confirmed by aspiration of joint fluid. The needle remained in the joint and the syringe with synovial fluid was removed and replaced with a 2nd syringe pre-loaded with a standard volume of PurePRP® per joint,

Figure 1: PurePRP® (30 ml), post first spin demonstrating separation of red blood cells and plasma made from a whole blood collection.

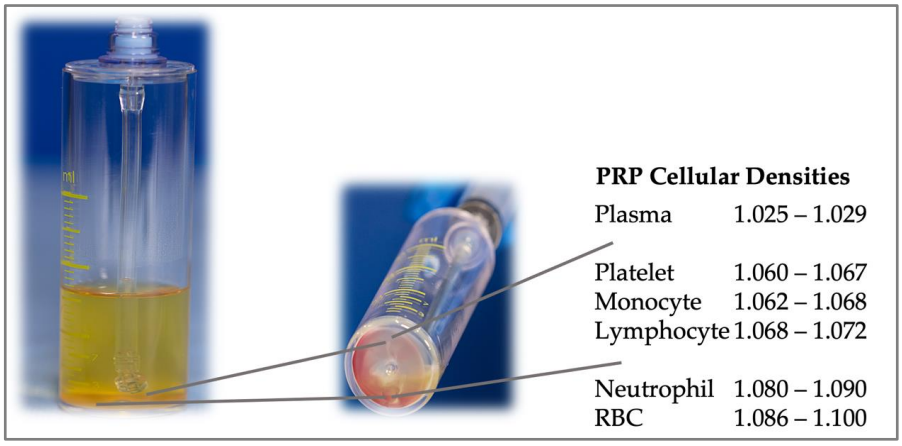


Figure 2: PurePRP® 30 ml, post second spin – a concentrated platelet buffy coat is noted on the bottom of the concentration device.

which was then injected (Table 2). The injection was then confirmed to be smooth and without resistance. After the injection was complete, the limb was placed in a series of range of motion (ROM) movements to disperse the PurePRP® within the treated joint space. Specific procedures of IA injection for different joints, which were followed in this study, are described in the literature.[6,11]

Table 2: Intra-articular injection volumes of PRP used for treatment of OA joints in the Control Group

Size of Dog	Total Injection Volume Guidelines for Joints (ml)	
	Elbow Joint	Shoulder, Stifle, Hip Joints
Miniature (<10 lbs.)	¼ – ½	½
Small (10-25 lbs.)	½ – 1	½ – 1
Medium (25-50 lbs.)	1 – 1½	1 – 1½
Large (50-100 lbs.)	1½ – 2	1½ – 2
Giant (>100 lbs.)	2 – 2½	2 – 3

Aqua-acupuncture Delivery Technique

The Test Group received aqua-AP using PurePRP® delivered to acupuncture points 1) local/periarticular to OA joints (shoulder, elbow, hip, stifle and/or hock) as well as, 2) non-local acupoints such as Master (thoracic and pelvic) and Influential (bone) points. Five acupoints were selected for each joint treated. The specific selection of the acupoints for either the thoracic or pelvic limbs were based on their given location and functionality of acupoints via TCVM principles. Using a 23-gauge ½ inch needle, 0.35 ml of PRP per acupoint was injected using aqua-AP methodology (Tables 3 and 4).[3,24,25,28,39-42]

Table 3: The sites treated with aqua-acupuncture using PRP for the Test Group

OA Joint	Acupuncture Points
Shoulder joint	SI-9, BL-11, GB-21, LI-15, TH-14
Elbow joint	SI-9, BL-11, LI-10, LI-11, *Zhou-shu*
Hip joint	BL-11, BL-54, GB-29, GB-30, BL-40
Stifle joint	BL-11, BL-54, GB-34, ST-35a, ST-36

The volume of PurePRP® injected per acupuncture point was 0.35 ml, with the use of a 23-gauge ½ inch needle. OA = osteoarthritis

Table 4: Acupuncture points used for aqua-AP injections in the Test Group to treat OA; anatomic location, indications and actions for each point are listed.[24]

Acupoint	Anatomic Location	Attributes, Indication and Actions
SI-9	Located caudal to the humerus in the large depression along the caudal border of the deltoid muscle at is juncture with the lateral and long heads of the triceps brachii muscles at the level of the shoulder joint	Local point for shoulder pain, thoracic limb pain, thoracic limb lameness or paresis/paralysis, generalized thoracic limb pain
BL-11	Located at the cranial edge of the scapula, 1.5 *cun* lateral to the dorsal spinous process of T1 (first palpable dorsal spinous process)	Influential point for bone, used for osteoarthritis, intervertebral disk disease, cervical pain, thoracolumbar pain, shoulder pain, thoracic limb weakness, cough, and fever
GB-21	Located in a groove in the muscle joint cranial to the scapula midway between GV-14 and the acromion (GV-14 is on the midline between C7-T1)	Local point to the shoulder, used for pain, thoracic limb pain or paralysis, dystocia, liver, and gallbladder disease (the crossing point of TH, GB, and *Yang-wei* Channels)
LI-15	Located at the shoulder region just cranial and distal to the acromion on the cranial margin of the acromial head of the deltoid muscle	Local point for shoulder pain, used for lameness, cervical pain, and intervertebral disk disease
TH-14	Located at the shoulder, caudal and distal to the acromion on the caudal margin of the acromial head of the deltoid muscles	Local point for shoulder and thoracic limb and used for limb pain and lameness
LI-10	Located on the craniolateral aspect of the thoracic limb 2 *cun* distal to LI-11 in the groove between the extensor carpi radialis and the common digital extensor muscles	The thoracic limb "three-mile point", a local elbow point for elbow pain, thoracic limb lameness, thoracic limb used for paresis, paralysis, *Qi* Deficiency or limb weakness
LI-11	Located on the lateral side of the thoracic limb at the lateral end of the cubital crease, halfway between the lateral epicondyle of the humerus and the biceps tendon with the elbow flexed	A local elbow point, *He*-sea point (Earth), used for tonification point for Deficiency disease patterns, elbow pain, thoracic limb paresis or paralysis
Zhou-shu	Located on the lateral side of the elbow between the lateral tuberosity of the humerus and the olecranon	Local elbow point for thoracic limb and used for lameness and paresis or paralysis
BL-54	Located at the coxofemoral joint at the level of the sacro-coccygeal hiatus, just dorsal to the greater trochanter of the femur	A Master point for the pelvic limbs, coxofemoral joint pain, used for osteoarthritis pain, pelvic limb paralysis or paresis, lameness, and muscle atrophy, *Qi* and Blood Stagnation to the pelvic limb
GB-29	Located at the coxofemoral joint in a depression just cranial to the greater trochanter of the femur	A local hip point for osteoarthritis of coxofemoral joint, used for pelvic limb pain, paresis or paralysis and gluteal muscle pain
GB-30	Located at the depression midway between the greater trochanter of the femur and the tuber ischii	A local hip point for osteoarthritis of coxofemoral joint, pelvic limb pain, paresis or paralysis and gluteal muscle pain

Table 4: Cont

Acupoint	Anatomic Location	Attributes, Indication and Actions
BL-40	Located in the center of the popliteal crease	A Master point for the caudal back and the coxofemoral joints, used for stifle disorders, pelvic limb paresis or paralysis, thoracolumbar-intervertebral disk disease, relieves local *Qi* and Blood Stagnation
GB-34	Located on the lateral side of the pelvic limb at the stifle, in a small depression cranial and distal to the head of the fibula	A local stifle point for tendon and ligament disorders, pelvic limb pain, paresis, or paralysis
ST-35a	Located in a depression distal to the patella, lateral to the patellar ligament	Local point for stifle pain, local *Qi* and Blood Stagnation and ligamentous disorders
ST-36	Located on the craniolateral aspect of the pelvic limb 3 *cun* distal ST-35, 0.5 *cun* lateral to the cranial aspect of the tibial crest, in the belly of the cranial tibialis muscle	Local stifle point for stifle disorders, *Qi* and Blood Stagnation

SI = Small Intestine; BL = Bladder; GB = Gall Bladder; LI = Large Intestine; TH = Triple Heater; ST = Stomach

Collection of Data

In addition to the baseline assessment (at Week 0), each patient returned at Week 4 and Week 8 for collection of objective outcome data for goniometry (non-blinded), lameness score (non-blinded) and digital thermal imaging of the treated joints (blinded). The clients (same person for all assessments) were given the Canine Brief Pain Inventory (CBPI) questionnaire to assess a subject's pain conditions (severity and interference scores) at Week 0, Week 2, Week 4, and Week 8.[43-46]

Goniometry

Goniometry, as defined for use in this study, is the use of an instrument (goniometer) to measure the ROM of joints in degrees relative to each joint angle measured.[3,4,47] It provides an objective measurement of joint motion. The arms of a transparent plastic goniometer were aligned with anatomic landmarks on the limbs (axis) and 1-degree gradations were used for measurements (Table 5).[48] All joints were measured in lateral recumbency except for shoulder abduction. Initial ROM measurements provided a basis for developing a treatment or therapeutic plan and pain assessment throughout the course of this study. All goniometry measurements were performed by a CCRP who was unblinded to the subject groups.

Orthopedic Lameness Grading Score

A lameness score, which provided a graded assessment of gait, was performed for each dog at Week 0 (baseline), Week 4 and Week 8. The small animal orthopedic lameness grading scale used for this study was based on a scale of 0-5 (Table 6).[14,3,43,47] The scores correlated a dog's gait with sound/normal = 0, or degree of lameness severity scored from 1-5, representing slightly lame (1) to severely lame/ reluctant to rise (5). A gait video was made of each dog at each assessment and played in slow motion via use of a smart phone to assess the patient's degree of lameness over time and response to therapy. A head or hip "bobbing" (when painful joint strikes the ground to lessen the weight on the affected limb) was noted and the affected limb associated with it. Forelimb lameness was evaluated when the dog walked towards the investigator and the hind limb lameness as the dog walked away from the investigator. This exam was not blinded as each CVA or CCRP had a treatment relationship with study dogs.

Table 6: Osteoarthritis lameness score criteria used for this study

Grade	Clinical evaluation
0	No detectable lameness at any gait
1	Walks normally
2	Slightly lame when walking
3	Moderately lame when walking
4	Severely lame when walking
5	Reluctant to rise and will not walk more than five paces

Table 5: Anatomical references used for correct positioning (goniometer) for goniometry measurements for each evaluated joint*

Joint	Static Arm	Vertex	Mobile Arm
Shoulder	Spine of the scapula	Subacromial space	Lateral epicondyle of the humerus
Elbow	Major tubercle of the humerus	Lateral epicondyle of the humerus	Lateral border of the radius
Carpus LL	Radius axis	Carpi axis	Longitudinal axis of the III and IV metacarpal bones
Carpus CC	Lateral epicondyle of the humerus	Styloid process of the ulna	Metacarpus V lateral axis
Hip	Iliac spine	Greater trochanter	Femoral longitudinal axis
Stifle	Femoral longitudinal axis	Lateral epicondyle of the femur	Lateral malleolus
Tarsus	Longitudinal axis of the tibia	Space between talus and calcaneus	Metacarpus V lateral axis

Abbreviations: CC = craniocaudal: for sagittal plane movements; LL = laterolateral: for transversal plane movements. *Adapted from "Goniometric Evaluation and Passive Range of Joint Motion in Chondrodystrophic and Non Chondrodystrophic Dogs of Different Sizes" by Reusing M, Brocardo M, Weber S et al.[48]

Infrared Thermal Imaging

An IRTI camera[b] was used to provide an accurate, objective, and quantifiable evaluation of study dog physiological status. The equipment detected radiant energy in degrees of temperature emitted from the patient's target tissue or region of OA. All patients had the selected OA joint prepared by a surgical clip with a #40 blade at least 7 days prior to the first IRTI collection. If the hair was noted to grow back during the study period, an additional clipping (medial and lateral) was repeated 60 minutes prior to any capture of IRTI. The preparation, with a time delay of 60 minutes, was necessary to prevent iatrogenic heat within the tissue from general limb handling and heat from the clippers. Each dog was allowed to acclimate to the treatment room temperature for 20-25 minutes on any given treatment date, prior to capturing IRTI. Similarly, for each subject, the IRTI data measured on the left joint was performed and compared to the corresponding opposite limb. The IRTI procedures were performed by a trained veterinary assistant or trained clinical project coordinator who was blinded to a subject's group assignment.

Canine Brief Pain Inventory

The same owner each time, who was not blinded to the group assignments, was asked to score their dog's pain during the study at Week 0 (baseline), 2, 4, and 8, respectively, using the University of Pennsylvania Canine Brief Pain Inventory (CBPI).[4,43,47] The CBPI grades two domain aspects consisting of pain severity and activity interference (Table 7). The CBPI contained four items (severity domain) pertaining to a dog's pain level to generate the average pain severity score (APSS). Owners graded each item on a scale of 0 (no pain) to 10 (extreme pain). Six activities (interference domain) were graded to generate the average pain interference (APIS) with the dog's daily activities. Each activity was scored 0 (no interference) to 10 (complete interference).

An APSS mean score for each week was generated from the daily pain scores given by the owner from the severity domain. The APIS mean score for each week was generated from the daily interference of activities (interference domain) score given by the owner. The APSS and APIS data was compared across both groups and over time throughout the study.

Table 7: Summary of the Canine Brief Pain Inventory (CBPI) scoring recorded by owners at 0, 2, 4, and 8 weeks of the study

Severity Domain	Interference Domain
Worst Pain	General Activity
Least Pain	Enjoyment of Life
Average Pain	Rising to Standing
Pain Now	Walking
	Running
	Climbing

Adapted from "Ability of the canine brief pain inventory to detect response to treatment in dogs with osteoarthritis" by Brown D, Boston R, Coyne J et al.[43]

Statistical Analysis

The study hypothesized that, with PurePRP®, both the proposed aqua-AP injection and the standard IA injection would improve the conditions associated with canine OA and that the proposed aqua-AP injection would result in better improvement than the standard IA injection. Based on the study design described above, the following statistical hypotheses were formulated: (1) H_0: The mean flexion outcome within the aqua-AP injection subject group remains the same over time (Week 0, Week 4, and Week 8); vs. H_A: There exists a change over time (Week 0, Week 4, and Week 8) on the mean flexion outcome within the aqua-AP injection subject group. This hypothesis set was also tested for extension outcome, thermal outcome, and CBPI pain outcome, respectively, and for the IA injection subject group. (2) H_0: There is no difference between the two injection groups with respect to the flexion outcome; vs. H_A: There exists a difference between the two injection groups with respect to the flexion outcome. The second hypothesis set was also tested for extension outcome, thermal outcome, and CBPI pain outcome, respectively.

To test the hypothesis sets for each outcome measurement, two-group repeated measure ANOVA using the ranked data was applied. Post hoc analysis that compared between the two groups at a specific assessment time was performed by the Wilcoxon Rank Sum test. A significance level of 0.05 was used for making all statistical conclusions. All statistical analysis was conducted with R software[e].

Sample Size and Power Analysis

To test the aforementioned repeated measure ANOVA, it was estimated that a sample size of at least 12 subjects in each group was required. This sample size would offer an 80% power to conclude time or group effect with a 0.05 significance level and effect size equal to 1.0

RESULTS

Animals

A total of 16 canine patients diagnosed with OA in the appendicular skeletal joints and meeting the inclusion and exclusion criteria were enrolled in the study. Half of the subjects were assigned to the aqua-AP injection group (Test) and the remaining half to the IA injection group (Control). All 16 subjects completed the 8-week partially randomized controlled trial and all subject data from study dogs were included in the data analysis. There were no adverse events associated with any of the Test or Control treatments recorded during the 8-week study.

In each subject, two affected joints, one from each side, were identified (Table 8). In the Test Group, all subjects had the same affected joint on both sides; 5 with left and right stifle joints, 2 with left and right elbow joints, and the remaining one with left and right hip joints. For the Control Group, 6 subjects had the same affected joints on both sides; 4 with left and right stifle joints, 1 with left and right elbow joints, and 1 with left and right shoulder joints. One control subject had left elbow and right stifle joints

affected, and another control subject had left shoulder joint and right hip joint affected. Combining the two groups (16 subjects), 10 had affected stifle joints (5 in each group), 4 had elbow joints affected (2 in each group), 2 had hip joints affected (1 in each group), and 2 subjects in the Control Group had their shoulder joints affected.

To confirm the comparability between the two treatment groups for comparing the treatment outcomes, group comparisons on a subject's signalment data including sex proportion, age, and weight were conducted (Table 9). Half of the subjects (4/8, 50%) in the Test Group were female, whereas 3 out of 8 (37.5%) in the Control Group were female. The group difference in proportion of sex was not significant (p = 1.000). All subjects were spayed/ neutered. The mean±SD age in the Test Group was 10.1±2.3 years old and was 10.4±2.2 years old in the Control Group (p = 0.590). On subject's body weight, the mean ±SD in the Test Group was 52.0±22.4 lbs., which was significantly smaller (p = 0.015) than that in the Control Group (77.5±11.8). Both groups consisted of multiple breeds. The Test Group included 1 West Highland Terrier, 2 Labrador Retrievers, 1 English Springer Spaniel, and 4 mixed-breeds. The Control Group had 6 Labrador Retrievers, 1 German Shorthaired Pointer, and 1 mixed-breed.

Based on the radiographic findings (scored 0 to 4; 0 = clinically normal; 1 = clinically normal with risk of OA; 2 = mild OA; 3 = moderate OA; 4 = severe OA); 6 out of 8 (75%) test subjects and 7 out of 8 (87.5%) control subjects, respectively, had at least moderate OA; the difference was not significant (p = 1.000). Other subject information included: (1) all subjects in both groups had a body condition being considered ideal (scored 3 or above on a 0 to 5 scale); (2) all subjects except one (a control subject) were treated with a Chinese herbal medicine, Body Sore[f] (*Shen Tong Zhu Yu Tang*), prior to and during the study and, (3) there were 4 and 5 dogs in the Test and Control groups, respectively, treated with an NSAID prior to and during the study.

Table 8: Affected osteoarthritis joints in each subject by study group

Subject	Group	Left Side	Right Side
1	Test	Stifle	Stifle
2	Test	Elbow	Elbow
3	Test	Stifle	Stifle
4	Test	Elbow	Elbow
5	Test	Hip	Hip
6	Test	Stifle	Stifle
7	Test	Stifle	Stifle
8	Test	Stifle	Stifle
9	Control	Shoulder	Shoulder
10	Control	Elbow	Stifle
11	Control	Stifle	Stifle
12	Control	Shoulder	Hip
13	Control	Elbow	Elbow
14	Control	Stifle	Stifle
15	Control	Stifle	Stifle
16	Control	Stifle	Stifle

Test: aqua-AP injection; Control: IA injection

Table 9: Summary statistics of study dog signalment data

	Test (n = 8)	**Control** (n = 8)	***p*-value**
Sex (Female; %)	50% (4/8)	37.5% (3/8)	1.000
Age (mean±SD; years)	10.1±2.3	10.4±2.2	0.590
Weight (mean±SD; lbs.)	52.0±22.4	77.5±11.8	0.015*

Sex proportion was compared by Fisher's Exact test; age and body weight were compared by Wilcoxon Rank Sum test; *=statistically significant $p<0.05$

Flexion Angles

At Week 0 (baseline), the flexion angles were not significantly different between the two subject groups (mean±SD): Test Group mean flexion angle of 51.3±5.9 vs. Control Group 51.1±9.7; p = 0.900). In the Test Group, baseline of 51.3±5.9 changed to 50.6±6.4 (p = 0.656) at Week 4, and was 51.9±6.3 (p = 1.000) at Week 8. In the Control group, baseline of 51.1±9.7 changed to 51.8±10.1 (p = 0.719) Week 4 and was 50.8±8.8 (p = 0.813) at Week 8. Within either treatment group, the changes (compared to Week 0) on flexion outcome were not statistically significant. Comparison of group differences for flexion change were also not statistically significant at Week 4 (p = 0.593), Week 8 (p = 0.741) and overall (p = 0.645).

Extension Angles

At Week 0 (baseline), the extension angles (mean±SD) in the Test Group was 152.4±9.5 and was 155.8±5.3 in the Control Group (p = 0.522). In the Test Group, baseline (152.4±9.5) had little change at Week 4 to 152.4±9.1 (p = 0.938) and was slightly improved at 154.1±8.8 (p = 0.203) at Week 8. The Control Group (baseline 155.8±5.3), changed to 159.2±3.2 (p = 0.141) at Week 4 and was 158.4±6.4 (p = 0.094) at Week 8. The overall group differences in extension change were not statistically significant (p = 0.217), nor for each assessment at Week 4 (p = 0.167) and Week 8 (p = 0.489).

Lameness Scores

At Week 0 (baseline), the lameness scores (mean±SD) were not significantly different between the two subject groups: Test Group 2.56±0.94 vs. Control Group 2.63±0.69; p = 0.556 (Figure 3). The Test Group's mean lameness score significantly reduced to 1.75±1.16 (p = 0.008) at Week 4, and further significantly decreased to 1.31±1.44 (p = 0.008) at Week 8. For the Control Group, the mean

lameness score also improved significantly at Week 4 (1.88±0.83; $p = 0.016$), and further significantly improved to 1.19±0.92 ($p = 0.008$) at Week 8. Overall, the group differences in lameness score improvements were not statistically significant ($p = 0.816$), nor for each assessment at Week 4 ($p = 0.870$) and Week 8 ($p = 0.789$) (Table 10).

Infrared Thermal Imaging

At Week 0 (baseline), the thermal outcomes (mean±SD) were not significantly different between the two subject groups: Test Group 101.1±11.7 vs. Control Group 103.3±9.1; $p = 0.575$ (Figure 4). After treatment, the Test Group mean baseline (101.1±11.7) temperature significantly reduced to 98.8±10.5 ($p = 0.039$) at Week 4, and further significantly decreased to 96.9±10.3 ($p = 0.016$) at Week 8 (Figure 5). On the other hand, the Control Group mean baseline (103.3±9.1) temperature did not change significantly at Week 4 (102.1±12.4; $p = 0.742$) but was significantly dropped to 95.4±10.0 ($p = 0.008$) at Week 8. The overall group differences in thermal outcome change were not statistically significant ($p = 0.575$), nor for each assessment at Week 4 ($p = 0.458$) and Week 8 ($p = 0.665$) (Table 11).

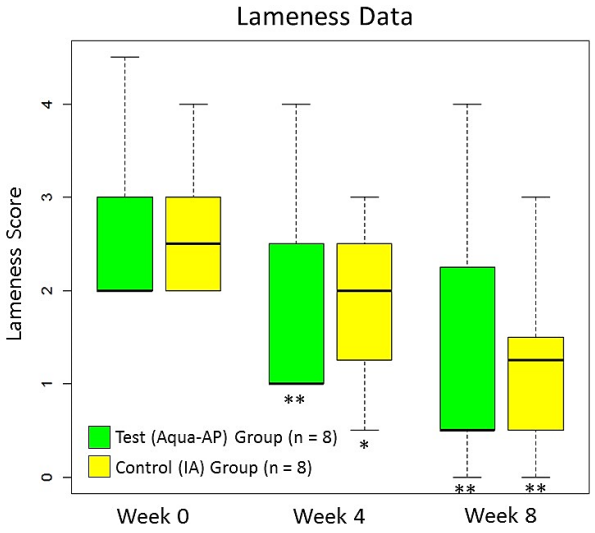


Figure 3: Distribution (by box-plot) of lameness score data in each subject group at the three assessment times; * = statistically significant $p < 0.05$, ** = statistically significant $p < 0.001$ compared to Week 0.

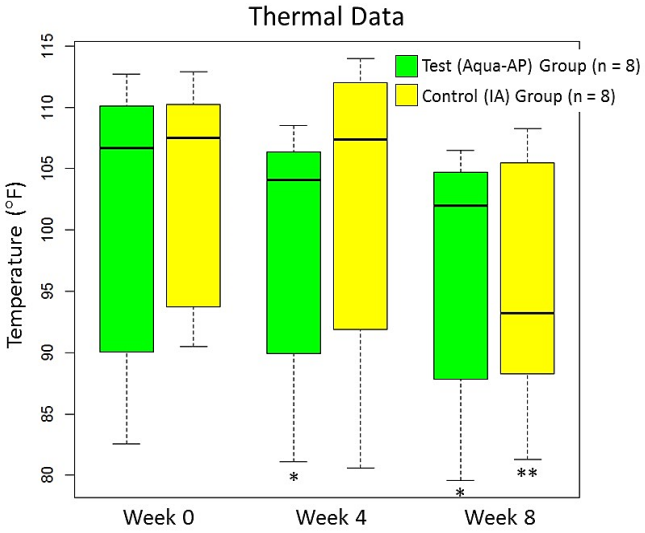


Figure 4: Distribution (by box-plot) of thermal imaging data in each subject group at the three assessment times; * = statistically significant $p < 0.05$, ** = statistically significant $p < 0.001$ compared to Week 0.

Table 10: Summary statistics (mean±SD) of lameness score data

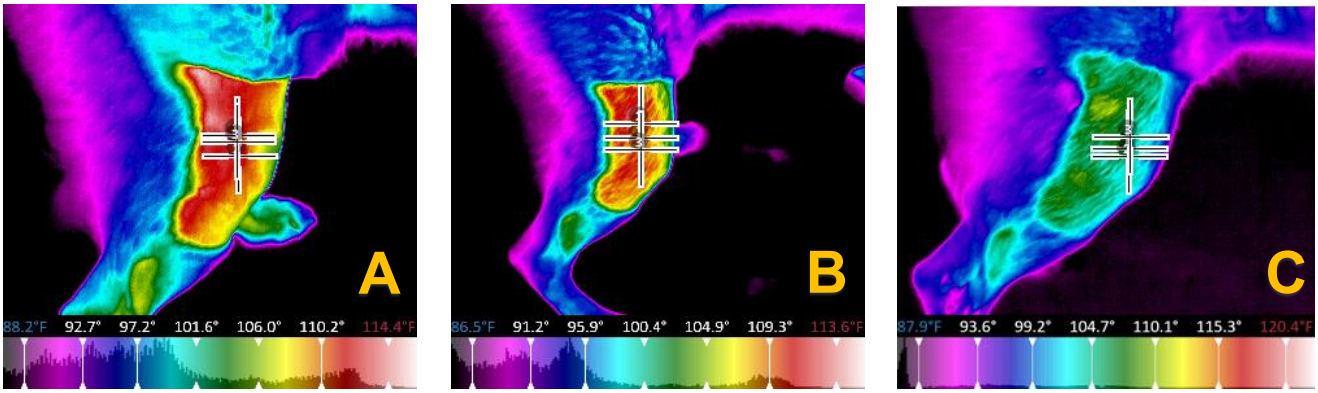
	Week 0	Week 4	Week 8
Test (aqua-AP)	2.56±0.94	1.75±1.16	1.31±1.44
	Change from Week 0	-0.81±0.26 ($p_1 = 0.008$)**	-1.25±0.53 ($p_1 = 0.008$)**
Control (IA)	2.63±0.69	1.88±0.83	1.19±0.92
	Change from Week 0	-0.75±0.46 ($p_1 = 0.016$)*	-1.44±0.56 ($p_1 = 0.008$)**
Difference between Test and Control groups	$p_0 = 0.556$	$p_2 = 0.870$	$p_2 = 0.789$

p_0 = p-value of Week 0 comparison between groups; p_1 = p-value of lameness score change compared to Week 0; p_2 = p-value of lameness score change comparison between groups; lower score = improvement

Table 11: Summary statistics (mean±SD) of infrared thermal imaging data (temperature in degrees Farenheit)

	Week 0	Week 4	Week 8
Test (aqua-AP)	101.1±11.7	98.8±10.5	96.9±10.3
	Change from Week 0	-2.3±3.0 ($p_1 = 0.039$)*	-4.2±3.6 ($p_1 = 0.016$)*
Control (IA)	103.3±9.1	102.1±12.4	95.4±10.0
	Change from Week 0	-1.2±4.4 ($p_1 = 0.742$)	-7.9±8.1 ($p_1 = 0.008$)*
Difference between Test and Control groups	$p_0 = 0.575$	$p_2 = 0.458$	$p_2 = 0.665$

p_0 = p-value of Week 0 comparison between groups; p_1 = p-value of temperature change compared to Week 0; p_2 = p-value of temperature change comparison between groups; lower temperature = improvement



Average temperature (in °F)

Zone 1	Zone 2	Zone 3
110.1	110.2	110.6

Average temperature (in °F)

Zone 1	Zone 2	Zone 3
107.7	107.4	108.0

Average temperature (in °F)

Zone 1	Zone 2	Zone 3
104.0	103.6	103.8

Figure 5: Infrared thermal images from the right stifle of aqua-AP Test Dog #1 starting with Week 0 on the left (5A), then Week 4 in the center (5B) showing decreasing joint temperature, and Week 8 on the right (5C) demonstrating continued decreasing joint temperature.

Average Pain Severity Scores

For each subject, an APSS within the previous 7 days was calculated (Figure 6). At Week 0 (baseline), the APSS (mean±SD) in the Test Group was 4.5±1.2 and was 4.5±2.3 in the Control Group. The group difference was not significant (p = 0.946). The range of APSS in the Control Group was much wider than that in the Test Group. Both treatment groups had statistically significant APSS improvement over time. In the Test Group (baseline 4.5±1.2), the Week 2 APSS decreased to 3.1±1.0 (p = 0.063), to 2.5±1.3 (p = 0.016) at Week 4, and to 1.8±2.1 (p = 0.031) at Week 8. Similarly in the Control Group (baseline 4.5±2.3), the Week 2 APSS reduced to 2.8±1.0 (p = 0.063), to 2.3±1.0 (p = 0.016) at Week 4, and to 1.3±0.5 (p = 0.016) at Week 8. The overall difference between the Test and Control groups in APSS improvements were not statistically significant (p = 0.413), nor for each assessment at Week 2 (p = 0.679), Week 4 (p = 0.863), and Week 8 (p = 0.607) (Table 12).

Average Pain Interference Scores

For each subject, an APIS was assessed (Figure 7). At Week 0 (baseline), the mean±SD APIS in the Test Group was 5.7±2.0 and was 5.1±1.9 in the Control Group. The difference between the two groups at baseline was not significant (p = 0.425). Like the pain severity scores (APSS), both treatment groups' APIS significantly improved over time. In the Test Group (baseline 5.7±2.0), the Week 2 APIS significantly reduced to 3.2±2.0 (p = 0.016), and then to 2.3±2.2 (p = 0.008) at Week 4, and to 1.9±2.9 (p = 0.016) at Week 8. In the Control Group (baseline 5.1±1.9), the Week 2 APIS significantly reduced to 2.5±1.6 (p = 0.008), then to 1.9±0.3 (p = 0.008) at Week 4, and to 1.2±0.6 (p = 0.008) at Week 8. The overall difference between groups in APIS improvements were not statistically significant (p = 0.708), nor for each assessment at Week 2 (p = 0.879), Week 4 (p = 0.645), and Week 8 (p = 1.000) (Table 13).

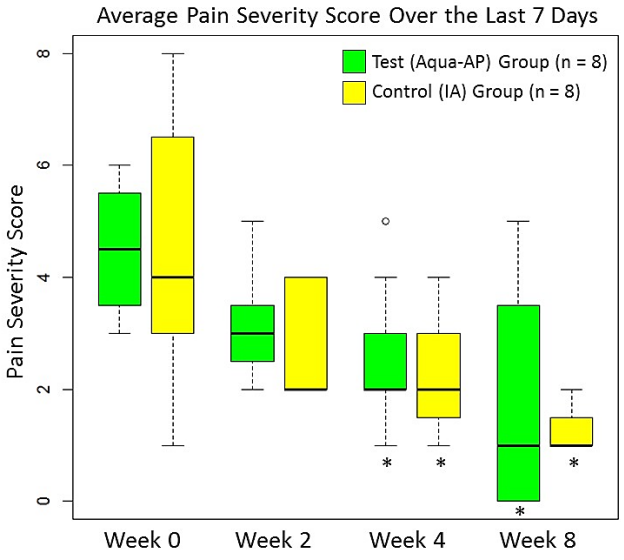


Figure 6: Distribution (by box-plot) of the average observed pain severity score (within the last 7 days) in each subject group at the four assessment times; * = statistically significant $p < 0.05$ compared to Week 0.

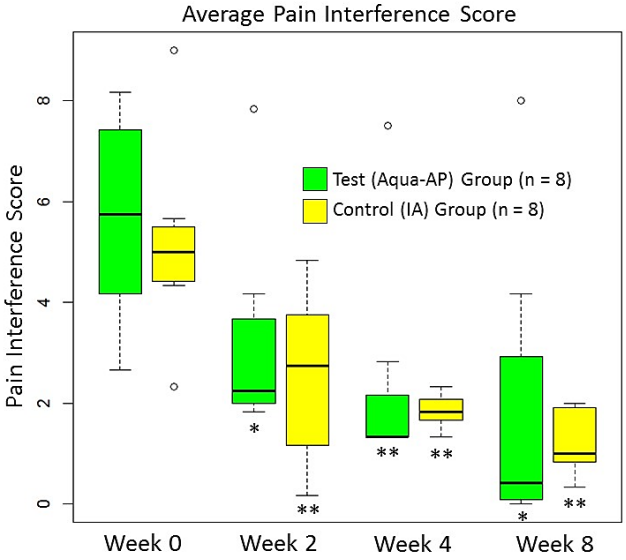


Figure 7: Distribution (by box-plot) of the APIS in each subject group at the four assessment times; * = statistically significant $p < 0.05$; ** = statistically significant $p < 0.001$ compared to Week 0.

Table 12: Summary statistics (mean±SD) of APSS data

	Week 0	Week 2	Week 4	Week 8
Test (aqua-AP)	4.5±1.2	3.1±1.0	2.5±1.3	1.8±2.1
	Change from Week 0	-1.4±1.5 (p_1 = 0.063)	-2.0±1.4 (p_1 = 0.016)*	-2.8±2.4 (p_1 = 0.031)*
Control (IA)	4.5±2.3	2.8±1.0	2.3±1.0	1.3±0.5
	Change from Week 0	-1.8±1.8 (p_1 = 0.063)	-2.3±1.6 (p_1 = 0.016)*	-3.3±2.3 (p_1 = 0.016)*
Difference between Test and Control groups	p_0 = 0.946	p_2 = 0.679	p_2 = 0.863	p_2 = 0.607

p_0 = p-value of Week 0 comparison between groups; p_1 = p-value of pain severity change compared to Week 0; p_2 = p-value of pain severity change comparison between groups; lower APSS score = improvement

Table 13: Summary statistics (mean±SD) of APIS data

	Week 0	Week 2	Week 4	Week 8
Test (aqua-AP)	5.7±2.0	3.2±2.0	2.3±2.2	1.9±2.9
	Change from Week 0	-2.5±1.8 (p_1 = 0.016)*	-3.4±2.1 (p_1 = 0.008)**	-3.8±2.3 (p_1 = 0.016)*
Control (IA)	5.1±1.9	2.5±1.6	1.9±0.3	1.2±0.6
	Change from Week 0	-2.6±1.4 (p_1 = 0.008)**	-3.3±1.8 (p_1 = 0.008)**	-3.9±1.6 (p_1 = 0.008)**
Difference between Test and Control groups	p_0 = 0.425	p_2 = 0.879	p_2 = 0.645	p_2 = 1.000

p_0 = p-value of Week 0 comparison between groups; p_1 = p-value of pain interference change compared to Week 0; p_2 = p-value of pain interference change comparison between groups; lower APIS score = improvement

DISCUSSION

Osteoarthritis is a chronic inflammatory disease riddled with joint morbidity. It has engendered a wide variety of treatments with varying efficacy. Orthobiologics have gained popularity within the past fifteen years as they aid in the repair of local tissues while avoiding the unwanted side effects of conventional OA medications. Platelet-rich plasma, the focus of the current study, is an autologous preparation from whole blood with uncomplicated processing, which offers the convenience of point of care therapy for OA. This clinical study evaluated and compared OA treatment protocols administering PRP in the typical fashion as an IA injection with a novel technique, periarticular aqua-AP injection. The study findings demonstrated improved APSS scores at all time points (2, 4, 8 weeks) with statistical significance at 4 and 8 weeks for both techniques. The APIS scores were similar but reached statistical significance at all measured study time points (2, 4, 8 weeks) for both study groups. The IRTI outcome data demonstrated statistically significant decreased temperature in the inflamed joints for both injection techniques, however, aqua-AP technique was statistically significant at both 4 and 8 weeks while IA technique was slower with a significant temperature decrease at 8 weeks post-treatment. Flexion and extension goniometry of OA joints did not show any significant improvement for either technique.

The infrared camera used to assess study dog OA joints utilizes specialized medical software which provides a pattern of emitted energy that is converted to a visible image. The information from this image is objective and can be analyzed, measured, and compared to other images of the same patient over a period of time, as demonstrated in Figure 5 in one of the Test Group dogs.[44,49]A direct correlation exists between increases within the thermal gradients and increases in the blood flow and inflammation within that anatomical area.[42-44,49] The use of IRTI, through identification of inflammation, can identify areas eliciting OA pain, and additionally provide the TCVM practitioner a view of Stagnation within specific anatomic regions. The thermal image data in the present study clearly showed marked temperature increases in affected joints of each patient suffering from OA joint pain. After treatment, the Test Group showed significant temperature reduction of the inflamed joint at Week 4, whereas the Control Group was slower with significant reduction not apparent until the Week 8 assessment. It may be inferred from the data that both injection techniques are effective for OA treatment. The temperature reduction delay in the Control Group (IA), may be related to iatrogenic joint injection trauma. This created an increased inflammatory response which slowed healing mechanisms versus the Test Group (aqua-AP) which created less technique-associated inflammation by using a periarticular technique.

Intra-articular regenerative therapies are generating interest as OA treatment options for providing effective pain relief and improved joint function in dogs.[50-52] The use of aqua-AP injections with orthobiologics to treat OA has been less commonly investigated. A canine study (n=9) using adipose derived stem cells (i.e. autologous adipose stromal vascular fraction or allogeneic adipose-derived stem cells) as a regenerative therapy for dogs with hip dysplasia, investigated aqua-AP delivery of the treatment. The investigators injected the stem cell preparation into acupuncture points (BL-54, GB-29, GB-30) of study dogs.[40] After the first week, clinical evaluation showed marked improvement, compared to baseline; and at days 15 and 30, all dogs but 1 showed improvement of ROM, lameness at trot, and pain when joints were manipulated. The authors concluded that the therapy appeared to safely improve hip function in dogs affected with hip dysplasia and represents an important therapeutic alternative.[40] Similarly, in a rodent hindlimb ischemia model, mesenchymal stem cell injection into acupoints increased levels of vascular endothelial growth factor, transfer growth factor-B1 and nitric oxide, improving angiogenesis and arteriogenesis.[53] A mouse model for Duchenne muscular dystrophy was treated with acupoint (BL-47, BL-49, BL-52) injections of mesenchymal stem cells.[54] Treated groups demonstrated decreased muscle damage, improved strength, and decreased creatinine phosphokinase levels. The results of these studies, similar to the present study, suggest that acupuncture points represent a potential administration route for orthobiologics as acupoint treated groups showed clinical improvements.

Recent advances in magnetic resonance imaging (MRI) technology have provided new insights and understanding into acupuncture point anatomy, their Meridian association, and mechanism of pharmacopuncture (i.e. aqua-AP). Kim et al. investigated the permeation and migration behavior of MRI contrast agent and tracer after injection at acupoints of small animals.[55] The distribution of injected material was reconstructed in 3-dimensional images. Kim's group found that a recently developed fluorine compound was effective for imaging the migration of the agent after injection into the acupoints BL-18, BL-20, and BL-23. The final distributions of the agent from each acupoint injection corresponded to the local fascial anatomy and the respective organs of the acupoints. The results suggested different migration paths and destinations for pharmacopuncture drugs is possible.[55] As future research is conducted and employs the use of more sophisticated imaging equipment (CT/MRI), the authors anticipate that a broader understanding of the local fascial anatomy and the mechanism of action of aqua-AP for delivery of cellular therapies within the Meridians will be developed.

The study did present unexpected findings. Both study groups did not have significant changes in goniometry data after PRP injection, even though overall patient observations demonstrated clinical improvements. These study findings support the conclusion that OA not only involves the bone, but also the periarticular supporting soft tissue structures, whose pathology may ultimately limit joint biomechanics. Future studies might investigate the additional benefit of aggressive physical therapy programs, photobiomodulation (laser), shock wave and hydrotherapy in various combinations with regenerative medicine therapeutics. Another finding, which has been reported, but was present in a much greater portion of study dogs (9/16, 53%) than expected, was bilateral stifle joint OA.[56-58] The proportion of subjects with bilateral OA disease of other joints was markedly smaller and included elbow (3/16, 18.8%) and one dog each with bilateral hip or bilateral shoulder OA (1/16, 6.6%).

Limitations to the study included less than planned enrollment numbers, primarily due to timing of the study (coronavirus pandemic) and owner reluctance to incur the expenses necessary to participate in the study (e.g. costs for professional time/anesthesia, PRP test kits, radiographs, bloodwork). The study was also limited by partial randomization, as many of the owners with an elderly animal wanted treatment for OA, but were hesitant to subject their dog to a procedure requiring general anesthesia and joint injection trauma; therefore, they were enrolled in the aqua-AP group. The study was only partially blinded, as the CCRP practitioner, who assessed goniometry measurements, also performed IA injections (Control Group) and owners who filled out the CBPI assessment were unblinded. The veterinary assistant who performed the thermal imaging, however, was blinded to group assignments, making thermal findings an objective assessment to compare to findings from non-blinded assessments.

Confounding study findings included a statistically significant (p=0.015) greater mean body weight for controls (77.5±11.8) versus test dogs (52.0±22.4). This could contribute to a difference in response to treatment, as generally smaller dogs (i.e. lower body weight) are less clinically affected by OA than larger dogs. Additionally, the study evaluated multiple joints, rather than limited to one joint, which likely contributed to differences in ranges across groups. Finally, different subject dog characteristics [e.g. innate platelet numbers, presence of inflammation, drug use that may affect platelet function (i.e. NSAIDs)] could contribute to mild PRP variation.[21] Overall, a fully funded study with a larger sample population, completely blinded/randomized, and more objective measurements, would have been superior. An additional element to include in future studies would be an aggressive post-PRP-therapy physical rehabilitation protocol, as there is a need for sound research in this area.

In summary, study results demonstrated that both treatments resulted in a statistically significant reduction of both OA pain and activity level interference scores with no adverse effects. There was no significant difference between treatment groups concluded ($p > 0.05$), however, the novel PRP delivery technique suggested several advantages over current delivery method. The new intervention had more rapid temperature decrease of inflamed OA joints, reduced sedation requirements and patient stress, eliminated iatrogenic trauma to joints and decreased procedure cost. Overall, client satisfaction was

extremely high. Geriatric dog owners in particular were interested in a treatment option that could be offered to canine patients with comorbidities. The study findings, under the experimental conditions of this study, suggest that PurePRP® treatment delivered with either the proposed novel aqua-AP or the standard IA injection may improve OA pain with a favorable treatment response.

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Declaration of Interest and Funding

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FOOTNOTES

[a] PurePRP®, EmCyte Corporation®, Fort Myers, FL, USA
[b] IRTI, WellVu Veterinary Imaging, Ocala, FL, USA
[c] Dexmedetomidine hydrochloride injection (solution), Zoetis Inc, Parsippany, NJ, USA
[d] Butorphanol tartrate, Dechra, Fort Worth, TX, USA
[e] R version 4.2.1 (64-bit). The R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org
[f] Body Sore (concentrated 90g); Dr. Xie's Jing Tang Herbal, Ocala, FL, USA

REFERENCES

1. Wright A, Amodie D, Cernicchiaro N et al. Identification of canine osteoarthritis using an owner-reported questionnaire and treatment monitoring using functional mobility tests. J Small Anim Pract 2022; 63(8):609-618. https://doi.org/10.1111/jsap.13500
2. Johnston S. Osteoarthritis. Joint anatomy, physiology, and pathobiology. Vet Clin North Am Small Anim Pract 1997; 27(4):699-723. https://doi.org/10.1016/s0195-5616(97)50076-3
3. Perkins P. TCVM for canine sports medicine. Practical Guide to Traditional Chinese Veterinary Medicine, Small Animal Practice. Xie H, Wedemeyer L, Chrisman C (eds). Reddick, FL: Chi Institute Press 2014:3-34.
4. Gingerich D, Strobel J. Use of client-specific outcome measures to assess treatment effects in geriatric, arthritic dogs: controlled clinical evaluation of a nutraceutical. Vet Ther 2003; 4(4): 376-386.
5. Zoetis PetCare. Tools and Resources, Rimadyl (Carprofen) prescribing and dosing quick downloads. https://www.zoetispetcare.com/products/rimadyl
6. Carr B, Canapp S, Mason D et al. Canine platelet-rich plasma systems: a prospective analysis. Front Vet Sci 2016; 2:73. https://doi.org/10.3389/fvets.2015.00073
7. Elanco. Our Product, How it works, Targeted Canine Osteoarthritis Pain Relief. https://my.elanco.com/us/campaign/galliprant
8. Verma V, Sheikh Z, Ahmed A. Nociception and role of immune system in pain. Acta Neurol Belg 2015; 115(3):213-220. https://doi.org/10.1007/s13760-014-0411-y
9. Shimberg M. The use of amniotic-fluid concentrate in orthopaedic conditions. J Bone Joint Surg Am 1938; 20(1):167-177.
10. Caplan A, Correa D. The MSC: an injury drugstore. Cell Stem Cell 2011; 9(1):11-15. https://doi.org/10.1016/j.stem.2011.06.008
11. Companion Animal Health. What is regenerative medicine? https://www.companionanimalhealth.com/page/regenerative-medicine
12. Murphy M, Moncivais K, Caplan A. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med 2013; 45(11):e54. https://doi.org/10.1038/emm.2013.94
13. Caplan A. Adult mesenchymal stem cells: when, where, and how. Stem Cell Int 2015; 2015:628767. https://doi.org/10.1155/2015/628767
14. Carr B. Platelet-rich plasma as an orthobiologic: clinically relevant considerations. Vet Clin North Am Small Anim Pract 2022; 52(4):977-995. https://doi.org/10.1016/j.cvsm.2022.02.005
15. Black L, Gaynor J, Gahring D et al. Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter, controlled trial. Vet Ther 2007; 8(4):272-284.
16. Black L, Gaynor J, Adams C et al. Effect of intraarticular injection of autologous adipose-derived mesenchymal stem and regenerative cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs. Vet Ther 2008; 9(3):192-200.
17. Jain N, Gulati M. Platelet-rich plasma: a healing virtuoso. Blood Res 2016; 51(1):3-5. https://doi.org/10.5045/br.2016.51.1.3
18. Vilar J, Manera M, Santana A et al. Effect of leukocyte-reduced platelet-rich plasma on osteoarthritis caused by cranial cruciate ligament rupture: a canine gait analysis model. PLoS One 2018; 13(3):e0194752. https://doi.org/10.1371/journal.pone.0194752
19. Everts P, Devilee R, Mahoney C et al. Exogenous application of platelet-leukocyte gel during open subacromial decompression contributes to improved patient outcome: a prospective randomized double-blind study. Eur Surg Res 2008; 40(2):203-210. https://doi.org/10.1159/000110862
20. Boswell S, Cole B, Sundman E et al. Platelet-rich plasma: a milieu of bioactive factors. Arthroscopy 2012; 28(3):429-439. https://doi.org/10.1016/j.arthro.2011.10.018
21. Kaneps A. A one-health perspective: use of hemoderivative regenerative therapies in canine and equine patients. J Am Vet Med Assoc 2023; 261(3):301-308. https://doi.org/10.2460/javma.22.12.0556
22. Everts P, Onishi K, Jayaram P et al. Platelet-rich plasma: new performance understandings and therapeutic considerations in 2020. Int J Mol Sci 2020; 21(20):7794. https://doi.org/10.3390/ijms21207794
23. Schoen A. Acupuncture for musculoskeletal disorders. Probl Vet Med 1992; 4(1):88-97.
24. Xie H, Preast V. Xie's Veterinary Acupuncture. Ames Iowa: Blackwell Publishing 2007:256.
25. Langevin H, Yandow J. Relationship of acupuncture points and Meridians to connective tissue planes. Anat Rec 2002; 269(6):257-265. https://doi.org/10.1002/ar.10185
26. Baker-Meuten A, Wendland T, Shamir S et al. Evaluation of acupuncture for the treatment of pain associated with naturally-occurring osteoarthritis in dogs: a prospective, randomized, placebo-controlled, blinded clinical trial. BMC Vet Res 2020; 16(1):357. https://doi.org/10.1186/s12917-020-02567-1
27. Li L, Liu H, Shi W et al. Insights into the action mechanisms of traditional Chinese medicine in osteoarthritis. Evid Based Complement Alternat Med 2017; 2017:5190986. https://doi.org/10.1155/2017/5190986
28. Carlson E, Perez-Abadia G, Adams S et al. A novel technique for visualizing the intralymphatic primo vascular system by using hollow gold nanospheres. J Acupunct Meridian Stud 2015; 8(6):294-300. https://doi.org/10.1016/j.jams.2015.09.003
29. Fry L, Neary S, Sharrock J et al. Acupuncture for analgesia in veterinary medicine. Top Companion Anim Med 2014; 29(2):35-42. https://doi.org/10.1053/j.tcam.2014.03.001
30. Huntingford J, Petty M. Evidence-based application of acupuncture for pain management in companion animal medicine. Vet Sci 2022; 9(6):252. https://doi.org/10.3390/vetsci9060252
31. Bordoni B, Lagana M. Bone tissue is an integral part of the fascial system. Cureus 2019; 11(1):e3824. https://doi.org/10.7759/cureus.3824
32. Cheng K. Neurobiological mechanisms of acupuncture for some common illnesses: a clinician's perspective. J Acupunct Meridian Stud 2014; 7(3):105-114. https://doi.org/10.1016/j.jams.2013.07.008
33. Mier H. Effectiveness of aqua-acupuncture for reducing stress of canine patients in veterinary clinics. Am J Trad Chin Vet Med 2021; 16(1):41-50. https://doi.org/10.59565/001c.82230
34. Hsu W, Mishra A, Rodeo S et al. Platelet-rich plasma in orthopaedic applications: evidence-based recommendations for treatment. J Am Acad Orthop Surg 2013; 21(12):739-748. https://doi.org/10.5435/JAAOS-21-12-739

35. Houard X, Goldring M, Berenbaum F. Homeostatic mechanisms in articular cartilage and role of inflammation in osteoarthritis. Curr Rheumatol Rep 2013; 15(11):375. https://doi.org/10.1007/s11926-013-0375-6
36. EmCyte Corporation. PurePRP® supraphysiologic nature's regenerative powerhouse: defining the clinical criteria for excellence in patient care. Accessed 11/27/22, https://emcyte.com/about-us/
37. McLellan J, Plevin S. Does it matter which platelet-rich plasma we use? Equine Vet Educ 2011; 23(2):101-104. https://doi.org/10.1111/j.2042-3292.2010.00185.x
38. Caplan A, Dennis J. Mesenchymal stem cells as trophic mediators. J Cell Biochem 2006; 98(5):1076-1084. https://doi.org/10.1002/jcb.20886
39. Soh K. Bonghan circulatory system as an extension of acupuncture Meridians. J Acupunct Meridian Stud 2009; 2(2):93-106. https://doi.org/10.1016/S2005-2901(09)60041-8
40. Marx C, Silveira M, Selbach I et al. Acupoint injection of autologous stromal vascular fraction and allogeneic adipose-derived stem cells to treat hip dysplasia in dogs. Stem Cells Int 2014; 2014:391274. https://doi.org/10.1155/2014/391274
41. Sha T, Gao L, Zhang C et al. An update on acupuncture point injection. QJM 2016; 109(10):639-641. https://doi.org/10.1093/qjmed/hcw055
42. Xie H. Chinese Veterinary Herbal Handbook. Reddick, FL: Jing Tang Publishing 2011:80,136,187.
43. Brown D, Boston R, Coyne J et al. Ability of the canine brief pain inventory to detect response to treatment in dogs with osteoarthritis. J Am Vet Med Assoc 2008; 233(8):1278-1283. https://doi.org/10.2460/javma.233.8.1278
44. WellVu. WellVu 640 veterinary specific camera. https://wellvuimaging.com/veterinary-products-cameras
45. Ramirez-GarciaLuna J, Rangel-Berridi K, Bartlett R et al. Use of infrared thermal imaging for assessing acute inflammatory changes: A case series. Cureus 2022; 14(9):e28980. https://doi.org/10.7759/cureus.28980
46. Aulakh K, Dongaonkar K, Barnes K et al. Influence of orthopedic examination on lameness scores and interobserver and intraobserver agreement in dogs with naturally occurring elbow osteoarthritis. Vet Surg 2020; 49(3):455-462. https://doi.org/10.1111/vsu.13390
47. Rialland P, Bichot S, Moreau M et al. Clinical validity of outcome pain measures in naturally occurring canine osteoarthritis. BMC Vet Res 2012; 8:162. https://doi.org/10.1186/1746-6148-8-162
48. Reusing M, Brocardo M, Weber S et al. Goniometric evaluation and passive range of joint motion in chondrodystrophic and non-chondrodystrophic dogs of different sizes. VCOT Open 2020; 3(2):e66-e71. https://doi.org/10.1055/s-0040-1713825
49. Wright B. Acupuncture for the treatment of animal pain. Vet Clin North Am Small Anim Pract 2019; 49(6):1029-1039. https://doi.org/10.1016/j.cvsm.2019.07.001
50. Ip H, Nath D, Sawleh S et al. Regenerative medicine for knee osteoarthritis – The efficacy and safety of intra-articular platelet-rich plasma and mesenchymal stem cells injections: a literature review. Cureus 2020; 12(9):e10575. https://doi.org/10.7759/cureus.10575
51. Pawitan J. Various stem cells in acupuncture Meridians and points and their putative roles. J Tradit Complement Med 2018; 8(4):437-442. https://doi.org/10.1016/j.jtcme.2017.08.004
52. Cuervo B, Rubio M, Sopena J et al. Hip osteoarthritis in dogs: a randomized study using mesenchymal stem cells from adipose tissue and plasma rich in growth factors. Int J Mol Sci 2014; 15(8):13437-13460. https://doi.org/10.3390/ijms150813437
53. Zhu C, Dong J, Li J et al. Preliminary study on the mechanism of acupoint injection of bone marrow mesenchymal stem cells in improving blood flow in the rat of hind limb ischemia. J Tradit Chin Med 2011; 31(3):241-245. https://doi.org/10.1016/s0254-6272(11)60050-2
54. Esper G, Pignatari G, Rodrigues M et al. Aquapuncture using stem cell therapy to treat mdx mice. Evid Based Complement Alternat Med 2015; 2015:132706. https://doi.org/10.1155/2015/132706
55. Kim J, Bae K, Hong K et al. Magnetic resonance imaging and acupuncture: a feasibility study on the migration of tracers after injection at acupoints of small animals. J Acupunct Meridian Stud 2009; 2(2):152-158. https://doi.org/10.1016/S2005-2901(09)60048-0
56. Buote N, Fusco J, Radasch R. Age, tibial plateau angle, sex, and weight as risk factors for contralateral rupture of the cranial cruciate ligament in Labradors. Vet Surg 2009; 38(4):481-489. https://doi.org/10.1111/j.1532-950X.2009.00532.x
57. Guthrie J, Keeley B, Maddock E et al. Effect of signalment on the presentation of canine patients suffering from cranial cruciate ligament disease. J Small Anim Pract 2012; 53(5):273-277. https://doi.org/10.1111/j.1748-5827.2011.01202.x
58. Gilbert S, Langenbach A, Marcellin-Little D et al. Stifle joint osteoarthritis at the time of diagnosis of cranial cruciate ligament injury is higher in Boxers and in dogs weighing more than 35 kilograms. Vet Radiol Ultrasound 2019; 60(3):280-288. https://doi.org/10.1111/vru.12718