Universidade de Évora – Instituto de Investigação e Formação Avançada

Programa de Doutoramento em Ciências Veterinárias

Tese de Doutoramento

Evaluation of the efficacy of four intra-articular therapeutic protocols for the control and treatment of osteoarthritis in a *Canis familiaris* model

João Carlos Agostinho Alves

Orientador(es) | Luis Miguel Alves Carreira

Catarina Falcão Trigoso Vieira Branco Lavrador

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Título: Avaliação da eficácia de quatro protocolos terapêuticos intra-articulares no controlo e tratamento da Osteoartrite em modelo *Canis familiaris*.

RESUMO

A osteoartrite (OA) é uma doença que afecta que afecta todos os mamíferos, com uma expressão clínica e económica muito importante. No cão, a sua fisiopatologia clínica, médica e terapêutica são muito semelhantes às do Homem, tornando esta espécie num modelo natural de excelência para o estudo da OA no Homem. Associando-se a doença predominantemente ao aparecimento de sinais e sintomas clínicos envolvendo a(s) articulação(ões) afetada(s), o uso de terapêuticas locais administradas por via intra-articular (IA), possibilitam reduzir a quantidade da substância activa a utilizar para obter o efeito médico desejado, assim como os potenciais efeitos sistémicos colaterais. Desde há muito tempo que o uso de corticosteróides e ácido hialurónico têm assumido um papel relevante na terapia IA da OA. Actualmente, terapias inovadoras como o uso de concentrados de plaquetas autologas ou de anabolizantes, como o Estanozolol, têm-se revelado promissoras no controlo local da doença. Contudo, os seus mecanismos de ação não estão ainda totalmente esclarecidos, o que se traduz na dificuldade em obter consenso entre os clínicos para o estabelecimento de protocolos padronizados, fazendo-se assim a sua utilização com base na medicina de evidência resultante da experiência individual do clínico. A monitorização da OA no que respeita à sua evolução e resposta à terapeutica instituída é actualmente monitorizada com o recurso a marcadores de inflamação, técnicas de imagem como a radiografia e termografia digitais, ao estudo e análise da biomecânica do doente em placas ou plataformas de força, que adicionam informação muito importante à colhida durante o exame clínico objectivo do doente.

O presente estudo realizado na espécie *Canis familiaris* com OA na articulação coxofemoral apresenta como principais objetivos: 1) Determinar o efeito de quatro substâncias administradas por via IA no maneio da OA da anca; 2) Avaliar a variação na concentração dos marcadores inflamatórios Proteína C-Reativa (PCR) e Interleucina-1 (IL-1) no líquido sinovial de doentes com OA coxofemoral; 3) Avaliar a relação entre a medicina laboratorial, a medicina de imagem e o exame médico objectivo do doente; 4) Validar o uso da termografia digital e da plataforma de pressão na avaliação da OA; e 5) Delinear um protocolo terapêutico para os doentes com OA coxofemoral utilizando o cão como modelo animal, sob o conceito geral de - Uma Única Saúde (*One Health*). O estudo foi desenvolvido numa amostra de cem articulações coxo-femorais (N = 100), de doentes com OA de ocorrência natural. A amostra estudada foi dividida aleatoriamente em cinco (5) grupos cada um com vinte (20) articulações, de acordo com o tipo de terapêutica IA administrada. Assim, consideraram-se: grupo de controlo (GC, n=20), grupo de hexacetonido de triancinolona (THG,

n=20), grupo de concentrado de plaquetas (Grupo PCG, n=20), grupo de estanozolol (SG, n=20) e o grupo de acido hialurónico - Hylan GF 20 (HG, n=20). Cada doente foi avaliado em nove (9) tempos diferentes ao longo do estudo: dia 0 (dia do tratamento) e dias 8, 15, 30, 60, 90, 120, 150 e 180 após tratamento. As avaliações de cada doente e articulação foram realizadas com a análise da biomecânica do doente em plataforma de força, goniometria da articulação (amplitude articular em flexão e extensão), perímetro da coxa, termografia digital, radiografia digital, análise do líquido sinovial e quatro instrumentos de metrologia clínica, nomeadamente: Escala Rápida de Avaliação de Dor Canina (*Canine Brief Pain Inventory*), Escala de Osteoartite Canina de Liverpool (*Liverpool Osteoarthritis in Dogs*), Índice Ortopedico Canino (*Canine Orthopedic Index*) e Escala Analógica Visual de Hudson *(Hudson Visual Analogue Scale*). O trabalho estatístico dos dados foi realizado com o programa IBM SPSS Statistics version 20. Foram realizados vários testes estatísticos, de acordo com a análise pretendida: teste t de amostras emparelhadas, ANOVA de medidas repetidas, com correcção Huynh-Feldt, ou o teste de Wilcoxon. O teste de Kaplan-Meier, comparado com o teste log-rank, e a regressão de Cox foram conduzidos para avaliar sobrevivência. Todos os resultados obtidos forma considerados como estatisticamente significativos para o valor de $P<0,05$.

Os grupos PCG e HG foram aqueles que registaram melhorias mais significativas e duradouras, de acordo com os resultados da regressão de Cox para as diferentes avaliações realizadas. Considerando as avaliações objectivas, PCH e HG apresentaram uma melhora de 81% e 69%, e de 61% e 57% para os índices de simetria e diminuição de suporte de peso em estação, respetivamente. De acordo com os resultados obtidos, o uso de concentrado de plaquetas autologas e de ácido hialurónico de alta densidade parecem ser os tratamentos preferenciais para a melhoria das alterações registadas nos doentes com OA, melhorando a qualidade de vida dos doentes. Os resultados obtidos permitiram verificar também uma redução no nível da dor nos doentes do grupo THG, o que pode ser atribuído ao marcado efeito anti-inflamatório dos corticosteróides.

Palavras-chave: Cão; Osteoartrite; Dor; Modelo animal; Hexacetonido de triancinolona; Estanozolol; Ácido hialurónico; Concentrado Plaquetário; Proteína C-Reactiva; Interleucina-1; Placa ou Plataforma de Força, Termografia digital; Radiografia digital; Goniometria; Metrologia clínica.

Title: Evaluation of the efficacy of four intra-articular therapeutic protocols for the control and treatment of osteoarthritis in a *Canis familiaris* model.

ABSTRACT

Osteoarthritis (OA) affects all mammals, being an important and costly disease. The pathologic process, clinical presentation, and response to treatment are very similar in humans and dogs, making the naturally occurring canine osteoarthritis model the closest to a gold standard for the study of human osteoarthritis. Since OA is mainly symptomatic in the affected joint while lacking obvious extra-articular manifestations, it is well suited to have a local therapy administered by intra-articular (IA) injection, reducing the total amount required to produce an effect as well as systemic adverse effects. There are several substances used in the IA management of OA, some used for a long time, like corticosteroids and hyaluronan, while others have gained more recent attention, as autologous platelets and stanozolol. In common, regardless of how long they have been used, their action mechanisms and effects are not fully known, as the protocol for their use is usually based on the clinician's individual experience. Disease evolution and response to treatment can be monitored through inflammation markers, different clinical evaluation modalities, as digital thermography, digital radiography, or stance analysis, which all add relevant information to the clinical examination.

This study set four goals: 1) to determine the effect of four IA substances in the management of hip OA; 2) assess variations in C-reactive protein and IL-1 in the synovial fluid of patients with OA; 3) evaluate the relationship between laboratory medicine, with imaging results and clinical assessment; 4) validate the use of digital thermography and weight bearing evaluation in OA assessement; and 5) to outline a treatment protocol for patients with OA, with the dog as an animal model, under the One Health concept. One hundred (N=100) hip joints were selected from patients with naturally occurring osteoarthritis and randomly assigned to five groups: control group (CG, n=20), triamcinolone hexacetonide group (THG, n=20), platelet concentrate group (PCG, n=20), stanozolol group (SG, n=20) and Hylan G-F 20 group (HG, n=20). Evaluations were conducted on days 0 (treatment day), 8, 15, 30, 60, 90, 120, 150, and 180 days post-treatment. They consisted of the evaluation of weight distribution, joint range of motion at flexion and extension, thigh girth, digital thermography, radiographic signs, synovial fluid analysis, and four clinical metrology instruments were collected: Canine Brief Pain Inventory, Liverpool Osteoarthritis in Dogs, Canine Orthopedic Index and the Hudson Visual Analogue Scale. All results were analyzed with IBM SPSS Statistics version 20. Several statiscal tests were conducted, according to the intended analysis: Paired Samples T-Test, Repeated Measures ANOVA, with a Huynh-Feldt correction, or Wilcoxon Signed Ranks Test. Kaplan-Meier estimators were conducted and compared with the log-rank test. Cox

proportional hazard regression analysis was performed to determine treatment survival. A significance level of $P<0.05$ was set.

PCG and HG registered longer lasting effects, and better improvements according to the Cox hazard regression with the different assessments made. Considering objective parameters, PCH patients showed a 69%-81% improvement in symmetry and weight-bearing reduction, respectively, while HG showed 61% and 57% improvements. These seem to be the preferred treatments for functional impairments due to OA. In addition to these evaluations, PCG and HG also registered more significant improvements in several scores as Hudson Visual Analogue Scale, stiffness, function, gait of the Canine Orthopedic Index. Better impact on pain interference was observed in THG, which could be attributed to the high anti-inflammatory effect of corticosteroids, and the relation between pain and inflammation.

Keywords: Animal model; Dog; Osteoarthritis; Pain; Triamcinolone Hexacetonide; Stanozolol; Hylan G-F 20; Platelet concentrate; Stance Analysis; Digital Thermography; Goniometry; Digital radiography; Clinical Metrology Instruments.

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ABBREVIATIONS LIST

CBPI – Canine Brief Pain Inventory;

CG – Control Group;

CMI - Clinical metrology instruments;

COI – Canine Orthopaedic Index;

COX – Cyclo-oxygenase;

CRP – C-reactive protein;

CS – Corticosteroids;

ECM – Extracellular matrix;

GAG – Glycosaminoglycan;

GRF – Ground Reaction Forces;

HD - Hip dysplasia;

HG – Hylan GF-20 Group;

HVAS - Hudson Visual Analogue Scale;

IA – intra-articular;

IL – Interleukin;

IL-1ra – IL-1 receptor antagonist;

LOAD – Liverpool Osteoarthritis in Dogs;

MMP – Matrix metalloproteinase;

MSC – Mesenchymal stem cells;

NO – Nitric oxide;

PCG – Platelet Concentrate Group;

PG – Proteoglycan;

PGE_2 - Prostaglandin E_2;

PIS – Pain Interference Score;

PRP – Platelet Rich Plasm;

PSS – Pain Severity Score;

PVF – Peak Vertical Force;

ROM – Range of motion;

SF – Synovial fluid;

SG – Stanazolol Group;

THG - Triamcinolone hexacetonide group;

TNF-α – Tumour necrosis factor-α.

À minha Mulher, Ana

I. INTRODUCTION AND BIBLIOGRAPHIC REVISION

INTRODUCTION AND MAIN GOALS

Osteoarthritis (OA) affects all mammals, being an important and costly disease in humans, horses, and dogs[1,2]. It is a source of chronic pain, with an estimated prevalence of 20% in dogs[3–5]. This value is expected to rise due to a simultaneous increase in life expectancy and obesity across species. In dogs, osteoarthritis is a common process in the hip joint, and it has been registered in 98% of animals at the end of life[6]. The physiopathologic process, clinical presentation, and response to treatment are very similar between humans and dogs, making the dog a frequent animal model for the study of osteoarthritis[7]. Both the surgical and naturally occurring models have been widely studied, with anatomical resemblance, similar disease progression and result translation to human medicine, with the advantage of providing a faster disease progression, making it easier to study. For those reasons, the dog is the closest to a gold standard model[8–11]. While histopathology remains the gold standard for outcome assessment in OA animal models, there has been an increasing call for less invasive measures of disease onset and progression and response to treatment[11]. For that same reason, the search for biomarkers that would be a good indicator of OA's development and evolution, have also been under significant attention[12].

Since OA is symptomatic mainly in the affected joints and lacks obvious extra-articular manifestations, it is well suited to be addressed by administering local therapy by intra-articular (IA) injection[13–15]. IA treatments present several advantages: applying the treatment directly at the intended place of action provides a higher concentration of the medication within the joint space, usually accompanied by higher efficacy, while minimizing systemic exposure, thus avoiding several systemic side effects. Also, systemically administered drugs have difficulty reaching the articular cartilage due to its avascular nature, raising the need for higher doses[15–17]. Many substances have been used in the treatment of OA for quite a long time, as corticosteroids and hyaluronic acid. Currently, innovative therapies as autologous platelet concentrates or anabolic steroids such as Stanozolol, have shown promise in the local control of the disease. However, its mechanisms of action are not yet fully understood, which translates into the difficulty in reaching consensus among clinicians for the establishment of standardized protocols, thus making their use based on evidence medicine resulting from the individual experience of the clinician´s[18].

In human reports, wide discrepancies in results can be observed, maybe because prospective, randomized, double-blind, placebo-controlled studies, with an intetion to treat, are rare[13]. At the same time, systematic reviews of the efficacy of OA treatments in dogs highlight the poor quality of study

design and reporting, limiting their authors' ability to make strong therapeutic recommendations. As a result, it may be difficult for practitioners to choose the most appropriate treatment for their patients[18]. Disease diagnostics and monitoring can be achieved through various methodologies, with an increasing interest in non-invasive modalities. These include digital radiography, gait analysis, thermography, and others, alongside molecular techniques such as Interleukine-1 (IL-1) and C-reactive protein (CRP) concentration level determinations[19–23].

Considering the above, the study of therapeutics and each option's effects available for OA management shows of crucial importance. The present study developed in dogs with hip joint OA naturally occurring disease aimed to:

1. Determine the effect of four substances delivered by intra-articular route (IA), in patients with hip joint osteoarthritis;
2. Evaluate the variation in the synovial fluid of patients with hip joint OA of inflammatory markers levels such as Protein C-Reactive (CRP) and Interleukin-1 (IL-1) through time after the IA administration of the different substances used for the OA treatment;
3. Assess the relationship between laboratory and imaging medicine data, and the patient's objective medical examination;
4. Validate the use of digital thermography and weight bearing evaluation in OA assessement;
5. Outline a therapeutic for IA treatment protocol for patients with hip OA using the dog as an animal model, under the general concept of One Health.

1. THE JOINT

Joints are intricate musculoskeletal structures, currently considered a complex organ, composed of multiple components with different characteristics[1,24]. They permit the movement of bony structures in relation to one another and, as a consequence, the movement of an individual in relation to the environment, translated in locomotion[24]. Joints must be as robust as the remaining musculoskeletal system, endure the forces generated during movement, reduce friction, promote a smooth motion, and should dampen the impact generated during contact with the ground[24].

1.1 Classification of joints

There are three categories of joints: fibrous joints (in which bone is connected through dense connective tissue), cartilaginous joints (in which cartilage is an interface between structures), and synovial joints (in which there is a cavity, filled with fluid)[24,25]. An example of a fibrous joint is the one between the vertebrae bodies, forming the *nucleous pulposus* of the intervertebral disc. Cartilaginous joints have an interface that comprises hyaline or fibrous cartilage, such as the one in the pubic symphysis. In synovial joints, the bony ends are covered with hyaline cartilage and glide over each other inside a joint capsule filled with the viscous synovial fluid (SF)[24].

Functionally, joints can also be classified accordingly to the amount of motion they allow. Synarthroses are joints that allow very little mobility, most of which are fibrous in nature (such as the ones that connect the bones of the skull). Amphiarthroses are joints that allow for more movement than synarthroses but still minimal (for example, the intervertebral joints). Diarthrodial joints are the ones that allow for maximal motion, with the range of movement being limited by intra or peri-articular structures, such as ligaments or joint capsule[24]. The primary plane of motion can also be used to characterize joints. Hinge-type joints, such as the elbow, move in the sagittal plane, while ball-and-socket joints, such as the hip, can move in sagittal, frontal, and transverse planes[26]. Still, three parameters should be used to define a joint movement fully: location of the axis (including location changes during movement), the amount of rotation that occurs, and the amount of translation (displacement along the axis) that occurs[27]. It is a challenging task to complete with absolute precision since most joints do not have a fixed axis of motion but rather a quite intricate movement[28]. Since joints exhibit three-dimensional movements, it can be useful to consider them as rotating in three orthogonal planes: sagittal (yaw), frontal (pitch) and horizontal (roll), and three translation components – caudocranial, mediolateral, and dorsoventral[27,28].

1.2 The synovial joint

The majority of tissues that compose the musculoskeletal system are classified as dense connective tissue. The skeletal muscle is the exception to this rule[29]. Synovial joints have a typical basic structure (represented in Figure 1) and should be considered an organ[1,30]. The ends of at least two articular bones that constitute the joint are covered with a layer of articular cartilage, and, under this cartilage, there is a layer of subchondral bone. Joint limits are encompassed by a joint capsule, a structure that restrains the SF within the joint space and holds the articular surfaces in place. Additional structures may be present, such as collateral or intra-articular ligaments, that aim to stabilize the joint and limiting movements in undesired directions, in a number that depends on the specific motion of that particular joint[24,31].

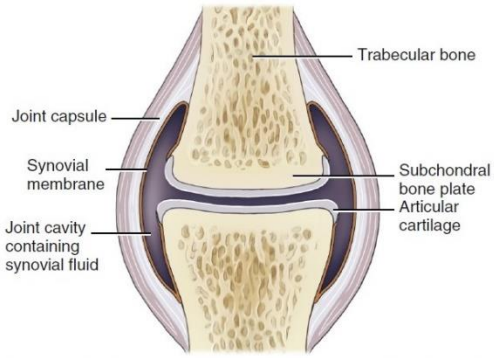

Figure 1 – A schematic representation of the basic structure of a synovial joint (adapted from van Weeren, 2015).

Cartilage is an avascular connective tissue composed of chondrocytes suspended in an extracellular matrix (ECM). The ECM contains type II collagen (50% of dry weight), elastin, variable amounts of type I collagen, a variety of proteoglycans (PGs) (35% of dry weight), and water (70-80%). There are other minor components, consisting of glycoproteins, minerals, lipids, and others[24,29]. The hyaline, fibro, and elastic types of cartilage differ in the ECM composition and organization. Hyaline cartilage forms the contact surface of synovial joints. Fibrocartilage is a tougher form with dense ECM adapted to compressive stress (like the one found in the menisci). The elastic cartilage is found in the larynx or the pinna[29]. Dense connective tissues are predominantly composed of ECM and have a relatively small number of cells[29]. These components are arranged in four layers: the superficial, the intermediate and deep zones (in combination, forming the hyaline cartilage), and the calcified cartilage. This last layer sets the demarcation between hyaline cartilage the subchondral bone[24]. The superficial layer is composed of flattened chondrocytes, dense collagen fibrils (type II), high water content, and relatively few PGs[32]. The transitional zone shows an increase in PGs content (compared with the superficial layer), with lower amounts of water and collagen. The deep zone has

the highest percentage of PGs, and the lowest of water, having chondrocytes arranged perpendicularly to the subchondral bone[33].

Chondrocytes are cells with a mesenchymal origin, with the ability to differentiate into fibroblasts. The environment is of significant importance to maintaining the phenotype. Unlike what happens with cells in other tissues, chondrocytes do not seem to be connected with each other. Their appearance varies with its location within the cartilage, ranging from a round shape in the deep layer to a flattened appearance in the superficial layer. These variations in appearance seem to be related to shifts in metabolic profiles[24]. Even though they represent a small portion of the cartilaginous tissue, they are responsible for the synthesis, maintenance, and turnover of the ECM[29]. A reduced number of progenitor cells are also present, representing several development stages from the multipotent mesenchymal stem cell to the cell-specific to the tissue. These cells are essential for the processes of regeneration, adaptation, and remodelling[29]. The joint capsule comprises two layers. The outer one is composed of stiff fibrous tissues, frequently intimately connected with extra-articular structures, to provide mechanical stability.

The joint capsule's inner layer is the synovial membrane or *synovium*, divided into the intimal and subintimal layers. It also covers the walls of bursa and tendon sheaths[29]. The intimal is composed of a layer of a couple of cells, with many blood vessels. It enables an easy passage of components from the blood to the synovial cavity, and vice-versa and accounts for SF's description as an ultrafiltrate of blood plasma, replaced several times over 24 hours[14,24]. There are two types of synoviocytes. Type A synoviocytes are similar to macrophages and have a phagocytic role, while type B synoviocytes are similar to fibroblasts and have the function of producing and excreting hyaluronan[34]. The synovial lining does not have a basal lamination, making it an inefficient barrier, providing no type of constraint to cells and mediators' movement. For this reason, periarticular tissues are easily and often affected by primary joint pathology while also being the source of secondary joint injury[29]. Subchondral bone supports the cartilage and connects to it through a layer of calcified cartilage. It also has an additional layer of compact bone and a greater distance of the cartilage, trabecular bone. This conformation bears mechanical characteristics, with the subchondral plate providing firm support, which maintains some rigidity (increasing with sclerosis), while the trabecular portion has some elasticity[24]. During athletic activity, the subchondral bone plays an important role in attenuation forces generated that load and impact the articular cartilage. Subchondral sclerosis, with its reduced elasticity, is a distinct characteristic of osteoarthritis (OA)[35,36].

Collagen molecules have an exceptionally long life when incorporated into the ECM. The collagen network is responsible for the tensile and shear stiffness of cartilage[37,38]. The largest amount of collagen in articular cartilage is collagen type II, with its large fibrils that give structure and

mechanical properties to the cartilage. Other types of collagen fibres are present in other structures of the musculoskeletal system, namely types I and III. Collagen type I, for example, is responsible for the high tensile strength of tendons and ligaments. Type II fibrils are arranged in a three-dimensional arcade structure, with a shape that depends on the joint and main direction of loading while providing deformation resistance in multiple directions. These triple helices are made up of three polypeptides called alpha chains[24,39]. The remaining types of collagen fibrils have different characteristics, from interrupted triple helices (types IX, XII, XIV, XVI, XIX, XX, XXI and XXII) to short-chains (types VIII and X), which can be filamentous in nature (type VI) or a constituent of the basement membrane (types IV, VII, XV and XVIII). While all these types do not form fibrils and are called nonfibrillar collagen, they associate with fibrillary collagen and regulate its assembly, interaction, and diameter[40,41]. Elastin is another essential component of musculoskeletal tissues, being part of tendons, ligaments, joint capsule, and articular cartilage. It is particularly abundant on the ECM, in the structures that undergo repeated elongation cycles and elastic recoil, such as the joint capsule[29].

PG molecules (comprising a protein and a sugar component, hence glycosaminoglycan or GAG) in the ECM form aggregates that disperse between the fibrils of collagen type II and connect to them directly through hyaluronan molecules. They also exhibit some side chains of chondroitin sulphate[24]. The basic structure of a PG consists of a core protein with a variable number of GAG side chains attached[29]. The length of the core protein and side chains decreases with age, which affects the structure and properties of the ECM[42]. They are negatively charged, therefore having a high affinity for water. It is this characteristic that accounts for the turgidity and resistance to compression of hyaline cartilage[29]. Hence, PG is responsible for the biomechanical properties of cartilage in compression[38,43]. Hyaluronan belongs to the same family of chondroitin sulfate but has a unique characteristic since it is never sulfated nor covalently linked to proteins. Besides being found in the ECM, it is also present in SF. Although in a smaller amount, other PGs are found in the ECM, such as decorin, lumican, and chondroadherin[24,29]. In particular, the latter is also present in bone, regulating its metabolism, decreasing the production of the cytokines interleukins IL-1 and IL-6, which, in turn, activate osteoclasts[44]. Synovial fluid (SF) comprises a high percentage of blood plasma, but with the distinct difference of having a high concentration of hyaluronan, which gives it its highly viscous character[24,45]. This fluid is in constant exchange with the blood plasma through the effect of the hydrostatic forces generated by locomotion.

Considering the joint as an organ by itself, and not just a system of ropes, pulleys, gliding surfaces, and lubrication, it is important to see it as part of the musculoskeletal system, working together and under the influence of other elements of the system[1,6]. For example, the support muscles

provide for joint function, and prevention of instability is critical. For this reason, during rehabilitation to correct joint pathologies, a great deal of attention is given to strengthening the muscle structures around the joint[46]. Also, a good muscle function is indispensable for processing proprioceptive information and thus ensuring correct joint loading. Like tendons and ligaments, the joint capsule provides constant information regarding the position, location in space, and state of loading, which allows for corrections in body position[6,47].

1.3 Joint physiology

Joints depend on each of its components' relation and health to fulfill its role in the body. For that purpose, all its components must be in an equilibrium between anabolic and catabolic processes. This homeostasis among different components (cartilage, synovium, subchondral bone, and possible intra-articular structures) is too complicated due to a wide variety of metabolic characteristics and turnover rate [24]. For example, collagen type II has an extremely long turnover rate, making the network's restoration following injury a complicated task, and playing a crucial role in the poor healing capacity of cartilage[24]. On the other hand, the chondrocyte is the only cell found in normal articular cartilage and is the sole responsible for producing all of the ECM components,and mounting the complex network in response to biomechanical challenges. They are arranged in close coordination with the orientation of the collagen fibrils with the matrix[29].

Several components regulate this metabolism, but interleukin-1 (IL-1) seems to be the key player on it, being regular considered as the cytokine associated with the OA development[48,49]. IL1 can influence other molecules' activity, such as matrix metalloproteinases (MMPs), which are responsible for cleaving collagen[24]. MMPs play an essential role in healthy joints and maintaining homeostasis. Under normal conditions, chondrocytes maintain a highly balanced control over matrix synthesis and degradation, adjusted to meet the tissue’s functional requirements[29]. Tumour necrosis factor-α (TNF-α) acts in synergy with IL-1 and inhibits proteoglycan synthesis[50].

Other anabolic and catabolic growth factors, together with cytokines and mechanical loading, have a role of mutual influence to keep the balance of this metabolism. They affect nearly every biological process, from embryonic development, disease pathogenesis, the progression of the degenerative processes, and stem cell differentiation[51]. A wide range of growth factors has been identified in joint metabolism, and several have simultaneous anabolic and catabolic effects. The major ones with anabolic effect are transforming growth factor-β and insulin-like growth factor-1[52,53]. transforming growth factor-β stimulates PG synthesis by chondrocytes and expression of collagen type II, possibly counteracting the effect of IL-1[54]. It also stimulates osteophyte formation after prolonged periods of exposure[55]. Insulin-like growth factor-1 stimulates matrix production and

inhibits degradation, and when in deficiency leads to OA-like lesions[53,56]. Cytokines are soluble or cell surface molecules, with a role in cellular communication through paracrine, autocrine, and endocrine signalling[57]. They can have a catabolic role, regulating cartilage remodeling by acting on target cells to increase products that enhance matrix degradation; anticatabolic or inhibitory action, inhibiting or antagonizing the catabolic activity of cytokines; and anabolic activity, acting as growth factors on chondrocytes to increase synthetic activity[58–62]. In the IA environment, cytokines and growth factors may diffuse from the SF towards chondrocytes and vice-versa[57]. The role of different cytokines, and their interaction with chondrocytes, are summarized in Table 1.

Catabolic	Modulatory	Anti-catabolic	Anabolic
IL-1	IL-6	IL-4	IGF-1
TNF-α	LIF	IL-10	TGF-β1,2,3
IL-7	IL-11	IL-13	BMP-2,4,6,7,9,13
IL-8	Basic FGF	IL-1Ra	
IL-17			
IL-18			
OSM			

Table 1 - Cytokines and their interaction with chondrocytes (adapted from Mary B. Goldring, 2000; Rutgers et al., 2009). BMP - bone morphogenetic proteins; FGF – Fibroblast Growth Factor; IGF- Insulin-like growth factor; IL – interleukin; IL-1Ra – interleukin-1 receptor antagonist; LIF – leukemia inhibitor factor; TGF – transforming growth factor; TNF – tumor necrosis factor.

Motion or movement is a foundation of life, and domestic species are no exception[63]. The effects of mechanical loading on musculoskeletal structures in general and joints, in particular, are more evident during an individual's young phase, with a higher growth phase. Adult and mature individuals have a long turnover phase, and extensive remodeling does not seem to occur. These changes occur via a cellular response, derived from the nucleus and cell's stimulation, and by changes in hydrostatic pressure osmolality and flow of interstitial fluid[24]. This flow affects the supply of nutrients and removal of the metabolic waste and stimulates the production and activity of transforming growth factor-β, for example[64]. It is through the movement of fluid from the ECM to SF and vice-versa that nutrients are delivered to chondrocytes. This mechanism is one reason why loading is indispensable to the joint's health, and immobilization is so detrimental. During rehabilitation following joint trauma and/or surgery, correct joint loading is one of the focus of the therapy program[29].

Articular cartilage has a good adaptation capability to increased in response to mechanical loading, and physical training has been considered to have beneficial effects on its properties[65]. This cyclic mechanical loading of joints is essential for the maintenance of cartilage tissues' integrity[66].

The overall effect depends on the degree of loading and relies primarily on the PG matrix content adjustment. The femoral head's articular cartilage in young Beagle dogs has shown to undergo a remarkable adaptation to long-distance running, exhibiting minor biochemical changes after one year of training. This type of exercise increased the amount of GAG, particularly Chondroitin Sulphate, more extensively in the less weight bearing tissue and may have elicited a tendency opposite to degeneration[65,67,68]. There is a strong correlation between the thickness of the calcified cartilage and the joint's mechanical loading. Subchondral bone also becomes ticker with loading, a process that may not be available when subchondral sclerosis is advanced[69]. It is responsive to mechanical loading, changing its thickness, reducing its resorption when loading increases and effects depend mainly on the degree of compression and, in non-contact areas, the ingrowth of connective tissue articular surface may take place[70,71].

Bone is known to remodel and adapt through life, but cartilage and collagen, in particular, are much less reactive. In the juvenile individual, however, exercise will have significant effects when the joint matures, but will also influence the process of joint development[72]. An increase in PG and enhanced biomechanical properties were observed with moderate exercise (4km/day) in Beagles dogs while maintaining an intact surface and normal histological appearance of the articular surface. A partial loss of these gains is observed when the exercise load increases (20km/day)[65,66,73]. If the joint is not stimulated during this period, it will still be responsive to loading later in life, but the tissue formed will be of inferior quality and more susceptible to lifelong wear and tear[72]. Exercise, on the other hand, predisposes to fewer gross lesions, less ECM staining, greater bone fractions and high bone formation rate, having a protective effect on joints, not just against OA but also others such as osteochondritis dissecans[74,75]. In horses, exercise has been shown to increase the amount of newly synthesized PG in cartilage. Interestingly, it has no effect on collagen content of the ECM but sites known to be predisposed to injury contained significantly less collagen in exercised horses[76].

Low-level activity (walking exercise for six months) does not seem to produce detectable PG synthesis changes by chondrocytes, while cartilage regularly submitted to high levels of stress are stiffer and may not be well adapted to high biomechanical demands[73,77]. Immobilization and disease place a significant toll on articular cartilage, with long-term immobilization causing alterations of its bio-mechanical properties in young animals, possibly permanent, with PG loss and cartilage softening probably as a result of a decrease in PG synthesis due to immobilization[43,71,78]. Changes in joint metabolism also occur, with a decrease in IL-1α, tissue inhibitors of matrix metalloproteinase, and Chondroitin Sulphate levels, although these changes are reversible in its early stages[79].

The need for continuous, adequate loading is paramount to joint health. A long-term deleterious effect in joint tissues was observed in a group of horses with the combination of a

sedentary life with bouts of high-intensity exercise[72]. A similar effect has also been described in tendons[80].

1.4 Joint biomechanics

Musculoskeletal tissues are anisotropic and viscoelastic. Anisotropic tissues have mechanical properties that vary depending on the direction of applied stress. Viscoelastic materials have mechanical properties that vary with the rate at which stress is applied. Under physiological stress, the primary response of these tissues is elastic[29]. Between articular surfaces, smooth motion is paramount, creating the need for a lubricant. Cartilage provides this smooth surface with low friction, maintaining an efficient glide during joint motion. When healthy, cartilage provides an extremely low-friction bearing surface. Friction increase is expected from the beginning of a pathological disease process due to microstructural alterations driven by biochemical and environmental effects[81]. A boundary layer of lubricants also aids in this process through hyaluronan and lubricin[1,82]. There are two mechanisms involved in this process, boundary and fluid-film lubrication. The first occurs in all gliding surfaces of the joint, while the second is additional lubrication between articular surfaces. Boundary lubrication is primarily provided by hyaluronan, adherent to gliding surfaces, providing a protective layer, and preventing excessive wear and tear[83,84]. Fluid-film lubrication occurs in various forms: hydrodynamic lubrication, when all surfaces retain their shape and remain completely separated from each other by the fluid when moving; elastohydrodynamic lubrication, if at least one of the surfaces has some elasticity and deforms; if no motion between surfaces occurs and the fluid film is squeezed out, a squeeze film lubrication takes place; finally, the fluid also can remain entrapped between surfaces, constituting hydrostatic lubrication. In a joint, the situation is more complicated since both surfaces deform when loaded, but also fluid from the ECM will be squeezed and added to the synovial fluid already present.

Due to this variety of mechanisms associated with the musculoskeletal system's loading shifts to any given joint, synovial fluid's viscosity changes over an activity, diminishing with increasing joint motion[24]. The ECM has an intrinsic pressure, generated by a balance of osmotic pressure (originated by the negative charge of chondroitin sulfation, which attracts water) and the restraint imposed by the collagen fibrils. These characteristics account for articular cartilage's viscoelastic properties. When subjected to load, water will be squeezed out, and it will yield. When the external forces diminish, water will be drawn back in. If these characteristics are compromised, the described mechanism will not work, increasing vulnerability to damage[24]. This biomechanical concept requires an entirely homogenous tissue, which may be the reason for articular cartilage being completely

avascular and aneural. Nutrition and removal of waste are guaranteed by assisted diffusion from the SF to counteract the lack of blood vessels[85].

The articular cartilage goes through significant deformation during regular activity, but this deformation is completely reversible due to its strains' elasticity. The ability to be deformed enhances congruity and contact area of articular surfaces during loading, improving stability, and reducing stress *per unit* area[29]. The joint's fluid component first sustains the impact and then transferred to the solid component before the fluid is squeezed out of the compressed region[86,87]. Due to the complex nature of joint loading, different joint areas are subjected to different types of loading with varying intensity. This variability can only be addressed by cartilage with different characteristics in different sites and is not determined by the genetic background but instead develops in the early phase of life under the strong influence of biochemical loading[88,89]. The most relevant joint loading occurs during the weight-bearing phase of the stride. Forces placed by the ground on the limb during this contact phase are called ground reaction force (GRF)[90,91]. The influence of these forces on joints depends on their location, congruity, and existing surrounding tissues. Besides, lesions or limitations on any given part of a limb can affect other joints due to changes in dissipating or transfer forces[26].

Muscles transmit forces to joints through tendons, storing energy during the loading phase of the stride. Lesions to a tendon can affect its ability to store energy, thus increasing forces acting on the limb. Unlike the one in ligaments, their collagen structure has a very organized parallel arrangement, with a characteristic appearance. Ligaments also contribute to the force generation mechanism while restricting joint movement in given plains, affecting force distribution as well[90,91]. The majority of the times, tendons and ligaments are subject to forces and loads below their failure limit, so lesions tend to occur due to chronic stress and cumulative failure of individual collagen fibers. When enough fibers are damaged, tissue failure can occur, even with physiological loadings[26]. These facts stress the importance of physical activity, during which submaximal, controlled loads are applied to tissues, raising stiffness and strength of the fibrous joint capsule, tendons, ligaments, and their interface, by increasing the total amount of collagen fibers, their type, cross-links, and also diameter[92,93].

All structures surrounding a joint, (joint capsule, articulating bones, muscles, ligaments, and tendons) give it support and tend to restore it to its original position[63,94]. According to Wolff's law, these soft tissues adapt to loading with changes in their composition, in a fashion similar to bone ("bone in a healthy person or animal will adapt to the loads under which it is placed")[95]. The synovium has no known biomechanical function but reacts to the mechanical stress that influences surrounding tissues. It increases collagen production, alters trans-synovial diffusion, and synoviocyte metabolism. The release of enzymes to the SF affects all tissues of the joint[7,96,97].

When movement becomes altered or challenge imposed over joint structures exceeds the ability to adapt, pathology may develop[26,63,72]. One of the measures of this alteration is the range of physiological movement of the joint, referred to as a joint's range of motion (ROM). It is characterized by a neutral zone (region of low stiffness, produced with minimal internal stiffness) and an elastic zone (measured from the end of the neutral zone up to the physiological limit)[98]. Nevertheless, stress and challenges are a requirement for the health of all tissues that compose the joint[99].

1.5 The hip joint

The hip bone (*os coxæ*) is composed of four bones with different origin and development. By the twelfth week, the ilium, ischium, pubis, and acetabular bones fuse, forming the acetabulum. This cotyloid cavity receives the femur's head, forming the coxofemoral joint, also referred to as hip joint. It is a ball-and-socket joint with a band, with main movements of flexion and extension, although it permits abduction, adduction, and multidirectional movements[25,100–103]. The characteristic of the hip joint is summarized in Table 2.

Participating Bones	Form / Composition	Classification	Movements	Normal ROM for dogs (in degrees)
Acetabulum, formed by ilium, ischium, pubis and acetabular bone + head of the femur	Spheroid Joint / Compound Joint	Synovial / Diarthrodial Joint	Flexion	55
			Extension	160-165
			Abduction with a flexed hip	120 (Stifle at 90)
			Adduction with a flexed hip	65 (Stifle at 90)
			Abduction with an extended hip	85
			Adduction with an extended hip	63
			Internal rotation	55
			External rotation	50

Table 2 – Characterization of the hip joint (adapted from Budras et al., 2002; Levine, D., Millis, 2014; van Weeren, 2015).

In a medium-sized dog, the acetabulum is 1cm deep by 2cm in diameter and is further deepened by a band of fibrocartilage on the rim, the acetabular lip (*labrum acetabulare*). The acetabulum is deficient on its smooth articular circumference on the medial portion, called the lunate surface. It also has a thin quadrangular, non-articular area, consisting of a depression that extends laterally from the acetabular notch, forming the acetabular fossa[100]. The acetabular fossa is an important landmark when performing ultrasound (US) guided intra-articular administrations. The joint has various mechanisms of stability. The femoral head ligament, the joint capsule itself, and the dorsal acetabular rim give primary stability. Secondary stabilization is achieved by the acetabular

labrum, hydrostatic pressure resulting from a minimal amount of synovial fluid, and the periarticular muscles of the gluteal complex[101].

The femur (*os femoris*) is the skeleton's heaviest bone and articulates with the hip bone proximally. Distally, it articulates with the tibia. It consists of a head, neck, prominent muscular processes proximally (the trochanters), and a body or shaft, which is continued distally with the femoral trochlea and condyles[25]. The bones of both pelvic limbs are in parallel sagittal planes[100]. Concerning the hip joint, the femur's relevant components are the head, neck, and two trochanters. The head is smooth, almost spherical (*caput ossis femoris*), and caps the neck's dorsocaudal and medial parts. It has a fovea (*fovea capitis*), where the ligament of the head of the femur attaches (*ligamentum capitis ossis femoris*, formerly round ligament). The neck (*collum femoris*) unites the head with the rest of the proximal femur[100]. Directly lateral to the femur's head and neck, there is the greater trochanter (*trochanter major*). Here is where the piriformis, gluteus medius, and profundus muscle insert, all of which are responsible for extending the hip joint and abducting the limb[25]. Between the neck and the greater trochanter, there is the trochanteric fossa (*fossa trochanterica*). The lesser trochanter (*trochanter minor*) is an eminence shaped like a pyramid that projects from the caudomedial surface. It is connected to the greater trochanter by the intertrochanteric crest (*crista intertrochanterica*), where the deep muscles of the hip joint insert[25,100]. The deep hip joint muscles are the gemelli, internal and external obturator, and the muscles' quadratus femoris. They are the supinators of the limb, turning the cranial aspect of the limb laterally[25]. Another essential muscle group comprises the hamstring muscles, the biceps femoris, caudal crural abductor, semitendinous and semimembranous muscles, which extend the hip joint and flexion the stifle[25]. These muscles are frequently, and visible atrophied in dogs with moderate to severe OA.

2. OSTEOARTHRITIS – EVOLUTION, SIGNS, AND BIOMARKERS

Synovial joints are subjected to a way range of challenges as a result of its functions. They have an ingenious way of facing this burden, even though it comes at the cost of its flexibility and capacity to repair, leading to long term problems[24]. Articular cartilage is an aneural and avascular tissue in mature animals, a very distinct characteristic compared to every other tissue in the organism. For this reason, any damage to the cartilage, which usually starts as small damage, progresses without clinical signs for long periods until the disease is an advanced stage. The vital issue with joint homeostasis is based on the possibility of tissue integrity being maintained or not in response to insult and, with it, biomechanical characteristics and functionality. If the tissue cannot maintain integrity, a

loss in capacity to withstand subsequent loading is inevitable, and, predictably, a vicious cycle of overloading and deterioration will ensue. This process will end in the development of a chronic condition, such as OA[24].

OA represents a significant burden to societies, as it affects individual's quality of life and implies an enormous cost in terms of healthcare, posing substantial welfare challenges and concern[24,104]. It is the most prevalent musculoskeletal disease in dogs and causes significant performance loss in working and sporting dogs[29]. OA seems to be as ancient as joints themselves since signs of OA have been found in ancient fossils, as of a saber-tooth cat 337.000-300.000 years old[105]. From this ancient nature, osteoarthritis currently displays high prevalence, duration, and severity, demonstrating particular welfare impact, and has emerged as a priority area for health-related welfare improvement in the human and canine populations[106].

2.1 Osteoarthritis pathophysiology

The diarthrodial joint should be considered an organ, and joint degeneration is a form of organ failure. OA is also referred to as a degenerative joint disease, but this terminology should be considered a misnomer. OA is not merely a process of wear and tear but rather a complex abnormal remodeling of joint tissues, driven by inflammatory mediators within the joint[1]. It is a relatively low-grade inflammatory disease, but the inflammatory process affects the disease's progression, without systemic manifestation or presence of neutrophils in the SF[107–109].

The inflammation in OA is distinct from that in rheumatoid arthritis and other autoimmune diseases, as it is chronic, comparatively low-grade, and mediated primarily by the innate immune system[110]. It constitutes the endpoint of the synovial joints disease process, interrupting articular surface or causing instability and mechanical injury[111]. Growing evidence suggests that there are different OA phenotypes, reflecting different mechanisms of the disease[112]. It is a common disease of diarthrodial joints, associated with the deterioration of six different structures within the joint: bone, articular cartilage, joint capsule, innervation, vascular supply and ligaments[113].

It is characterized by an imbalance between cartilage synthesis and degradation, leading to increased breakdown of matrix components, degeneration of articular cartilage, new bone formation at joint margins, pain, and loss of function[109,114,115]. It constitutes the primary cause of lameness in humans, dogs, and horses[31]. At least 80% of the cases of lameness and joint diseases in companion animals are classified as OA, with 20% of middle-aged and 90% of older dogs presenting OA in one or more joints[2,116,117]. It is estimated to affect around 200 000 dogs annually in the United Kingdom, with risk factors including breed, being neutered, of higher body weight, and older than eight years[5]. There is, however, conflicting information regarding the interactions between and relative weight of

risk factors, such as age and sex[118]. In OA, all joint tissues are affected to some extent due to their interaction as a functioning unit[69]. The osteoarthritic process usually begins long before the disease presents as a clinical problem since joint structure and function are typically substantially altered before symptoms are noticeable[12].

The initial wear and tear concept was based on the fact that OA is observed primarily in elderly individuals. This view is currently less favored since younger patients often display OA symptoms secondary to injury or genetic predisposition[119,120]. Changes seen in involved tissues are attributed to diffusible factors, various cytokines, and growth factors, including proteolytic enzymes, such as MMPs and members of the Disintegrin and Metalloproteinase with Thrombospondin Motifs (ADAMATS) family present in the joint environment during the disease[121–124]. These factors exert their effects by enhancing synthesis, secretion, or activation of proteinases[123]. The idea that OA is not one disease but a syndrome encompassing heterogeneous, stratified groups of patients with characteristic aetiologies has been gaining acceptance[111].

Hip OA has a large epidemiological representation in the canine population, with varying degrees of severity and many cases are subclinical[101]. The hip joint should be tight-fitting and congruent, capable of withstanding severe exercise throughout a dog's life without developing OA[125]. Risk factors for OA development are typically divided into two fundamental mechanisms, related to the adverse effects of abnormal loading on normal cartilage or normal loading on abnormal cartilage[126]. Changes to normal movement can lead to pathological development. In several cases, what makes way to OA is subluxation and/or laxity, leading to partial dislocation of a bone from a joint[63]. Loading of an unstable joint causes severe changes in the structure and function of articular cartilage[65]. Clinical instability of joints, also referred to as pathological hypermobility, leads to damage in the structures that restrain a joint's movement[27]. At the degree of functional subluxation, a corresponding increase in the volume of synovial fluid occurs. On the hip joint, this results in an increasingly more significant impact of the femoral head on the acetabular labrum upon foot strike, producing cartilage damage and erosion[6]. Hip dysplasia (HD) is a major example of this incongruity. Other risk factors include age, prior joint injury, obesity, genetic predisposition, and abnormal joint shape[1]. Individual activity levels are also relevant. Sporting and working animals are more exposed to chronic fatigue injuries, a term used to characterize the pathological processes that start at the molecular and biochemical levels, leading to histological evidence of tissue damage and ultimately tissue failure, which results in clinical signs[127].

Pathological changes that occur with OA include degradation of joint cartilage[128], subchondral bone thickening, osteophyte formation, synovial inflammation, and degeneration of ligaments. Besides, periarticular muscles, nerves, and bursa are also affected, contributing to OA or

its symptoms[1]. The subchondral bone remodeling has been assumed as an initiator of secondary changes in the articular cartilage, being more metabolically active in OA than in healthy joints[69,129,130]. Vigorous and intense exercise has also been suggested to decrease PG of the articular cartilage and cause erosion, pitting, and fibrillation of cartilage surface[68]. The failure of osteochondral defects to heal after injury or disease is the primary limiting factor of any rehabilitation attempt[131]. Cartilage damage can occur following traumatic injury, chronic overuse, or in consequence of physiological loads in the presence of anatomical incongruity[29]. With the development of modern imaging techniques, it is becoming evident that most joint insults and injuries, while acute in clinical appearance, develop into a chronic pathological process[127]. Electron microscopy revealed that impact causes rapid structural changes in mitochondria that are related to reduced function. Mitochondria has a vital role in mediating cartilage's per-acute response to traumatic injury. Thus, mitochondrial protection may be a therapeutic strategy for injury-induced cartilage damage[132]. In a traumatically injured joint, the synovium and joint capsule become inflamed, causing both physical and biochemical damage to the joint[126]. The continuous wellbeing of articular cartilage is vital for the joint, as it is almost totally unable to recover from trauma[65].

To evaluate clinical or functional instability, the neutral zone of a joint (a region of no or little resistance to motion in the middle of the joint's ROM) may be a good indicator of clinical of functional instability, more than ROM, for example. In the spinal column, the neutral zone increases with the injury or weakness of spinal muscle[98]. It is reasonable to assume that the same mechanics may occur in the remaining joints of the body. During high-intensity activities, a greater degree of deformation of the ECM may occur, leading to micro-injuries. These micro-injuries induce inflammatory responses in vascularized tissues, and the inflammatory mediators produced may trigger cellular responses. Depending on the degree, this may represent a net gain in tissue strength through anabolic metabolism or a loss through catabolism[133–135]. The resulting loss of articular cartilage is the hallmark of late-stage clinical OA[12]. At the cellular level, cartilages become thinner, irregular, and fissures. With this, the intermediary zone's mineralization ensues, and, finally, cartilage loss occurs with exposure of subchondral bone[29]. Other tissues of the musculoskeletal system have different abilities to regenerate. While bone and muscle can do so, tendons, ligaments, and cartilage tend to produce mechanically inferior tissues[29].

At a molecular level, initially, articular cartilage becomes depleted of PG[66,69,136], followed by progressive disruption of the collagen network, allowing water to accumulate[69,137]. Diseased cartilage experiences an increase in water content from a typical 60-85% to over 90%[138]. When imbalances between osmotic and tensile components occur, compromises to the cartilage's mechanical integrity take place, leading to ECM degeneration, decreased joint function, and OA[139]. This reaction sets OA

as an active response to injury rather than a degenerative process[1]. As a result, tissue generated during the healing process is usually unorganized. Cartilage overload can lead to the death of chondrocytes and rupture of the collagen network of the matrix[29]. Genes associated with OA, specifically Asporin and Calmodulin 1 genes, reduce chondrocytes' ability to express genes encoding Aggrecan and type II Collagen[140]. Once the cartilage's mechanical properties are compromised, there is a reduction in the ability to withstand the load, even at physiological levels. This stress causes further damage, leading cartilage to enter a vicious cycle[29]. The interplay between tissues is also altered, with cartilage and subchondral bone entering a self-perpetuating cascade of degeneration. Cartilage is highly sensitive to TNF-α, which works in strong synergy with IL-1. Physiological relevant concentrations as low as 0.25ng/mL are sufficient to increase the release of GAGs from OA cartilage in humans[109,141,142], and may also be more responsive to IL-1 than healthy cartilage[143,144] while being essential for its health[145].

In a healthy joint, the degradative process is usually opposed by the production of enzyme inhibitors and anabolic growth factors. Degeneration of joints results in forms an imbalance of this primary mechanism. While a moderate loss of PG can be restored, an extensive collagen network breakdown is irreversible and leads to progressive joint degeneration. Therefore, PG turnover is a significant target of the investigation since it represents a vital tissue adaption mechanism. The types and concentrations of PG within the ECM in response to stimulation are what allows for the tissue's adaptation according to the demand[29]. Initially, chondrocytes react to matrix disruption by increasing anabolic activity and producing collagen and PG. This reparative response is limited, and, after an initial period, the cell ultimately shifts to a catabolic phenotype[107]. They become activated and proliferate, forming clusters that start to produce matrix proteins and matrix-degrading enzymes. This process then leads to matrix remodeling, hypertrophic maturation, and cartilage calcification[146].

With the critical loss of matrix components, morphologic damage occurs, going through a cycle of chondromalacia, with cartilage softening and swelling (due to loss of water and GAG). Superficial fibrillation follows, which then progresses to the middle and, finally, deeper layers of the cartilage, leading to loss of articular cartilage and full-thickness erosions[126]. The paucity of chondrocytes in the ECM, their inability to migrate to the zone of injury, and their relative inability to regenerate large ECM amounts, meaning these defects will usually progress[30]. It is interesting to note that this progressive cartilage degradation is the result of prolonged exposure to pro-inflammatory cytokines, such as IL-1 and TNF-α. At an initial phase, these cytokines are part of a system for remodeling damaged ECM components in response to mechanical stress or other forms of injury[109]. OA is a common feature in overweight and obese animals. In obese people, it has been characterized not only as a consequence of the increased mechanical loading but of the generalized

low-grade inflammation that is always present[147]. The catabolic chondrocyte produces many pro-inflammatory mediators and MMPs, which degrade ECM components and contribute to tissue remodelling[148,149].

The precursor of IL-1α is then translocated to the nucleus of the cell and activates the transcription of proinflammatory genes[150]. MMPs are produced in an inactive preform, and activity can be regulated to a point where they are converted into the active form. Active MMPs also form clusters with tissue inhibitors of MMPs (tissue inhibitors of matrix metalloproteinase), which leads to its inactivation. After an insult to a joint, these two mechanisms can be affected, which leads to a rapid increase in MMP activity [24]. The MMP family includes Aggrecanases and Collagenases, besides other Serine and Cysteine proteinases[151]. In addition to these molecules' actions, at the early stages of OA, there is evidence of a general increase in synthetic activity. The ECM synthesis pattern is also altered, for example, in terms of types of collagen produced [109]. Chondrocytes from OA patients are deficient in glucocorticoid receptors, and this lack of response to circulating glucocorticoids may result in increased cytokine and metalloprotease synthesis levels degrading cartilage[152,153].

Pro-inflammatory mediators augment catabolic activity through feedback, both in a paracrine or autocrine manner. They can also be released in the SF and enhance gene expression of MMPs[148,154]. These mediators' synovium is rapidly activated, resulting in joint effusion and recruitment of leucocytes to the intra-articular space. MMPs produced by leucocytes and synovial cells begin furthering the cartilage through an attack on matrix components. Cytokines can stimulate the catabolic activity of chondrocytes, and matrix degradation results in the release of fragments of many of its components to the synovial space. These fragments can function as a biological mediator that increases the production of cytokines and MMPs[29]. IL-1β has been pointed as the most important pro-inflammatory cytokine responsible for the catabolism in OA, increasing the expression of inflammatory genes and mediators, and in relation to lameness duration[126,155,156] (figure 2), even though several studies have reported low or undetectable levels in OA patients[157–159]. Low innate production of IL-1β and IL-6 is associated with the absence of human OA in the old age[160]. A strong negative correlation between IL-1β and Norberg angles has been described, suggesting that SF's activity increases in proportion with laxity severity[161]. In the porcine knee, OA, the median concentration of IL-1α was 0.043 ng/mL in cases of mild OA and of 0.288 ng/mL in moderate OA. IL-1β concentrations were 0.109 ng/mL in knees with mild OA and 0.122ng/mL in moderate OA[162]. In dogs with HD, IL-6 bioactivity is correlated with the duration of clinical signs and radiographic signs of OA[161]. *In vivo*, likely, the presence of cytokines alone would not usually lead to joint pathology without any other predisposing factor, such as alterations in gait, stiffening of subchondral

bone, joint laxity, or some other altered joint parameter[123]. Comparing dogs with OA and normal joints, differences in pro-inflammatory and anti-inflammatory biomarkers have been detected, but no relationship was identified between biomarker concentrations and gait asymmetry in dogs with OA[163].

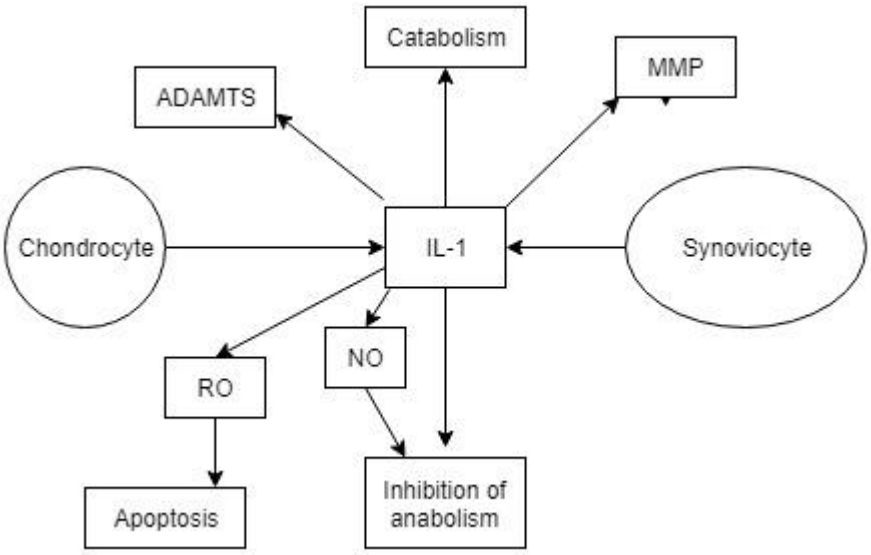

Figure 2 – The pivotal role of IL-1 in the cartilage metabolism in osteoarthritic joints (adapted from Chevalier & Kemta-Lepka, 2010). Legend: ADAMTS – A disintegrin and metalloproteinase with thrombospondin; MMP – matrix metalloproteinase; NO – Nitric Oxide; RO – Free radical.

Early phase matrix degradation might mainly be due to the actions of MMP-3, A Disintegrin, and Metalloproteinase with Thrombospondin Motifs 5. These degrade Aggrecan and activate Collagenases, particularly MMP-13, which degrades type II Collagen with excellent efficiency. Damage of the collagen network seems to be the state that, when reached, cannot be reversed[1,115,164]. Other MMPs are also active, such as MMP-2 and MMP-9, which play a role in tissue repair, angiogenesis, and inflammation, digest type-I, II, and IV collagen[165–167]. Elevated MMP-3 has been described in the hip joints of dogs with HD and stifles with Cranial Cruciate Ligament rupture[155,161,168]. MMP-9 concentration has been used to diagnose OA, differing between degrees of cartilage damage and described as elevated, along with MMP-2, in SF of canine OA joints[169–171]. The balance between MMPs and tissue inhibitors of matrix metalloproteinase is essential to prevent the progression of articular cartilage degradation[126,172]. MMPs can accumulate in tissues and play a prominent role in the degradation of tendons and ligaments as well[173].

Chondrocytes have receptors for ECM components, many of which are responsive to mechanical stimulation. When activated, these receptors induce the production of matrix-degrading proteinases and inflammatory cytokines and chemokines, as initiating or feedback amplification events[1]. Fragments found include fibronectin, small leucine-rich PG, and collagen[174–177]. As discussed previously, disruption of the ECM is a hallmark of OA, and the products of its catabolism

have been linked to inflammation through activation of the complement cascade and stimulation of Toll-like receptors[1]. PGE_2 produced by chondrocytes, under stimulation by IL-1 and TNF-α in joints induces vasodilation, enhances pain perception, proteoglycan depletion from cartilage, bone demineralization, and plasminogen activator secretion[126]. In rats, joint swelling showed to be proportional to TNF-α and TNF-R levels[178]. iNOS is induced by mechanical factors and inflammatory cytokines and is an inductor of chondrocyte apoptosis. Excess amounts produce both chondrocyte death and matrix degradation[179]. Chondrocytes express cytokine and chemokine receptors, IL-6, IL-8, MMPs, PGE_2, leukemia inhibitor factor (LIF), and several other genes that enhance or modulate inflammatory and catabolic responses, including COX-2, microsomal PGE synthase-1 (PGES-1), soluble phospholipase A2 and inducible nitric oxide synthase[1,180]. Chondrocytes can also have apoptosis induced by alterations in mitochondrial function, as a consequence of proinflammatory cytokines, prostaglandins, reactive oxygen species and NO action[181]. With age, they also present a secretory phenotype with senesce characteristics, marked by increased production of cytokines, chemokines and MMPs [182]. Joint cartilage can then become calcified, which in turn can stimulate chondrocytes and synovial cells to promote the production of more inflammatory mediators[183].

The nature and characteristics of synovial inflammation may change with the evolution of the disease. While macrophage seems to be present in an earlier phase[184], the prevalence of synovitis increases with the disease stage[184–186]. In post-traumatic synovitis cases, macrophage and lymphocyte infiltration are commonly observed, either diffusely or in perivascular aggregates[187]. More advanced stages are usually accompanied by villous hyperplasia, fibrosis, the debris of cartilage and bone, and increased vascularity[188]. These macrophages, along with activated monocytes, are the first to generate IL-1 and TNF-α in an immune response. Once those cytokines are released, they activate non-immune system cells such as endothelial cells, chondrocytes, and osteoblast to produce more IL-1 and TNF-α, thus amplifying cytokine concentrations resulting in severe inflammation[189]. In particular, macrophages that express tartrate-resistant acid phosphate are considered a key factor in promoting progressive, irreversible articular cartilage and joint destruction associated with many other production degradative enzymes, which has a significant impact on the development of OA[168,190–193]. The complete regulatory mechanism of this disease process is extraordinarily complex and involves a wide range of agents. Just regarding pro- and anti-inflammatory cytokines, cartilage explants produce IL-1β, IL-4, IL-7, IL-10, and IL-13 but do not secrete IL-6, IL-8, and IL-1Ra. In contrast, synovial tissues explants secrete IL-6, IL-8, and IL-1Ra and do not secrete IL-1β, IL-4, IL-7, IL-10, and IL-13[194]. In OA SF, several cytokine profiles are altered: IL-1β (irregularly present), IL-1Ra (present), IL-6 (irregularly present), IL-7 (present), IL-8 (present), IL-10 (irregularly present), and IL-13 (irregularly present)[195]. IL-6 is strongly induced by IL-1 and TNF-α and has been described as

having high activity in SF samples of dogs with experimental induced OA[50,189]. IL-11 shares several actions with IL-6, including stimulation of tissue inhibitors of matrix metalloproteinase production without reflex on MMP production[196,197]. IL-17 and IL-8 are potent inducers of catabolic responses in chondrocytes[164,198]. IL-17, in particular, increases degradation of cartilage PG independently of IL-1, and Aggrecanases are responsible for the IL-17 induced cartilage destruction[199–201]. IL-8 plays an essential role in the self-strengthening process of cytokine release and production[202]. Other cytokines play an anti-inflammatory and chondroprotective role, such as IL-10, which suppresses inflammatory mediators' release by macrophages (TNF-α, IL-6, and IL-1β), blocks elements of the inflammatory pathway, and prevents chondrocyte apoptosis[203]. A schematic representation of cytokine interactions in the intra-articular environment is presented in Figure 3.

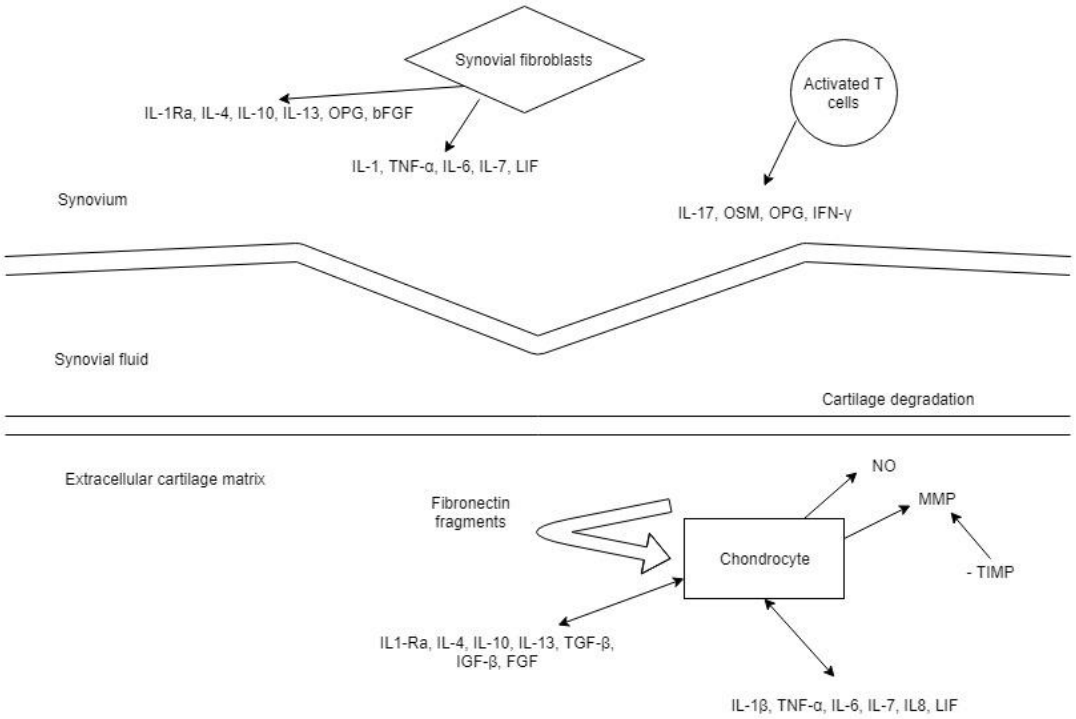

Figure 3- Schematic representation of the cytokine interactions in the intra-articular environment (adapted from Rutgers et al., 2009 and Sutton et al., 2009). IL – interleukin; IL-1Ra – interleukin-1 receptor antagonist; LIF: Leukocyte-inhibitory factor; MMP: Matrix metalloproteinase; NO: Nitric oxide; OPG: Osteoprotegerin; OSM: Oncostatin M; TIMP: Tissue inhibitor of matrix metalloproteinase.

Many cytokines and chemokines can promote synovitis. Many of them are increased in SF during OA, particularly IL-1 and TNF-α, which suppress matrix synthesis and promote cartilage catabolism[108,146]. Exposure to IL-1β stimulates chondrocytes and synovial cells to produce catabolic proteases with apocrine signaling, further enhancing MMP release and the resulting degradative cascade[180,204]. IL-1α also plays a role in bone pathophysiology, being known as osteoclast activating factor[205]. The level of several cytokines in SF, its sources and effects are presented in Table 3.

Cytokine	Source	Synovial fluid (pg/ml)	Effects
IL-1	Synoviocytes, chondrocytes, macrophages	<3.0	Increase cartilage and bone resorption Inhibit proteoglycan synthesis Up-regulate MMP expression Production of proteolytic enzymes Stimulate other cells to produce proinflammatory cytokines Stimulate other cells to produce chemotactic cytokines Stimulate pro-angiogenic factor release Stimulate NO production Induce chondrocyte apoptosis
IL-4	Synoviocytes, chondrocytes	0.1	Suppress gene expression of TNF-a and IL-1b Reduce pro-inflammatory cytokine production, inflammation, vascularisation, and cartilage degradation Inhibit IL-1b stimulated production of PGE2 Inhibit LIF synthesis by acting directly on OA synoviocyte IL-1b and TNF-a-induced LIF production Reduce MMP-3 transcription and activity in articular chondrocytes Up-regulate expression and production of IL-1Ra by human OA IL-1b-activated synoviocytes Decrease osteoclast formation, thereby reducing bone resorption Inhibit synoviocyte apoptosis, contributing to synovial hyperplasia in OA
IL-6	Synoviocytes, chondrocytes	0.5	Inhibit proteoglycan synthesis Reduce chondrocyte proliferation Increase MMP-2 activity Increase aggrecanase-mediated proteoglycan catabolism
IL-8	Monocytes, synoviocytes, chondrocytes, osteoblasts	89.9	Recruit leucocytes Neutrophil chemoattractant Stimulate the release of pro-inflammatory cytokines Hypertrophic differentiation and calcification of chondrocytes
IL-17	Activated T-lymphocytes	0	Induce NO synthesis Induce MMP synthesis Increase production of IL-1b, Il-6, and IL-8 Stimulate the release of pro-angiogenic factors
TNF-α	Synoviocytes, chondrocytes	<5.0	Increase cartilage and bone resorption Inhibit glycoprotein and collagen synthesis Up-regulate matrix metalloproteinase (MMP) expression Stimulate other cells to produce proinflammatory cytokines and growth factors Stimulate pro-angiogenic factor release Stimulate other cells to produce chemotactic cytokines Stimulate nitric oxide (NO) production Induce chondrocyte apoptosis
IL-1Ra	Synovial membrane	614	Competitive inhibitor of the IL-1b receptor Inhibit MMP production

Table 3 – Concentration of different cytokines in SF, its sources, and effects (adapted from Rutgers et al., 2009 and Sutton et al., 2009). IL – interleukin; IL-1Ra – interleukin-1 receptor antagonist; TNF – tumor necrosis factor.

Synovial inflammation and damage-associated molecular patterns (DAMP) or alarmins have also been implicated in the activation of inflammation and catabolic events in articular cartilage and collagen while also acting as ligands of Toll-Like Receptors. OA cartilage lesion areas, as the

synovium itself, also lead to increased expression of inflammatory and catabolic genes, including MMP-3, MMP-13, nitric oxide syntase, and C-reactive protein (CRP)[206–210]. Synovial inflammatory infiltration is observed in OA, secondary to the release of cartilage breakdown products, and it affects cartilage metabolism by reducing GAG production[194,211,212]. Acute synovitis may be one of the first changes in OA, as synovial tissue from early patients has been shown to over-express inflammatory mediators and may even be the initiator[31,213]. Recent studies have demonstrated a direct association between joint inflammation and OA progression, and its degree is usually associated with the disease's progression[108,214]. These changes occur from an early stage of the disease, after joint insult[185,209]. An association between this low-grade inflammation and disease manifestation has been made[215,216]. Acute synovitis and capsulitis have been described as the most common problem in high-motion joints of athlete horses and may contribute to the degradative process in articular cartilage by releasing enzymes, inflammatory mediators, and cytokines, being a driver of OA[126]. In humans, synovitis was found in more than 50% of patients with normal radiographic knees and connected with the continuation of the structural changes seen in OA[214,217]. It is reasonable to assume that this is also true in dogs. The accumulation and activity of immunological cell populations within the inflamed synovium are likely to depend upon pro-inflammatory cytokines' secretion and exhibit synovial lining cell hypertrophy and cellular infiltration[189,212]. In advanced cases of OA, changes in the synovial membrane can mimic those in rheumatoid arthritis[217].

CRP is an acute-phase protein, mainly synthesized in the liver during an inflammatory reaction or tissue injury, and is also produced at the inflamed tissues[19]. It has the advantage of being an objective, quantitative marker of inflammation that is not biased by treatment with NSAIDs or glucocorticoids[19,218–220]. Therefore, this acute-phase protein can be used to assess the innate immune system's systemic response to infection, inflammation, or trauma[221–223]. CRP is accepted as the most useful acute-phase protein in the dog, similarly to man, with the advantage of its shifts being noted from a very early stage[19,224]. At the joint level, its concentration in dogs' stifle with naturally occurring Cranial Cruciate Ligament rupture is higher than in normal stifles and lower in the stifles of dogs with OA from other sources. Its serum concentration is also higher in dogs with thoracic limb lameness than pelvic limb lameness, before and after treatment[171,225]. A study showed that dogs with OA correlated with SF IL-6 and serum CRP[226]. A small increase in CRP quantity has a potential predictive clinical value in rapid diagnosis progression of knee OA in humans[227]. Also, in humans, a reduction of CRP levels following therapy is predictive of clinical response within 12 to 24 weeks in over 50% of patients with OA[228]. It has also been associated with the severity of pain and the events that ultimately lead to radiographic progression of OA[229–231]. High sensitivity CRP is another biomarker that reflects systemic synovitis[187].

Lymphocytes compose most perivascular infiltrates[209], and many cytokines promote their activation, activity, and survival. For example, serum levels of IL-15 have been associated with the evidence and progression of radiographic OA[232]. Its synovial levels are also increased in early OA[233]. Lymphocytes also produce cytokines, being the predominant source of IL-17, which induces chemokine production by fibroblasts and chondrocytes in synergy with IL-1 or TNF-α[234]. A decrease in anti-inflammatory cytokines, such as IL-1 receptor antagonist (IL-1ra), soluble TNF receptor-I (sTNF-RI), and sTNF-RII, led to reduced pain and lameness scores in dogs with OA [235].

Subchondral bone changes in volume and density reflect loading history. Bone remodeling in OA may also be initiated at local bone damage sites, which occur due to excessive repetitive loading. This damage is usually associated with the appearance of microcracks that initiate targeted remodelling[236]. Additionally, changes occur at joint margins and entheseal sites, where new bone is added by endochondral ossifications. Although no definitive understanding of osteophytes' role, they may stabilize the joint rather than contributing to OA progression[237]. The formation of osteophytes has been linked to high transforming growth factor-β concentrations[238]. The properties of subchondral bone influence the characteristics of organic bone. The properties change during the progression of OA, being associated with a decreased bone modulus, possibly due to a decreased and incomplete mineral density, resulting from an increased rate of remodeling. This remodeling may have significant implications in treatment strategies for targeting subchondral and periarticular bone remodeling in OA[239]. It has a substantial role as a mechanical damper and a source of inflammatory mediators in the OA process, pain, and the degradation of the deeper layers of cartilage[119]. The subchondral bone itself is a source of inflammatory mediators and OA pain[240]. The bone then enters a high and continued mineral deposition phase, making it more resistant to deformation and, therefore, more fragile, adversely affecting the overlying articular cartilage[241]. Also, bone plays an important role in cartilage health. Removal of cartilage explants from the underlying bone tissue leads to a higher percentage of cell death in chondrocytes than if they were left attached to the bone[242]. During OA, calcified cartilage separates articular cartilage from the subchondral bone and goes through marked alterations. This process involves penetration by vascular elements recapitulating the growth plate's vascular event that occurs during development[243,244]. This complex interaction between cartilage, synovium, and subchondral bone leads to synovial hyperplasia and villous hypertrophy, decrease the quality of synovial fluid, subchondral bone sclerosis, and persistent capsular inflammation and fibrosis[29].

SF characteristics are also affected by the osteoarthritic process during the early stages. SF hyaluronan concentrations are reported to increase as a result of elevated synthesis in response to pathological stimuli[245]. As the disease progresses, hyaluronan concentrations decrease to amounts

below normal, as synthesis is overcome by degradation and loss [246–248]. All of this leads to joint stiffness with decreased ROM, further contributing to OA progression and continuously changing the patterns of cartilage loading[29].

2.2 *In vitro* and animal models

Several models have been developed to study OA in an attempt to mimic the factors and conditions which initiate the disease or dissect the pathways active during its progression[180]. They are divided into spontaneous and induced, surgically, or nonsurgically[249]. Slowly progressive spontaneous models of disease have the advantage of mimicking the course of primary OA in humans more closely, without the need for intervention. Surgical models have the advantage of repeatability, rapid onset, and progression. The rapid course of the disease, however, makes them less ideal models of spontaneous OA[11].

Most *in vitro* models use supraphysiological loads of cytokine concentrations compared with the naturally occurring disease to impart a timely response from cells or tissues. Although this helps to understand the role of a single stimulus, models that comprehend different physiological and molecular aspects of the disease and reflect the naturally occurring disease's pathogenesis are usually prefered[180]. A limitation often pointed to *in vitro* approaches is that they can't replicate the complexity of a multiple living tissue organ such as the joint[250].

Animal models were used to study human OA, providing valuable advantages and significant information in comparison with human OA research. Both the spontaneous disease and the surgical induced models have similar pathogenesis to that observed in humans[121,251,252]. Animal models also offer the opportunity to study early features of the disease's development, before the installation of a fulminant catabolic process, more difficult to dissect. *In vivo* studies suggest that different joints from different species are physiologically comparable[180]. Besides, companion animals share the same environment and suffer similar co-morbidities as humans, with OA usually being present for prolonged periods. These naturally occurring painful disease models may better reflect the complex genetic, environmental, temporal, and physiological influences present in humans[253].

The dog is a common and appropriate model for OA's study, and, significantly enough, spontaneous OA is common in dogs, in multiple joints, from various aetiologies. It is considered a nearly ideal species for human OA translation research and the most used research model. It has the advantages of being anatomically, biochemically, genomically, and molecularly similar to humans, with clinical progression and treatment similarities, where degenerative, trauma, and overuse aetiologies occur[7,11,253,254]. They also share many of the environmental variations that influence human OA. Along with their foreshortened lifespan and equivalent life stages to those in humans, the

dog holds the potential for a longitudinal evaluation of the disease and its treatment[111,255,256]. There are numerous studies published regarding spontaneous OA models (from HD, elbow dysplasia, to anterior cruciate ligament – ACL, deficiency), surgically induced models (ACL transection, abrasion, cartilage defect, joint distraction, and others), enzymatic/chemically induced OA (using iodoacetate, calcium pyrophosphate, and others) and impact models (acute patellofemoral loading)[7,50,168,257]. As referred, there are several reasons for this magnitude of interest, namely anatomical disease mechanism, response to treatment, and clinical similarities to humans. Other animals, such as the rat, are also commonly used, but they do not share the entirety of similarities with the human disease as the dog does, and naturally occurring disease is also very uncommon[7]. For example, in rodent models, in contrast to what happens in the canine model, the complement of MMPs and degradation products are not as consistent with humans, and rodent models are a poor predictor of drugs' efficacy or toxicity in human OA[115,258,259].

It is essential to determine to what extent induced OA represents the naturally occurring disease[172]. Most *in vitro* research mimic OA by adding inflammatory cytokines, such as IL-1 or TNF-α, to cartilage explants of chondrocyte cultures. However, OA is a slowly progressing disease, and, in naturally affected joints, cytokine concentrations are relatively small[180,194]. Despite this, animal models still provide evidence that IL-1 has a role in inducing PG loss after intra-articular (IA) injection and that IL-1ra has been able to slow down the progression of OA[212,260–264]. IL-1ra expression levels increase to limit IL-1 signaling in response to several inflammatory stimuli. The concentration in healthy canine joint fluid and dogs with OA may play an essential role in the pathogenesis and treatment of OA[265]. Other models have also provided insight into how the initial stages of the disease occur. In a chemical-induced OA model, sodium urate injection to a joint causes synovitis, a dramatic increase in synovial PGE2 concentration, and an initial infiltration of monocytes, macrophages, and mast cells. This process is followed by polymorphonuclear leukocytes into the synovial space and is generally accompanied by pain[266,267]. This infiltration by mononuclear leukocytes of the synovium is a process that is initiated at a very early stage after injury and remains present throughout the entire development of OA[268]. Suitably aligned studies of spontaneous OA in dogs, particularly hip and knee OA, could highlight new advances regarding the disease.

Developing cross-species collaborations will provide a wealth of research material and knowledge relevant to human OA, and that cannot be obtained from rodent models of experimentally induced dog models of OA. Changes in slowly progressive spontaneous dog OA closely match those of human OA while contrasting with those seen in rapidly advancing experimental surgical induced dog OA[269]. Dogs may, therefore, be embraced as a missing link in the translation of OA treatment from mice to men[270]. Animal studies could potentially reveal the underlying biochemical pathway(s),

refine treatment modalities, and provide opportunities for new treatment and prevention targets[256]. Therefore, the dog is an ideal species to study human OA and exploring spontaneous dog OA under the One Medicine initiative to improve the health and well-being of both humans and dogs[111,252].

The search for biomarkers measured in the SF, serum or urine, that would be a good indicator of OA's development and evolution, has been under significant attention, and can be measured in SF, serum, or urine[12]. A biomarker can be defined as a characteristic that can be objectively measured and evaluated as an indicator of normal biological or pathogenic processes or pharmacological responses to a therapeutic intervention. They can be anatomical, physiological, biochemical, or molecular parameters associated with the presence and severity of specific diseases, detectable by various methods including physical examination, laboratory essays, and imaging[271,272]. The best candidates for biomarkers are most likely to be structural molecules or fragments linked to cartilage, bone, or synovium and may be specific to one type of joint tissue or common to them all[273]. Although it is complex to assess the impact of different biomarkers on OA pathogenesis, it is recognized that a putative biomarker would offer the potential to monitor disease more easily since early structural changes will likely remain asymptomatic for years[172,272]. Markers of other musculoskeletal components have also been studied, as is the case of tenascin-C, an ECM glycoprotein of the myotendinous junction. SF levels have been correlated with the degree of inflammation, chondrocyte differentiation, cartilage degradation, and radiographic severity of OA[274]. Its synthesis is mainly regulated by IL-6, but also by IL-1 and TNF-α[275–277].

Given that, they can be categorized as direct or indirect molecular markers. Direct markers are measures of tissue synthesis or breakdown, reflecting anabolic or catabolic processes. Indirect biochemical markers, such as cytokines, inflammatory mediators, growth factors, and enzymes, are released with trauma and disease processes, affect tissue turnover, but are not themselves generated in the process of tissue synthesis or breakdown. While direct markers may more closely represent the status of joint degeneration, indirect markers can be used to learn more about the processes preceding or leading to the development of OA[254,272,278]. They can also be categorized using the five-point BIPED classification scheme developed by the Osteoarthritis Biomarkers Network, which stands for burden of disease, investigative, prognostic, efficacy of intervention and diagnostic[273,279]. Biomarkers can be pro-inflammatory mediators, typically produced in the early phase of inflammation or tissue destruction; enzymes and their inhibitors, which play a role in joint destruction; or extracellular cartilage products degeneration[172]. Several have been pointed out as sensitive and specific for early cartilage changes related to different joint components, such as the biomarker of intact proteoglycan (aggrecan 846 epitope) or cleavage (BC-3 and -14) and type II collagen degradation (Col2-3/4Cl$_{long\ mono}$ and Col2Ctx) and synthesis products (protocollagentype II C-protopeptide or one of its two

isoforms)[12,172,280]. These breakdown products are released into SF and cleared into the serum and urine, where they are measured as biomarkers for disease progression or response to therapeutic intervention[115]. High stromelysin production occurs in OA cartilage of dogs and, in cartilage explants, appears to correlate with OA severity[164]. One of the significant limitations in identifying specific biomarkers can be attributed to articular cartilage since most of the body's cartilage is located in the respiratory system and spine[12]. Others provide a real-time quantification of the formative and resorptive activities of bone[281].

Diagnostic biomarkers ideally identify patients at an early stage of OA where treatment may be most effective. Although there have been many promising studies in this direction, no single biomarker stands out for use in OA diagnosis[273]. C-terminal telopeptide of collagen type II has been used as a biomarker for the burden of disease due to its correlation with the presence of osteophytes and reflection of bone turnover[169,282,283]. It was also studied as a prognostic biomarker since high urinary levels are associated with radiographic progression, independent of age, sex and body mass index [284], and the efficacy of intervention biomarker, since several studies have linked its urinary levels to interventions in OA[285]. Serum lysophosphatidylcholines to phosphatidylcholines ratio can predict clinical response to licofelone and naproxen treatments in symptomatic knee OA patients, with a greater than optimal ratio cutoff of 0.088 corresponding to a 2.93 fold chance to respond to specific NSAIDs[286]. Experimental models have demonstrated an increase of total and active collagenase in OA cartilage and an imbalance in activation and inhibition of MMPs[123]. Increased GAG levels in SF samples have been described as markers of cartilage degradation, but their levels may be influenced by inflammatory activity, joint effusion, and cartilage OA stage [287–289]. Some genetic polymorphisms related to cartilage breakdown have been identified, and some provide interesting insight, like the fact that the risk loci are generally specific to hip or knee, indicating joint-specific disease pathways[290,291]. SF CRP is a possible useful biomarker of joint inflammation and a tool to monitor response to anti-inflammatory treatment. Although its origin is yet not fully known, it is very probably located within the joint[19]. The level of SF CRP is elevated in dogs with naturally occurring OA compared to control dogs, but not with a corresponding increase in CRP serum levels[292,293]. A different study found a contrasting relation, with dogs with chronic OA showing high plasma CRP levels, and serum vitamin B12 and folate concentrations, compared with normal dogs[294].

Several factors, like exercise and activity, may affect biomarker levels in SF. It appears to be dependent on the amount of exercise performed, and most of them will not react to light-moderate exercise but may increase in case of strenuous exercise. These results reflect the effect of heavy exercise on the metabolism of the ECM components[72]. The evaluation of biomarkers in the serum and urine is usually less rewarding than in SF since other systemic conditions not related to OA may

affect their concentration[254]. An additional constraint is a fact that even mild synovitis, as seen in OA cases, may significantly increase the clearance of a molecule from a joint, thus affecting its measurement[217]. Additional factors, such as activity or immobilization, also affect clearance rate[79]. Overall, this field is still characterized by many contradictions, and, to date, neither a general, universal biomarker of OA nor a joint/aetiology specific biomarker in synovial fluid is available for use in small animal practice[172,273]. The ones with the most consistent evidence appear to be those at the end of the pathways of tissue destruction, which may be more specific for a particular tissue, as is the case of C-terminal telopeptide of collagen type II[273]. An alternative to this limitation is the possibility of analyzing clusters of biomarkers related to specific pathogenic processes, as cartilage synthesis, synovium, or inflammation[295].

2.3 Joint pain

Pain is the most relevant clinical sign of OA and a hallmark of the disease[24,249]. It is a multi-dimensional experience, with a sensory, evaluative, and affective component. Like happiness, it is not directly measurable[296]. In dogs, OA related chronic pain is currently underdiagnosed and managed[297]. The symptomatic management of this pivotal symptom is central in both human and veterinary clinical practice, and the current therapeutic goal for both species is the management of pain and associated loss of function[270,298]. Pain may be classified according to its duration (acute, chronic, or intermittent), anatomic origin (somatic, visceral, or neuropathic), and severity (mild, moderate, severe, or excruciating). Chronic pain typically refers to pain with a duration greater than three months[299]. One clear difference between dogs and humans is the ability to self-report pain[111]. There is a lack of a gold standard in veterinary medicine to assess articular pain in patients with naturally occurring disease. Still, much progress has been made in developing methods to measure chronic pain via subjective and objective methods, particularly in owner assessment tools and limb use measurements and activity[300]. Others focus on clinical metrology instruments (CMI), with recent research areas extending to develop measures of activity, sensory function, and quality of life (QoL). In all of these areas, more data on validity is needed, and assessments are made by testing in field studies, which incur considerable time and expense[301]. Exploring peripheral mechanisms of arthritic pain in animals like dogs has the potential to reduce the veterinary burden of OA and to improve the translatability of pain research into the human clinic[302]. Intra-articular anesthesia is a commonly used tool to detect articular pain, but its limitations since the simultaneous contribution of various anatomic and pathological sources of pain concur in OA[272].

Serum cortisol levels may also be used to assess joint pain in dogs, with a cut-off set at ≥1.6μg/dL to detect most dogs with pain in a trial comparing cortisol levels with force plait

analysis[303]. The perception of pain is transmitted through afferent fibers, with peripheral neurons also exerting efferent functions, through the mediation of neuropeptides[304]. Similarities in neurophysiology across mammals strongly suggest that humans' and animals' type of pain is analogous[305]. It involves a multiplicity of pathways, mechanisms, and transmitter systems, which likely explains why a single analgesics class is usually unable to provide complete analgesia[306]. Neuropeptides are small molecules produced in the spinal cord's dorsal root and autonomic ganglion neurons capable of inducing the release of cytokines, prostaglandins, and nitric oxide (NO). They can also have an action similar to growth factors in a healthy joint, meaning that they play a role in joint homeostasis[307]. Canine studies of naturally occurring OA pain add valuable data supporting drug treatment mechanisms that may translate to humans[308].

Since the joint is a complex organ, affected by many pathological processes, the cause and precise origin of pain may be hard (if possible) to determine. The perception of pain and overall joint function will be influenced by sensory innervation of all composing tissues, from subchondral bone, periosteum, synovium to the capsule. When pathological processes take place, the exposure of subchondral bone, tissue remodeling with osteophyte formation, and marrow edema can all contribute to the perception of pain that is triggered when deterioration reaches the synovial membrane and/or the bone beneath joint cartilage by a self-perpetuating vicious cycle, which is schematically represented in Figure 4. Deep somatic pain originating in joints and tendons is a major therapeutic challenge [309,310].

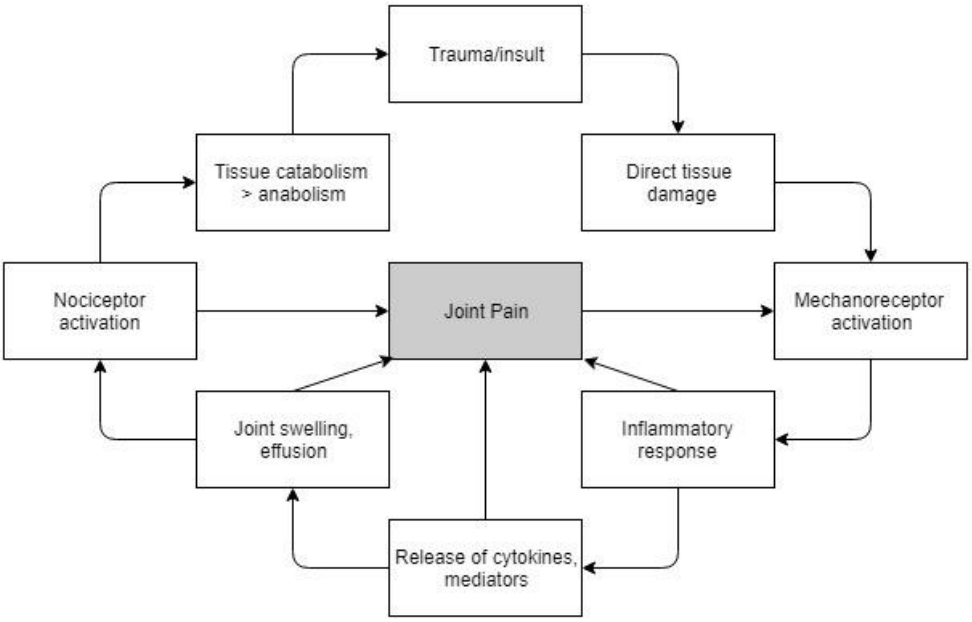

Figure 4 – Processes that may contribute to joint pain (adapted from van Weeren & de Grauw, 2010).

Joint swelling, a consequence of synovitis, together with fibrosis, activates the joint's mechanoreceptors, further increasing the perception of pain [24]. Muscle masses surrounding joints also contribute to pain perception, both as a primary source and because of compensatory mechanisms, and are invariably atrophied in chronic joint pain[312,313]. The pain generated by OA has the potential to alter the normal function of the limb, a phenomenon referred to as functional allodynia[314]. There is evidence that joint pain leads to central sensitization, which facilitates nociceptive output and amplifies signals, resulting in greater perceived pain. This process is due to the central nervous system's plasticity and functional alteration in response to peripheral nociceptive input[315–318]. In the spontaneous canine OA model somatosensory sensitivity occurs, which is an indicative of central sensitization. This a further validation of the canine spontaneous OA model as an appropriate model of the human OA pain condition[319]. Most nociceptors are polymodal, responding to noxious mechanical stimuli, mechanical stimuli (painful pressure, squeezing or cutting of the tissue), thermal stimuli (heat or cold), and chemical stimuli[320]. Nociceptors of joints respond to noxious mechanical stimulation of the joint or are silent[321]. During acute pain, the nociceptive processes and the subjective experience are closely related but can differ in chronic states[309]. With time, spinal sensitization occurs, increasing spinal cord neurons' excitability, making them more susceptible to peripheral inputs and, therefore, giving stronger signaling to stimulation[318,322]. With inflammation, chemical stimulation of nerve afferents induces pain, and polymodal nociceptors are then sensitized[309]. It is conducted by different endogenous mediators, with IL-1 and TNF-α at the head in OA, while also stimulating the production of other mediators, such as PGE_2, cyclo-oxygenase -2 (COX2), microsomal PGE synthase-1, and soluble phospholipase A_2. The cycle further perpetuates itself by the upregulation of NO production[109,241]. In particular, activated synoviocytes release a wide range of inflammatory mediators, including kinins, leukotrienes, prostaglandins, and acute-phase proteins such as serum amyloid A[272]. Many of these molecules can act on polymodal nociceptors, as free C-fiber and A-δ nerve endings[272]. These synoviocytes also have an inferior *in vitro* fibrochondrogenic potential compared to normal ones. However, given adequate conditions, they can produce ECM components at a comparable level of normal joints[198]. Inflammatory pain is linked to sensitization of sensory proteins at the nociceptive endings whereas pain originating from nerve damage (neuropathic pain) has been linked to axonal ion channels producing ectopic discharge in nociceptors as a source of pain. They show increased responses to normal stimuli, have a lowered threshold, and recruit silent nociceptors[309]. In humans, as in dogs, patients suffering from chronic pain conditions, such as OA, are deficient in controlling noxious input to the central nervous system[323]. This effect is associated with canine OA pain, and the naturally occurring canine OA model may be used to test drugs that normalize this function[324].

PGE_2 is also considered a central mediator of pain in OA, with the ability to sensitize neurons and reduce their activation threshold, enhancing response to other stimuli[325,326]. Its production requires the enzymes COX-2 and PGE synthase, both of which are induced by IL-1β [126]. TNF-α is another crucial mediator of mechanical joint hyperalgesia, being implicated in the initiation of neuropathic pain pathways[327–329]. Several other nociceptive molecules are recruited into the osteoarthritic joint, including nerve growth factor, calcitonin gene-related peptide, and chemokine ligand 2 causes activation of peripheral nociceptors[330–332]. Sensory nerve fibers that express nerve growth factor have been observed in the vascular channels associated with osteochondral angiogenesis in the calcified cartilage and maybe an additional source of symptomatic pain[243,244,333]. nerve growth factor has been pointed out as a critical driver of pain in OA, and antibodies directed at nerve growth factor are potent analgesics in humans[334]. Artemin, a neurotrophic factor, and its receptor, glial-derived neurotrophic factor family receptor alpha-3, have been identified as involved in pain and may play an important role in chronic pain associated with OA in dogs. Elevated serum levels of artemin from osteoarthritic humans compared to healthy individuals suggest translational relevance[335].

2.4 Physical examination

As a whole, orthopaedic conditions of the pelvic limb are some of the most frequently diagnosed causes of lameness in dogs[336]. Along with Cranial Cruciate Ligament disease, hip dysplasia and OA are the most commonly diagnosed problems[337]. Clinical signs of OA can vary significantly, both in presentation and history, ranging from mild discomfort to severe acute or chronic pain, restriction of activity level, limitations in the ability to perform, poorer proprioception, and loss of strength and flexibility[6,338]. Most animals with OA will probably live with the undetected disease for a large portion of their life, since identifying OA-associated pain signs may be very difficult, especially earlier in the disease process[339].

In dogs with hip dysplasia, the primary culprit is joint laxity, which causes joint subluxation and subsequent osteoarthritic changes. These animals will often exhibit a biphasic pattern of clinical signs throughout its life. Initially, at around 5-9 months of age, a period of lameness is observed and is usually a consequence of capsular stretching and tearing, with degeneration of the femoral head ligament, synovial fluid overproduction, subchondral bone sclerosis, and synovitis. Typically, a period of stability then ensues, with synovial fluid reduction, joint capsule hyperplasia, and bone remodeling. This phase is often associated with reduced or unapparent pain. Gait alterations developed during this period to minimize pain may lead to subsequent compensatory problems[340]. Particularly in large and giant breeds, most clinical signs are observed in the older population, with

the disease at a chronic stage. Lameness is usually due to OA's consequences, and patients exhibit a history of uni or bilateral pelvic limb lameness, difficulty raising, stiff gait with reluctance to run or jump[6,101,125,341]. Compared to other joints, hip OA seems to be better tolerated by animals, and dogs presenting with the disease often have slightly abducted pelvic limbs, easily noticeable when the animal is standing, increasing acetabular coverage[101].

It is necessary to conduct a neurological and orthopedic examination and rule out other conditions that might have a similar presentation to attribute clinical signs to the hip joint. In a retrospective report, 32% of cases referred to a teaching hospital for HD management had cranial cruciate ligament rupture[342]. Unlike those with musculoskeletal disease, patients with neurologic conditions do not have pain that worsens at different times of the day or with exercise, but rather signs that are always present. Some signs may be overlapping, as dogs with orthopaedic related pain will often have inadequate or slow paw replacement during proprioception testing[343]. Documenting the history of lameness is also essential, as the expectations for the animal's level of activity and use[344]. While owners can identify a wide range of acute and chronic OA clinical signs, typically subtle and intermittent, behavioural and demeanour changes in their dogs', few attribute these changes to OA. In some cases, many months can go by before these patients are presented to a veterinary practice[345].

During the static examination, protrusion of the greater trochanters, dorsal and laterally, may be observed. Dysplastic dogs may exhibit a wide-based stance (on an early phase, to reduce the hip joint) or a narrow-based stance (to reduce discomfort caused by reduction of the femoral head after subluxation). Muscular atrophy is a consistent finding and may be evident within a few weeks[6,101,336,346]. Pelvic limbs may also be tucked under the body, the back is maintained arched, and shoulders muscles may be hypertrophy due to a shift of body weight towards the thoracic limbs[341,346]. On palpation, pain may sometimes be elicited by applying pressure over the hip and also when placing the joint through its range of motion (ROM), mainly during extension. The joint should also be manipulated through flexion/extension, abduction/adduction, and internal/external rotation. While doing this, restrictions in range (figures 5 and 6), crepitus, and pain should be assessed. Due to its location, the effusion of the hip joint is impossible to detect[101].

Stage	Signs
Mild OA	Stiffness, decreased activity, lameness
Moderate OA	Pain, muscle atrophy, difficulty rising
Severe OA	Loss of range of motion, vocalization, crepitus, lethargy, inappetence

Table 4 – Common clinical signs of osteoarthritis (adapted from Towell & Richardson, 2010).

Goniometry is a reliable and objective method for determining the ROM of joints[348]. Coxofemoral ROM may also be diminished, particularly during extension, and crepitus may be present[6]. This restriction is not a universal finding since animals with poor function may have a reasonable ROM, and those with severely restricted ROM may function reasonably well[349]. Normal ROM of the hip joint in military working German Shepherd Dogs have been described as 44°±6 at flexion and 155°±6 at extension[350]. In Labrador Retrievers, a normal ROM of 50°±2 at flexion and 162°±3 at extension in one report, and 49°at flexion and 159° at extension in another has been presented[348,351]. Normal ROM values have some variation according to the dog size, breed and type[352].

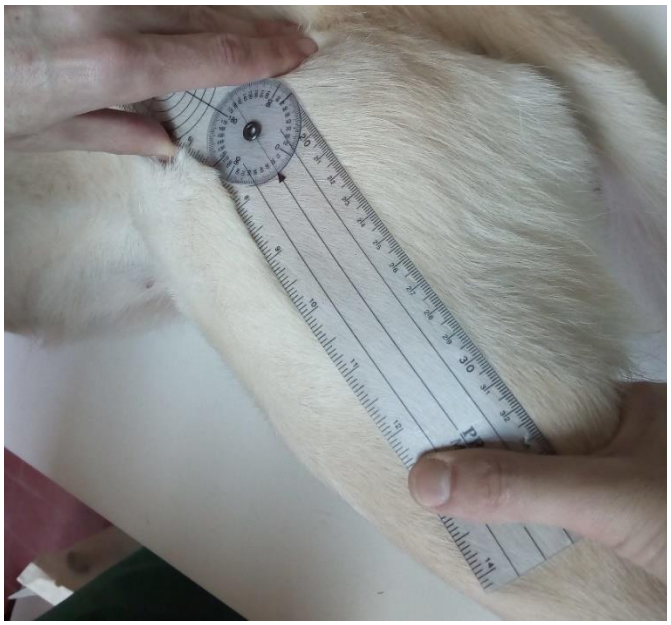

Figure 5 – Goniometry of the hip joint at an extension.

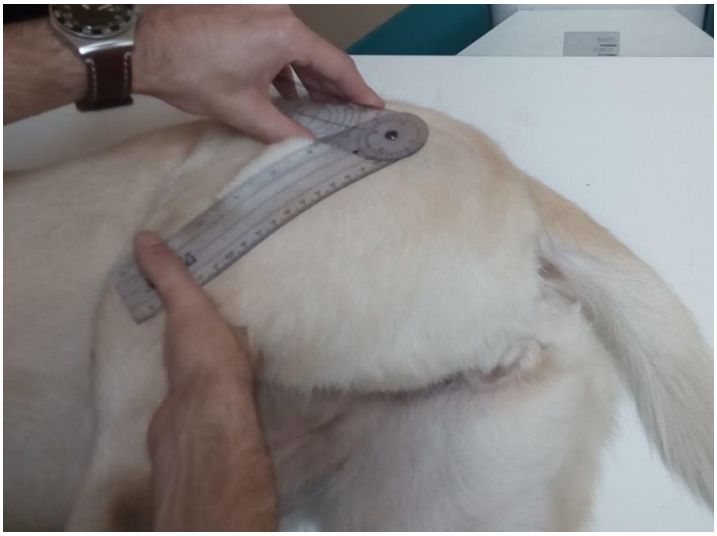

Figure 6 - Goniometry of the hip joint at a flexion.

Manipulation of the joint should isolate flexion-extension from adduction-abduction and assess internal and external rotations. At this point, it is important to differentiate between caudal lumbar/lumbosacral pain and pelvic limb pain. While hip extension will inevitably produce lumbosacral spinal pain and hip pain, pain during abduction will not generally be presented by a dog with the lumbosacral disease. Yelping is not a usual finding in OA cases[346]. In dogs with HD, hip laxity may be detected under general anaesthesia or heavy sedation by inducing the Ortolani sign, by which the femoral head is forced into subluxation, then returning to a normal position[125]. Measuring thigh girth may also be a useful measurement in the initial assessment and as an outcome measure since the quadriceps muscle group is particularly prone to atrophy secondary to decreased limb function[353]. This measurement, mainly when made with a Gullick II measure, was low intra- and inter-observer variation, with or without clipping the hair or stifle flexion or extension[353] (figure 7). It may also help distinguish a neurological from an orthopaedic condition since disuse atrophy is usually mild to moderate, taking weeks to months to ensue, while neurological atrophy sets over days or weeks[343]. The evaluation of asymmetry and muscle atrophy, measurement of static weight-bearing, and joint ROM have been described as the most valid and sensitive physiotherapeutic evaluation methods[354,355].

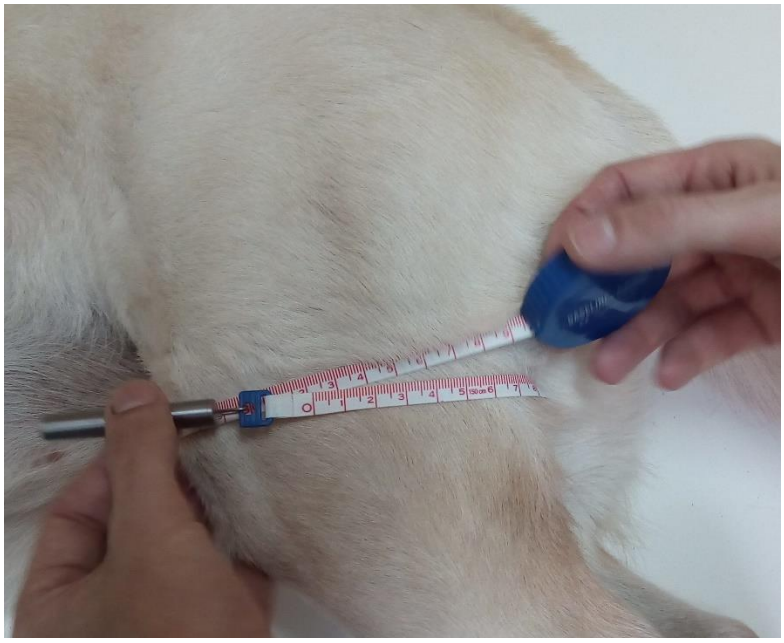

Figure 7 – Thigh girth measurement, using a Gullick II measure.

During the dynamic examination, a pelvic sway gait, which increases with exercise, is visible in an attempt to reduce hip flexion. Also, animals present a decreased and stiff stride with a possible weight shift to the thoracic limbs[101,346]. By placing a hand over the dog's hips during walking, it is sometimes possible to palpate the clunking that occurs with subluxation and reduce an unstable hip at stance and swing phases[346]. The animal is usually evaluated at a trot since it is a symmetric gait, which provides a good visual presentation of movement for the clinician to diagnose lameness[340]. Concerning the dynamic examination, the moment at which the physical examination is conducted does not change lameness scores[356]. The increased displacement of the hip ('hip drop' of the lame side during the swing phase) is a good indicator of hind limb lameness in dogs[357].

Lameness evaluation is an acquired ability, resulting from practice experience along with the integration of objective information. In working dogs, lameness often does not need to be obvious to limit performance. For this reason, the use of diagnostic analgesia can be an interesting tool to identify a lameness source correctly[344]. It is also interesting to note that dogs with mild lameness may be less prone to improve following treatment, emphasizing the need to manage OA dogs even with more subtle afflictions carefully[358]. It is well established that the hip OA radiographic signs poorly correlates with clinical signs[346]. Also, since OA's current diagnostic methods combine radiographic and clinical signs, the disease is usually only definitely diagnosed when the destruction of joint tissue is irreversible[273].

2.5 Hip dysplasia

In dogs, hip OA is commonly a consequence of HD. HD is the most common orthopaedic condition in dogs, with a prevalence of up to 71% in predisposed breeds, causing joint inflammation with variable degrees of clinical presentation[359,360]. It is defined as an inherited development disease of the hip joint, influenced by many genes specific for each breed (polygenetic trait)[361]. HD is developmental, and all dogs are born with normal hips, with instability emerging in the early stages of the disease, between 4 and 12 months of age[125,341]. Joint laxity leads to abnormal wearing of the coxofemoral joint and subsequent OA and means that, if left untreated, it will progress to OA[360]. It is comparable to human developmental dysplasia of the hip and is considered a pre-OA disease. Progression to OA is significantly faster in dogs than humans, occurring within 1–2 years of age, associated with dogs' shorter life span, making it valuable as a pre-clinical spontaneous animal model[256]. Canine HD resembles the hip's development dysplasia but progresses over a compressed time frame, adding to its utility as a model[111].

Although the etiology and pathogenesis of HD are still unclear, it has been accepted that it reflects the interaction of multiple genes with environmental influences, and joint laxity is central in the development of OA[6]. A study regarding the entire lifespan of a group of dogs showed that animals with coxofemoral subluxation developed OA, on average, 9 years earlier than those without subluxation. Still, 98% of all animals had developed OA by the end of their life, whether or not they had subluxation[362]. HD is known to affect any breed of dogs but is more common in large and giant breeds such as German Shepherd Dogs, Labrador, and Golden Retrievers, amongst others[363]. Similarly to human developmental dysplasia of the hip, it is characterized by delayed femoral capital ossification, underpinning instability continuum (detected by the Ortolani test), with severe forms characterized by complete subluxation, focal cartilage overload, and OA[364–366].

Many screening methods have been proposed and used, ranging from palpation to radiography. More recently, ultrasound, computed tomography, and magnetic resonance imaging are among the new imaging techniques that allow an HD accurate diagnosis[6]. The earliest signs can be observed at 30 days of age, consisting of a femoral head ligament with increased volume and increased synovial fluid (SF)[140,367,368]. The earliest radiographic signs can be observed around the 7th week of life, consisting of femoral head subluxation and underdevelopment of the acetabular rim[369]. During the development of the hip joint, the joint's functional subluxation is accompanied by an increase of forces that cross the joint and a decrease in the area over which the forces are exerted. These events promote cartilage damage, joint inflammation, and, ultimately, secondary OA[6,140]. Early subluxation of the femoral head, followed by shallowing and deformation of the acetabulum, is thought to be the first step in the pathogenesis, followed by varying degrees of arthrosis and

remodeling of the femoral head and acetabulum[361]. Secondary OA is also a complex trait influenced by several genes and environmental factors and is additively inherited, without dominance[140]. Several studies have focused on determining the genetic basis for the disease, without ever reaching a definitive conclusion[114,370,371]. Currently, none of the genetic analysis tests surpass the clinical utility of a phenotype-based selection program like the Norberg-Olson Angle, the Distraction Index,or a subjective Hip-Extended Score [6]. The Norberg-Olson angle is the angle formed by a line connecting the centres of both femoral heads and a second line, drawn between the centre of a femoral head and the acetabulum's craniodorsal rim on the same side. Lower angles have a high correlation with the development of OA[361]. A normal hip joint has been set to have an angle of 105° or more, although different values for different breeds have been reported, as 100.3° for German Shepherd Dogs[372,373].

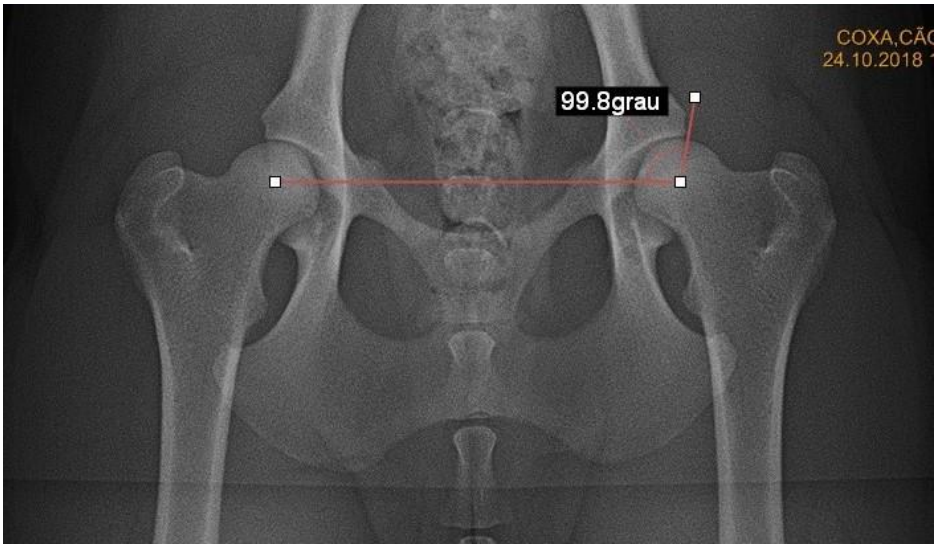

Figure 8 – Measurement of the Nordberg-Olson angle.

The Distraction Index is based on the measurement of the forces during the gait cycle, and this passive joint laxity is used as an estimation of functional hip laxity. This laxity leads to premature wear of the articular cartilage, microfractures to the subchondral bone, and, ultimately, osteophyte formation and OA[374,375].

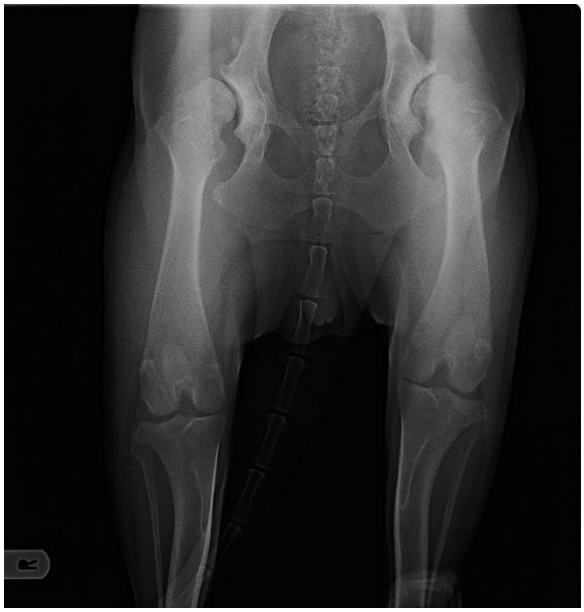

Figure 9 – Ventrodorsal extended leg X-Rays of dogs with severe hip dysplasia.

Significantly increased passive joint laxity and, consequently, HD, have been reported in dogs with significantly higher volumes of SF and thickened femoral head ligament[376,377]. It is not clear if these findings are a cause or a consequence of HD. The balance between production and removal of synovial fluid is maintained by the intracapsular veins and lymphatic vessels, a mechanism that becomes impaired with the inflammatory events of joint trauma and inflammation[367,378].

The amount of pelvic muscle mass was also found to positively correlate with the development of HD, with non-dysplastic dogs (such as Greyhounds) showing greater muscle mass and low prevalence, while dysplastic dogs (like German Shepherd Dogs) have less development muscle masses[379]. Hormones, such as oestrogen and relaxin, influence skeletal development. While oestrogen, within physiological levels, does not seem to induce significant amounts of laxity, relaxin has been associated with peripheral joint laxity. This hormone is produced in high levels and secreted through milk in bitches, and some breeds may have even higher levels, as is the case of Labrador Retrievers[380,381]. The effect of weight and growth rate on HD development has been studied from an early stage. At 60 days of age, heavier dogs, of a group of German Shepherd Dogs, had the highest incidence of HD at maturity[382]. Additional reports showed that heavier Labrador Retrievers develop radiographic OA 6 years earlier than thinner littermates and that heavier dogs required long-term treatment 3 years earlier than slimmer dogs[383]. The rate at which growth occurs can influence final phenotype, and dogs with an earlier acetabular growth plate fusion seemed more prone to have dysplastic joints[377]. For these reasons, an intentionally restricted feeding regimen, leading to a slower growth rate, has been proposed to delay OA's onset in predisposed dogs. Additional nutritional factors include excessively high dietary calcium, associated with delayed endochondral ossification and

skeletal remodeling, and, ultimately, HD[384]. Dietary restriction has been shown to reduce canine OA while also extending longevity, and weight only correlates moderately with disease severity[385].

Despite the attempts, there has been a failure to eradicate canine HD, based on the radiographic selection using the Norberg-Olson angle. One of the reasons for this fact may be that it is only valuable if used in relation to other members of the same breed due to breed-specific laxity profiles. Another reason may be a lack of honesty regarding HD's control and reporting, possibly for financial motivations[386–389].

3. DIAGNOSTICS AND TREATMENT MONITORING

In human and animal health, there is an increasing shift toward patient-centred healthcare[390]. The assessment of pain, is pivotal to measure the response to treatment and to determine the relevance and utility of translation research successfully[249]. Dogs are the gold standard species used as study models for OA , and the results obtained are more accurate in predicting clinical relevance than with other animal species[391]. An outcome assessment instrument can provide information on the effectiveness of interventions and guide clinical practice. Methods used to assess dogs' pain include direct observation of the degree of lameness, gait analysis, and subjective rating scales, with no consensus on which is the best[249,390]. Histopathology has been and will likely continue to be the gold standard for outcome assessment in OA animal models. However, there has been an increasing call for less invasive measures of disease onset and progression and response to treatment[11]. Even though OA is a common canine disease, adequate outcome measures are still lacking. Visual lameness assessment, radiographic appearance, and an evaluation of pain seem to be the most commonly used outcome measures[18]. When making these assessments, it is important to keep in mind that a placebo effect has been described in humans and animals, particularly in OA treatments. In dogs, GRF is not affected in placebo treatment groups. In humans, it is more associated with all subjective outcomes, but not with objective outcomes, such as ROM and muscle girth[392].

3.1 Digital radiography;

Digital radiography has exploded in the veterinary market and has significant advantages, such as centrally storing, searching, and sharing images with ease[344]. Pelvic radiographs are frequently performed in dogs, mainly to assess HD, OA, and fractures. They have been used for over four decades in several screening mechanisms worldwide and clinically and experimentally to determine outcome[393–395]. Imaging plays a vital role alongside the clinical review of patients with

joint disease since it can depict individual joint involvement while avoiding the need for invasive tissue sampling. It can also be done repeatedly and safely within recognized limits, which is important for following chronic conditions[278,396].

The most common radiographic view is the ventrodorsal hip extended view, for which sedation is required in all dogs[6,397,398]. For this projection, the animal is placed in dorsal recumbence, with the assistance of positioning aids. The limbs are internally rotated and abducted, at the point that the stifles are almost touching. The limbs are then extended, maintaining the internal rotation, while the femurs are parallel to the table. If the positioning is correct, the patella should be central over the femoral condyles, the femurs parallel to each other, and the obturator foramen uniform and of equal size. The sacrum, ilium wings, and 7th lumbar vertebra should be included in the image[361,397]. This position results in the joint's tightening, reducing the degree of visible subluxation and, consequentially, its score[125,399]. The ventrodorsal flexed view (also called frog-legged view) is also a valuable option, particularly in unsedated dogs with painful hips. It enhances the visibility of the cranial and caudal aspects of the femoral head and neck, helping in assessing circumferential femoral head osteophyte and caudolateral curvilinear osteophyte[361].

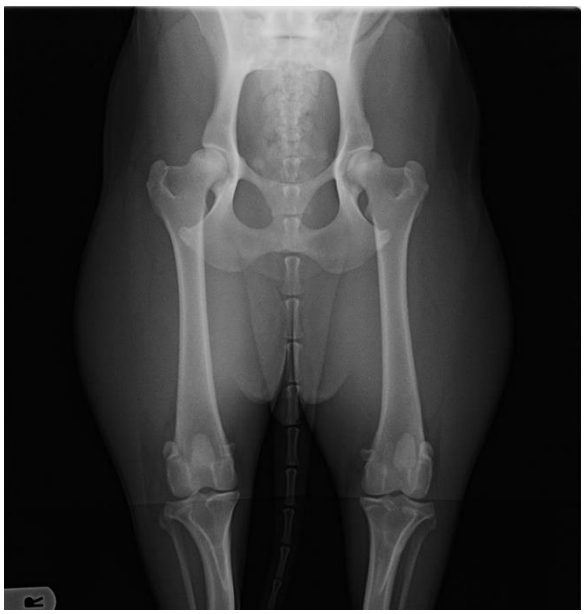

Figure 10 – The ventrodorsal extended legs X-view.

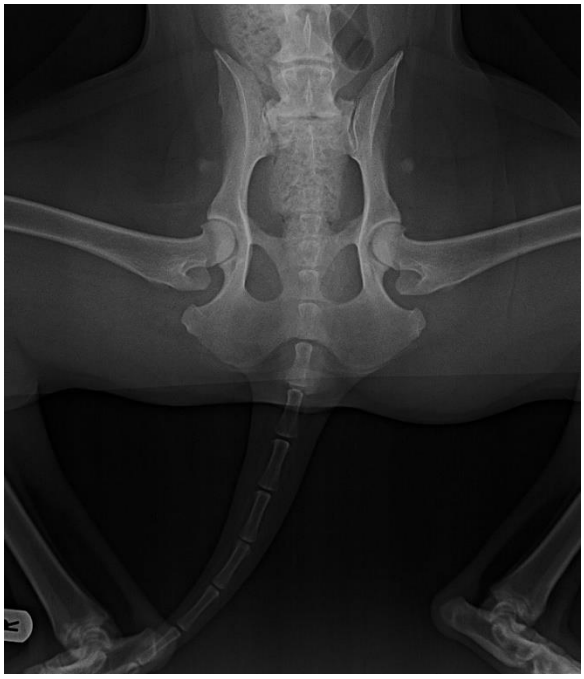

Figure 11 – The ventrodorsal flexed (frog-legged) view.

It is well established that radiographic signs' development occurs later than the structural changes associated with OA[6]. The bone must undergo a 30-40% change in density (for example, osteophytes must form) before OA-related changes can be detected radiographically[140]. Also, several accounts for low inter and intra-observed agreement of hip scoring have been published[398,400–402]. There is a low relationship between radiographic changes and score progression, with a progression or lack of progression of clinical signs, between limb function and radiographic severity signs, or between radiographic score and joint synovial markers[155,393]. Radiographic changes also do not account for the level of pain experienced by the animal, as they are affected by synovitis, osteochondral pathology, and sensitization, which are not detected by digital radiography[318,393,403]. Therefore, plain radiography has limitations concerning the evaluation and staging of OA, not assessing the result of the interaction of biochemical and biomechanical factors[404]. Detectable radiographic changes include femoral periarticular osteophyte formation, subchondral sclerosis of the craniodorsal acetabulum, osteophytes on the cranial/caudal acetabular margin, and joint remodeling from chronic wear. Specifically, curvilinear opacity in the joint capsule's attachment on the femoral head (Morgan's line), remodeling of the cranial and caudal acetabulum, flattening of the femoral head, and irregular widening of the femoral neck[346]. Besides bone changes, soft tissues may also experience transformations observable in the X-ray image[405]. In both cases, signs must be severe before being observed on the X-ray[140]. The features that have been deemed of major clinical importance are circumferential femoral head osteophyte, caudolateral curvilinear osteophyte, and subchondral bone sclerosis, representing early radiographic signs that predict the development of the

clinical signs of hip OA[361,406–408]. Caudolateral curvilinear osteophyte was first described in 1961, and arises at the caudodorsal part of the femoral neck because due to traction on the hip joint capsule. It manifests as a radiopaque line (Figure 12). It correlates with hip subluxation and, therefore, represents a risk factor for OA later in life[398]. Circumferential femoral head osteophyte is a marked radiopaque line, encircling the junction between the femoral neck and the epiphysis, along the insertion of the joint capsule (Figure 12)[409]. Subchondral bone sclerosis of the cranial acetabular edge is an alteration seen as a loss or increased density of the normal trabecular bone pattern. It is considered to occur when excessive or abnormal joint stresses arise because of the subluxation of the femoral head[398].

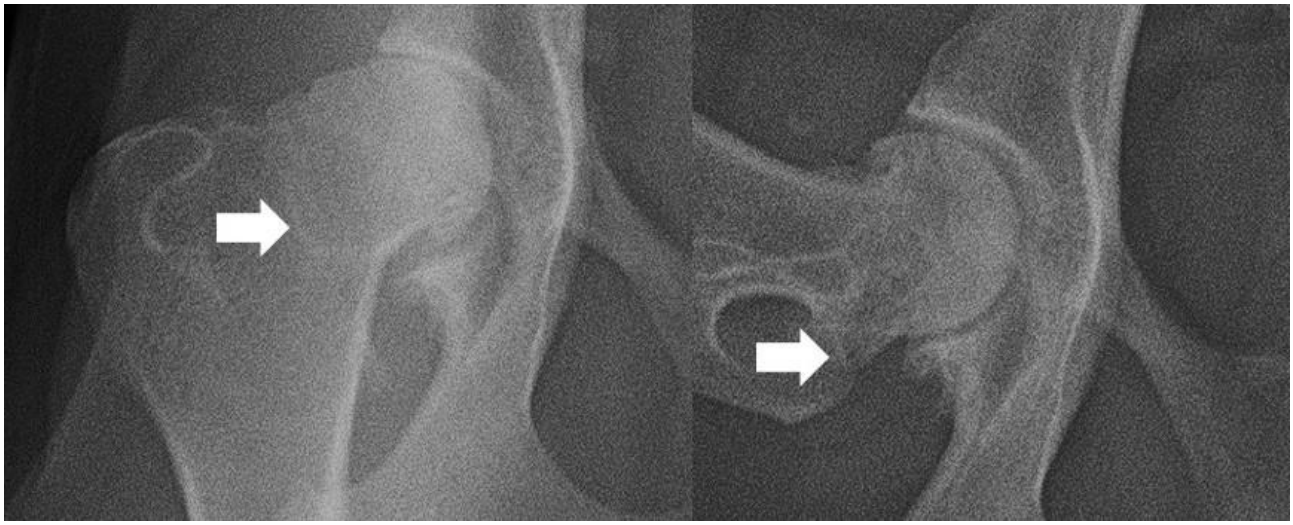

Figure 12 – Ventrodorsal (left) and ventrodorsal flexed (right) views of a hip joint of a dog with osteoarthritis. The arrow identify the caudolateral curvilinear osteophyte.

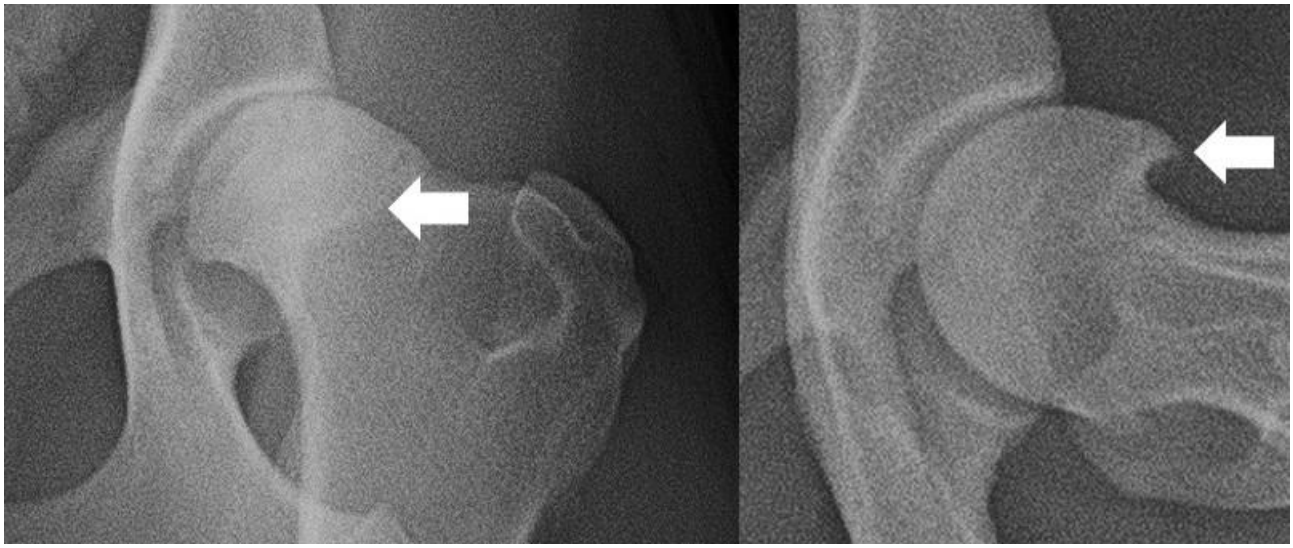

Figure 13 – Ventrodorsal (left) and ventrodorsal flexed (right) views of a hip joint of a dog with osteoarthritis. The arrow identify the circunferantional femoral head osteophyte.

It is beyond this work's scope to review the screening systems for canine HD, but due to their relevance and association between HD and OA, they will be summarily described. In 1961, the Orthopaedic Foundation for Animals assembled ten veterinarians to outline the standards for diagnosing and classifying canine HD[410]. They presented guidelines regarding animals' equipment and positioning, description of normal and dysplastic hips, radiographic and clinical signs[410,411]. The Orthopaedic Foundation for Animals score is based on three radiologists' consensus using a subjective seven-point grading system (excellent, good, fair, borderline, mild dysplasia, moderate dysplasia, and severe dysplasia). Dogs must be at least 24 months, and exam submission is voluntary by dog owners[6].

The Fédération Cynologique Internationale has developed a hip-grading system used throughout Europe, Asia, and parts of South America[412]. Hips are evaluated on the hip-extended position, with the possible addition of a frog-leg position. Animals must be 12 months old or 18 months old in large and giant breeds. Norberg-Olson angles are measured to quantify laxity, and a grade, ranging from A through E, is attributed, with A being the best score. A and B grades subdivide into 1 and 2[6]. With this method, the interobserver agreement has been reported to be low, even with experienced observers[400,401].

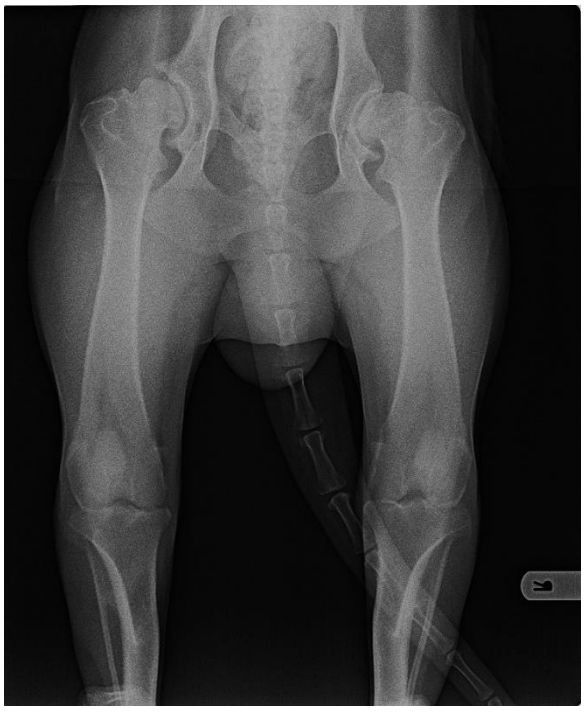

Figure 14 – Grade E hip with severe secondary osteoarthritis.

The British Veterinary Club/Kennel Club program was established in 1978 to control HD and applied for all breeds in 1983[413]. This system is used in the United Kingdom, Australia, and New Zealand and is based on the evaluation of hip-extended radiographs of dogs at least 12 months of age.

Individuals' hip features have then attributed a score that can amount to 53 points/hip (106 in total, with lowest scores being better)[413,414]. The scores estimate laxity, degree of subluxation, Norberg angle, and presence/severity of OA. These three methods have in common the fact that the Norberg angle is the only objective parameter observed[386]. Systems based on phenotype scoring were found to be only fairly reliable within and between experienced observers[398].

The University of Pennsylvania Hip Improvement Program was introduced in 1993 and is the most evidence-based hip screening method available[368,375]. This method requires the dog to be placed under heavy sedation or anaesthesia. Three radiographic images are obtained, a ventrodorsal hip-extended view, a compression view, and a distraction view with legs in neutral position and hips distracted[368]. The measurement of laxity is made by a distraction device, quantifying the femoral head displacement from the acetabulum using a distraction index[6]. The significant relation between distraction index and the development of hip OA has been documented in several studies[362,415]. Despite the screening scheme considered, the reliability of the result is also dependent on the experience and integrity of the scrutineers[361].

Computed tomography and magnetic resonance imaging can also be used for diagnostics and monitoring joint diseases[416]. They are non-invasive techniques, but that requires general anaesthesia. Its use is often a balance between access, availability, cost, and clinical indication[417]. They have the advantage over digital radiography of detecting the articular cartilage's morphology but not early OA compositional changes[418]. On OA, magnetic resonance imaging can be useful for detecting early osteophyte formation, full or partial thickness cartilage loss and subchondral bone lesion[419]. The association between radiography and hip joint measures dfrom computed tomography detected morphology changes as early as 16 weeks of age and improved animals predisposed to hip OA[373].

3.2 Gait analysis;

Gait is the result of the combined activity of the musculoskeletal and nervous systems. Pathological changes in these systems may lead to changes in locomotion[420], which encompasses two primary types, gait (walk, trot, pace, gallop and swim) and nonrepetitive motions (jump, sitting, and others) [340]. Gait analysis is the investigation of locomotion[421]. It can be done through visual analysis, a more traditional approach to lameness investigation, but still an essential part of the physical exam, which depends on the ability and perspicacity of the observer[422]. Instrument gait analysis is an objective method for analyzing gait, employing sophisticated equipment to measure spatial and temporal parameters, in addition to forces acting on the limb, allowing their quantification and interpretation[267,421]. Limb movement and use can be assessed by analyzing GRF (kinetics), limb movement (kinematics), or a combination of both[344].

The assessment of GRF is made through kinetic measurements, the gold standard, done with a force plait. This evaluation requires time and expertise to ensure correct data collection and interpretation[26,314,423,424]. Forces measured are generated each time the limb hits the ground, comprising two opposite forces, the action, and the reaction forces, equal in magnitude and opposite in direction[314]. This analysis can be used as a window into neurodegenerative disorders to identify markers of subclinical pathology, inform diagnostic algorithms of disease progression, and measure the efficacy of interventions[425]. The measurement of GRF is a well-established method to describe gait and severity of lameness, with a 90% sensitivity and specificity[22,426,427]. An increasing interest in this field is evident in assessing response to surgical procedures, treatment outcome, orthopaedic conditions, and differences between breeds[393,428–431]. Significant breed differences have been reported, both at a walk and trot, which may reflect human selection based on confirmation criteria, which would lead to an advantage in the breed's intended work function[432]. Changes observed with force plait analysis during the evaluation of a patient are probably the result of structural deficits and pain sensation, making it the most sensitive sole measure of pain in dogs[391]. Although measuring changes in limb function is not directly correlated to changes due to joint pain, one could expect function in the limb to have comparable changes due to it[433].

Evaluating quadrupeds may be challenging, as they can alter movement or the distribution of weight within the limb or paw to minimize pain, in a way that the abnormality may be unnoticeable[340,434]. Although objective gait analysis has a theoretical advantage over subjective measures, the ability to determine the sensitivity and specificity of any method to detect lameness is hampered by the lack of a criterion-referenced test for comparison[435]. Nonrepetitive motions are not usually assessed during gait analysis. These movements involve a wide ROM and segmental alignments with high force outputs and impacts and can be a source of injury of providing clinical information used to reveal injuries not noted on a routine clinical exam[340].

Kinematics involves the analysis of limb movement. Each limb has a stride characterized by a swing and a stance phase. The stance phase is defined as when the foot is in contact with the ground, whereas the swing phase is the period during which the limb is propelled through the air. The stance phase can further be divided into braking and propulsion, with braking occurring as the limb exits the swing phase and transitions into propulsion before the next swing phase in the gait cycle[421]. For data collection during kinetic gait analysis, a handler leads the animal across a force plate. For a trial to be considered valid, the dog's velocity must be within a defined range since it influences measured forces. Most systems use three photoelectric switches, positioned at a predetermined distance apart, and an accurate method for determining trunk velocity[436–438]. Force plaits can be integrated on a treadmill, making velocity precise and controlled[22,439]. Critics of this evaluation method argue that

gait may be altered and forces values on a moving surface compared with self-propulsion on a normal substrate[421]. As the limb contacts the ground, the reaction force at the ground is measured. For each valid trial, three orthogonal ground reaction forces are measured from ipsilateral thoracic and pelvic limb[440]. GRF have been described as outcome measures reflecting pain-related functional impairments in the context of OA, being abnormally lower[314,441,442]. Similar to what is observed with OA's clinical signs, no correlation has been found between radiographic OA score and vertical GRF[393]. Although the use of force plaits has become a standard method for this type of evaluation and research, it presents some disadvantages. The plates must be located over a leveled surface in a dedicated area and do not record successive footfalls over one passage, requiring multiple passes to collect data for each limb[21]. These requirements may turn force plaits into a tool difficult to use outside a research centre.

Forces vertical to the ground are represented on the Z-axis; mediolateral horizontal forces are depicted on the X-axis, and craniocaudal forces are plotted using the Y-axis. Vertical force is usually the largest of measured forces, with craniocaudal braking and mediolateral forces having smaller intensity[421,440,443]. GRF that act on the canine foot are represented in Figure 15.

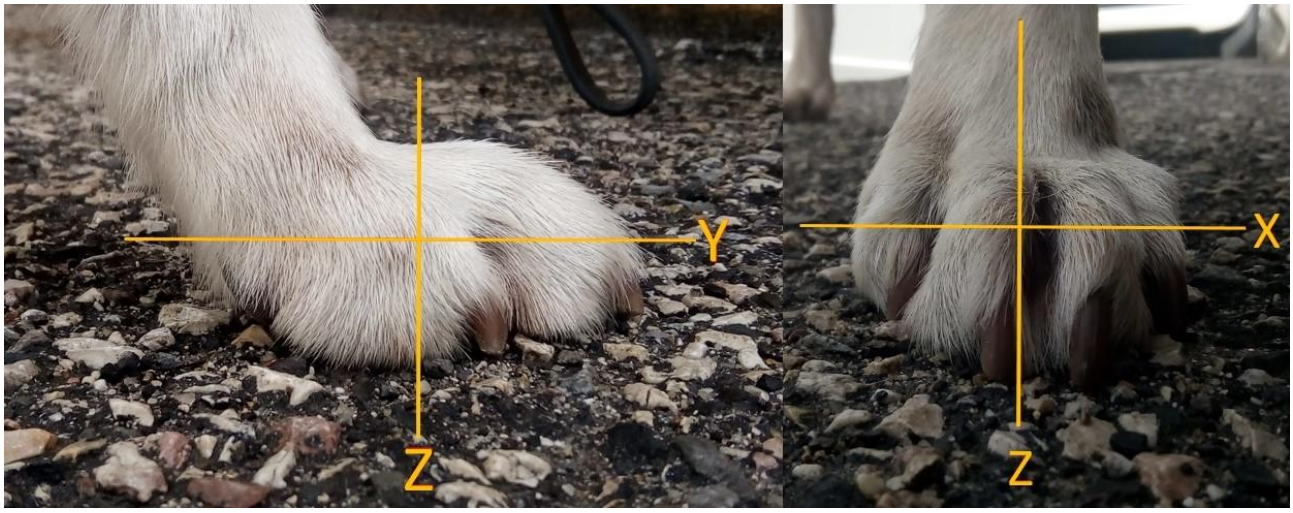

Figure 15 – Ground Reaction Forces that act on the canine foot in three orthogonal planes, X, Y, and Z**.**

Data obtained during kinematic analysis is usually normalized in concern to the animal's body weight so that animals of different sizes and conformations can be compared. Non-normalized Peak Vertical Force (PVF) of small and large dogs significantly correlate with body weight and is also influenced by body size, conformation, and type of gait [420,444]. Collecting data from multiples footfalls and limbs *per* trial improves data accuracy concerning detecting asymmetry or evaluating compensation for lameness or neurological impairment[439]. For this effect, walkways are better tools, as they provide a large area where footfalls may occur[421]. Still, a good correlation between results

obtained in walkways and a single force platform has been found in normal dogs[435]. Loss of weight and body fat and animal in the course of treatment can influence subsequent evaluations, as with greater weight, greater ROM is detected during the stance phase and greater GRF[445,446]. Growing animals, which experience rapid conformation changes, may present significant variability between sessions[447]. Older animals also experience changes, mainly related to increased joint stiffness and consequent decrease ROM, particularly in the forelimbs[448].

PVF is an optimal measure to differentiate sound from lame animals before and after surgical correction[449]. It is defined as the maximum force exerted perpendicular to the surface during the stance phase and is represented at the top of the peak vertical force curve. Along with vertical impulse, these are the two most commonly used parameters to compare lameness between groups or individuals[394,421,435,438]. The majority of canine biomechanical research focus on the walk and trot and since PVF is dependent on velocity. Further investigation is needed, particularly regarding different sports and activities. Velocity has an increased effect on lame dogs, as excessive trial repetition may exacerbate lameness during trial collection[427]. Trial repetition is one factor that causes more significant changes in results, as it is related to the variation of velocity[450]. Also, the influence of different gaits is essential. A walk may be better when dogs have marked lameness, as Cranial Cruciate Ligament rupture, while the trot may be more sensitive with low-grade lameness[451–453].

Additionally, the quantification of rate and location of limbs in space, comprising displacements, angular velocities, and ROM, are made[421]. Research regarding peripheral joints has focussed mainly on the stifle, specifically in a cranial cruciate ligament rupture and repair, both as a primary disease and as a model for human OA. Peak forces are the maximum forces generated in the described phase of the gait, and impulse is the area under the force-time curve[421]. Besides being used as a gold standard for determining the functional outcome, PVF is an indirect measure of pain[393,394,454,455]. When a limb is affected, significant adaptations occur on all limbs, both at stance and swing phases. Naturally, most pronounced differences occur in the affected limb, followed by the contralateral limb, opposing contralateral limb and, to a lesser extent, the opposing ipsilateral limb[456].

Chronic pain has been associated with permanent changes in gait, even though the correlation between the degree of lameness and pain, while logical, is impossible to prove[457]. When used to measure lameness, it involves two- or three-dimensional evaluation of limb and body motion[344]. During lameness, PVF is decreased because dogs tend to bear less weight on a painful limb. Vertical impulse typically decreases as well, since force and time in stance are decreased during lameness, reducing the area under the force-time curve[421]. Variations due to proximal *versus* distal dysfunction and the specific joint affected may occur, leading to an indication to which limb section is involved[456].

Although labor-intensive, gait analysis tends to be more objective than observation alone[458]. Kinetic asymmetry indices have shown a good correlation with visual gait assessment scores[459,460]. GRF determined using a pressure-sensitive walkway may be lower than those determined with a force plait, but they exhibit a similar trend, suggesting that they can be used for comparative studies[461]. Pressure plates may provide a more practical alternative to forces plates, as they can register simultaneous, consecutive, and collateral foot strides in a single passage, which requires fewer trials. It also allows for the evaluation of load distribution among foot pads[459].

The metatarsal pad is the one experience lower forces, compensated by contralateral limb pads[426]. However, the existence of a specific kinematic signature is still debatable since kinematic changes differ between reports[462,463], as animals with hip OA present complex changes in gait involving more joints than just the affected hip alone. Interestingly, GRF of the lame limb in dogs with hip OA can be similar to those of non-affected dogs' limbs, whereas the contralateral limb shows higher values[464]. Varying results have been found in dogs with OA when comparing kinematics and force plait analysis, with no differences in GRF but in joint kinematics[465,466]. When assessing animals after total hip replacement, no difference between the sensitivity of vertical impulse and PVF seems to exist[467]. Other factors may influence GRF, as carrying weight in the mouth, which likely produces additional stress on the forelimb joints, muscles, and connective tissues[23]. The knowledge of the fact is of particular interest in sporting and working dogs, in which a knowledge of specific tasks/missions may give insight to expected specific lesions. The calculation of a symmetry index is also a commonly used measure. Although a small amount of asymmetry is normal in healthy dogs, is asymmetry caused by pathology would is easier to detect when using a symmetry index calculation[468]. Symmetry indices can be used to detect lameness as limb symmetry is expected to change with lameness due to the force distribution pattern[468]. Research focusing on HD found subtle changes in dysplastic dogs compared to sound ones, including coxofemoral extension at the end of the stance phase, increased femorotibial flexion throughout the stance and early swing phase, and increased stride length with decreased PVF[267,469,470]. Significant differences were also observed in maximum angular velocity and maximum angle of hip joint, higher in dysplastic dogs. Even if no signs of lameness were observed in dysplastic dogs when trotting on a treadmill, joint kinematic alterations were observed on both pelvic and thoracic limbs, with more rapid extension of the hip in dysplastic German Shepherd Dog[458]. In comparison, Belgian Malinois with borderline HD had an earlier maximal flexion of the hip, along with less flexion and ROM of the stifle joint during the swing phase than clinically normal dogs[466,471]. Mean PVF is also lower in dogs with severe HD than mild dysplastic dogs, which suggests that HD degrees can affect lameness severity[471,472].

Subtle changes in posture or weight-bearing may occur in the early stages of the disease process, which can be easily missed with visual assessment, and are not improved through slow-motion video[235,447,473]. Weight distribution and off-loading or limb favouring at the stance are commonly used subjective assessments during the orthopaedic examination, yet lameness may be difficult to detect during gait evaluation[438,474]. Additionally, OA animals may not be overtly lame at a walk or a trot but exhibit subtle shifts in body weight distribution at a stance due to pain or instability associated with orthopaedic or neural disease[467,475]. Stance analysis has been reported as sensitive for detecting lameness in dogs, being more sensitive in large breed dogs (Figure 16 and Figure 17)[476]. If a dog presents pelvic limb-lameness, in general, a load redistribution occurs, more by side-to-side compensation rather than pelvic-to-thoracic has been described[465,477]. Normal weight distribution on the weight distribution plate is the same as for pressure-sensitive walkway total pressure index—30/30/20/20 (left thoracic limb/right thoracic limb/ left pelvic limb/right pelvic limb)[414]. Weight distribution platforms, as pressure-sensitive walkways, can provide accurate and consistent measures of weight distribution with no significant difference between devices[478,479]. It has been proposed that bodyweight distribution at a stance may be an equivalent or superior measurement of pain associated with hip OA than both VI and PVF, with the highest sensitivity and specificity being set at a cut-off of 2 (thoracic limb value less than 28 or pelvic limb value less than 18)[475,480]. There may exist a tendency to see fewer improvements in male dogs concerning bodyweight distribution with pelvic limb pain relief, as they naturally tend to carry more weight on the thoracic limbs[467]. A recent report analysing weight distribution within the paws of dogs suffering from hip osteoarthritis concluded that the weight distribution compensatory mechanisms are very complex, relieving weight in the affected limb, counterbalanced through higher loading of the caudal quadrants in all unaffected limbs[481].

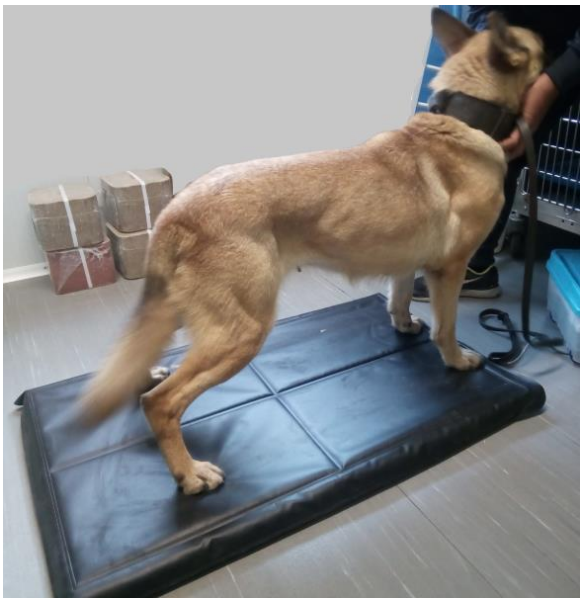

Figure 16 – A dog during stance analysis.

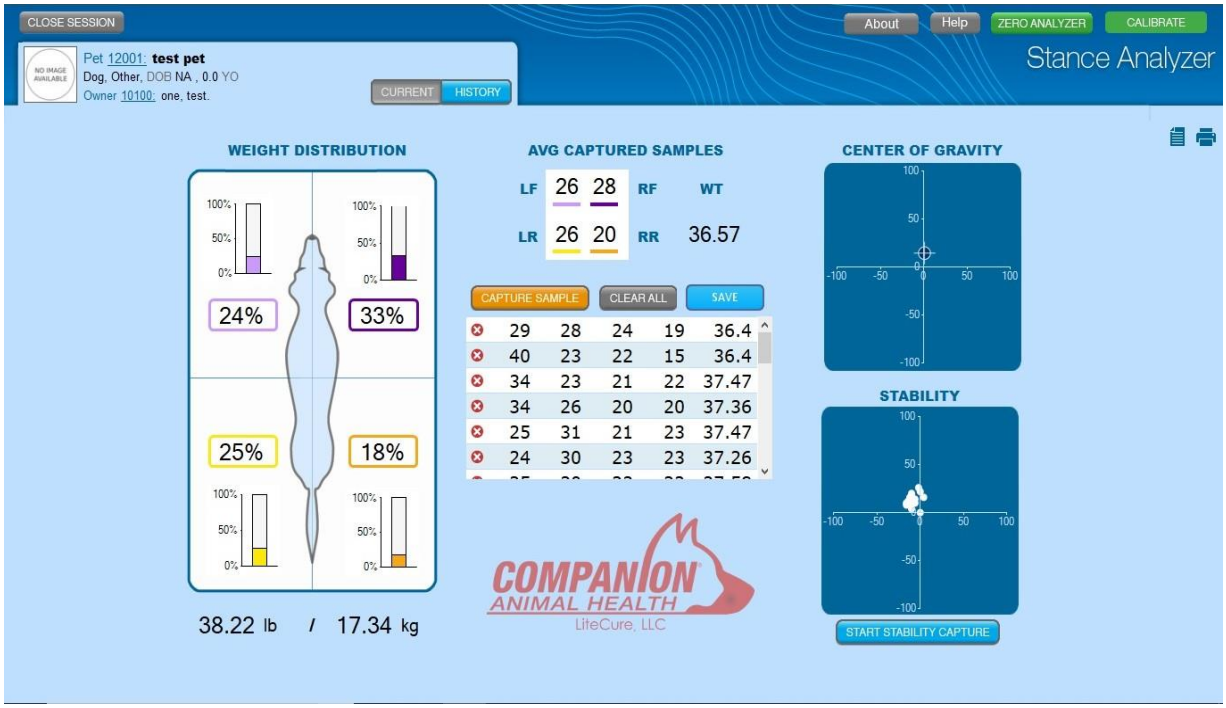

Figure 17 – Stance analysis results.

3.3 Digital Thermography;

Digital thermal imaging is a non-invasive, non-radiating, contact-free, physiologic diagnostic tool that depends on heat resulting from physiological functions related to skin temperature control[20,482,483]. It does not replace but rather augments a thorough physical examination, being an efficient, safe, non-invasive, and (compared with other diagnostic methods) relative low-cost. By correlating changes in temperature patterns with the various diseases, degenerative, or injury processes, digital thermography can provide a reproducible diagnostic tool, particularly in early phases[484–486]. Digital thermography uses non-ionising radiation, thus allowing for an unconstrained and harmless application in patient[487]. Skin temperature is a reflex of a complex system that depends on blood-flow rate, local structures of subcutaneous tissues, and the sympathetic nervous system's activity. This system is so complex that, to date, the mechanism of thermoregulatory adaptation to exercise not fully understood[20]. An injury is often related to variations in blood flow, which can affect skin temperature[488]. Superficial tissue temperature changes can reflect inflammation in subcutaneous and deeper tissues. During the inflammatory process, skin temperature rises due to changes in blood vessels' diameter and blood flow rate and increased capillary permeability[489,490].

Digital thermography provides a visual map of the skin temperature distribution but does not quantifies absolute temperature values. Although modern cameras provide an absolute temperature value, it still has a significant margin of error. Nevertheless, in a given evaluation moment, a

difference of more than 1°C between similar areas or tissues is considered significant. The identification of inflammation, characterized by, among others, an increase in temperature, is a critical step in determining the appropriate treatment[20,491]. With focal plane arrays of 320x240 pixels, high-resolution cameras, a thermal sensitivity less than 50mK, and a spatial resolution of 25-50µm, ensure useful thermal and spatial details[484]. It allows for the acquisition of detailed infrared thermograms, images of the temperature distribution of the target[483]. A 180x180 pixel resolution has been deemed enough to provide reliable results, but a higher resolution (320x240) means smaller changes can be detected[492]. It is crucial to standardize image collecting procedures, to obtain relevant information. The International Standards Organization recommends using the rainbow scale for medical thermograms (in which high temperatures appear in red and low temperatures in blue colours) since the temperature range does not usually exceed 10°C. The temperature scale should be displayed alongside the final image. The merger of a digital image (a traditional picture) and an infrared image is also recommended, as it allows for reliable mapping of anatomical landmarks. Some camera models conduct this procedure automatically. For image collection, room temperature should be controlled, and animals should be given a period to adjust to room temperature (30minutes), and all images should be adequately identified[20,483,492,493]. Horses are an exception to this rule, which may require no adjustment or equilibration time for performing thermographic imaging. This point has been attributed to the fact that the horse is a much bigger and more even heat source than a dog[494].

Digital thermography has been used to assess soft tissue injuries, including muscle strains, sprains, tendinopathies, and also OA, through the identification of tissue changes involved in subtle performance and gait abnormalities. It is also useful to monitor rehabilitation progress or training stressors before an injury occurs. Unlike other medical modalities, thermography is not related to morphology[20,495–497]. It has been described as useful in several species, from humans to horses and cats, but its clinical utility has rarely been studied in small animals[20,491,492,498]. Infrared imaging has also been used to monitor *in vitro* MMPs activity, major actors in OA[499]. Canine thermal imaging has been documented only recently[495]. Still, a growing interest in this modality has led to an increase in the number of studies evaluating thermography use to assess a wide range of pathologies in the canine hip, stifle, elbow, intervertebral disc, and bone neoplasia[492,500–504]. Thermography has also been used to monitor body temperature in exercising dogs and is useful for detecting surface temperature variations of specific body regions[505]. Each thermographic image to assess the hip should include the area from the last lumbar vertebra to the first coccygeal vertebra at a minimum, from a distance of 60 cm to simulate a clinical setting where the space around the patient could be limited (Figure 19 and Figure 20Figure 1)[492]. Fur clipping has not been proven as necessary for the thermographic evaluation of dogs' structures and can be harmful. Still, the coat's type and color are variables that must be taken

into account and its influence documented[496,500,501,505,506]. For example, short-haired dogs exhibit a more drastic increase in body surface temperature compared to other dogs in response to environmental changes[507].

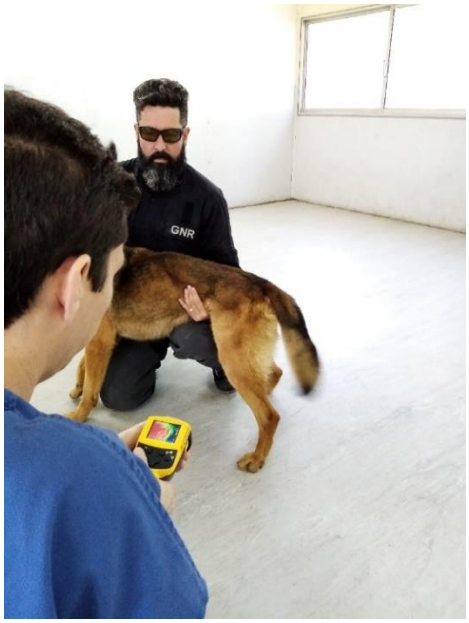

Figure 18 – Digital thermography image collection.

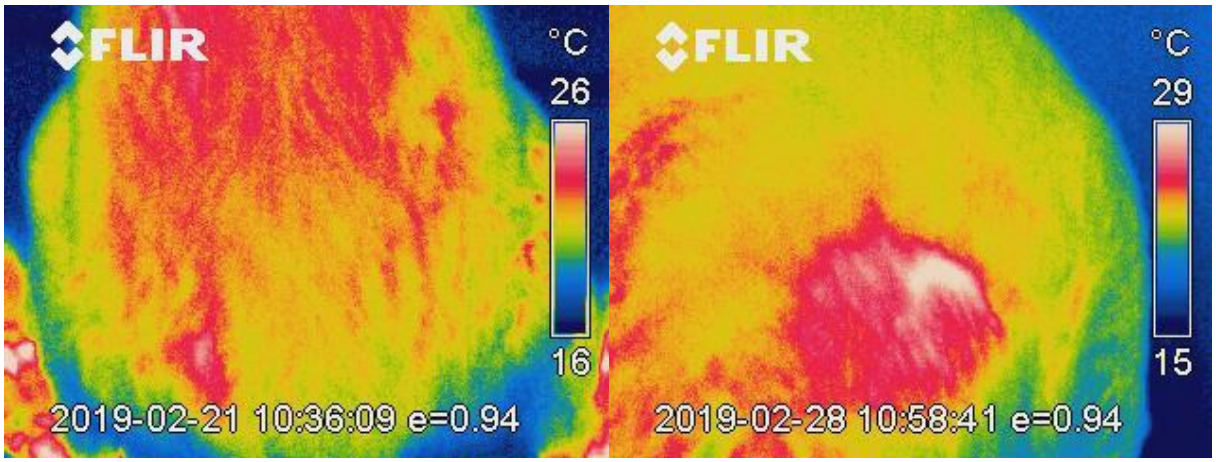

Figure 19 – Digital thermography of the canine hip, on a dorsoventral (left) and lateral (right) views.

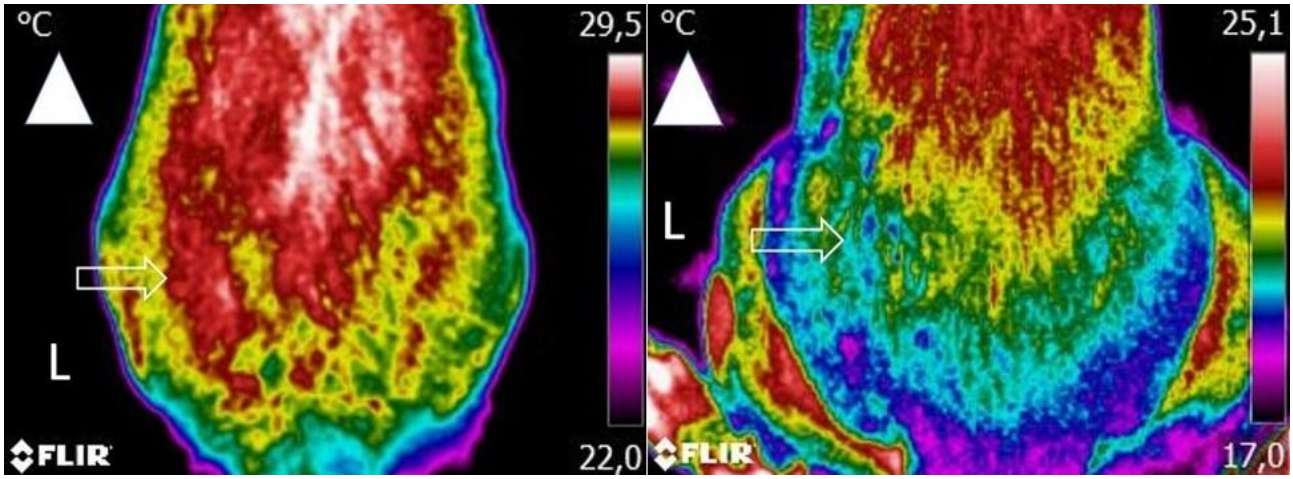

Figure 20 – A dorsoventral view of a dog with moderate osteoarthritis (left) and another with severe osteoarthritis (right), after image editing with the software Tools®. Arrowhead indicates cranial direction. Arrow indicates the anatomical location of the hip joint. An area of increased temperature is observed on the patient with moderate OA and of lower temperature on the patient with severe OA.

In humans OA studies, increased temperatures have been related to even slight degenerative changes and low temperatures in more severe disease cases[490]. In knee OA, a good correlation between an increase in temperature and more severe radiographic changes has been described[508,509]. Thermography has also been shown to be a reliable technique to assess inflammatory arthritis pain and differentiate normal, rheumatoid arthritis, and osteoarthritis subjects from each other[510,511].

3.4 Pedometers;

Mobility is important to health and wellbeing. Mobility impairment and decreased activity are associated with musculoskeletal pain in humans, and improved mobility results have been recommended to measure outcome[253]. Pedometers and accelerometers have been used in humans to measure ambulatory physical activity while also opening up new research opportunities. One of the advantages of activity monitoring includes gathering data over a prolonged period in the patient's home environment[349]. Pedometers, in particular, are inexpensive, simple devices that measure ambulatory activity with acceptable accuracy[512]. Accelerometers have also been used in veterinary medicine as activity monitors[513–516]. They have the advantage of portability, unrestricted movement, and direct measurement of three-dimensional accelerations[517,518], and have been used to assess improvements related to interventions in a canine model of OA[519], serving as objective measures of chronic orthopaedic pain[520]. As inertial measurement units, other systems have been developed for objective lameness evaluations in horses and are based on sensor technology comprising gyroscopes, accelerometers, and magnetometers. Its use has also been described in healthy dogs and with induced lameness[521,522].

Unlike accelerometers, pedometers are not designed to capture the pattern, intensity, or physical activity type. They also do not adequately capture the intensity of specific activities. Nonetheless, several reports have pointed out that simple and inexpensive pedometers agree acceptably with more expensive accelerometers[523]. They are highly adaptable and have been used in several reports with animals, from dogs to cows, horses, and turkeys, to assess multiple interventions and behaviours[524–529]. In a study designed to measure physical activity in dogs, pedometers showed to measure it with reasonable accuracy. The amount of activity that a dog experiences, particularly walking, is highly correlated with the owner's motivations and characteristics[530,531]. Animals with a higher body condition score and less active owners had a lower number of steps per day[523,532]. Activity counts also experience a clinical influence from environmental conditions, as average temperature and total daylight hours[533]. At a walk and a trot, pedometers tended to overestimate the actual number of steps by 17% in large and medium dogs and underestimated by >5% the number of steps of large dogs when running. Using a different mounting technique to position the pedometer, another report registered a smaller difference in medium and large dogs, amounting to 3.3%[532]. While presenting some inaccuracy degree, results are consistently inaccurate, which allows for result correction and to monitor overall activity on a daily basis[523]. In normal dogs, pedometer steps may reasonably estimate distance travelled[534]. Recording data over several days, in opposition to single-day recordings, has demonstrated to help avoid anomalies in other species[535].

3.5 Clinical metrology instruments;

Pain and functional ability are essential parameters in the evaluation of OA treatment efficacy[257]. Measuring and evaluating pain, in particular, is integral to assess the relevance and utility of any specific animal model on translation research, but also for effective pain management and a requirement of evidence-based medicine. Typically, OA pain is localized and related to movement or weight-bearing of the affected joints[249,296]. Historically, measuring pain intensity was the focus in human and veterinary medicine, but, more recently, a focus on the affective dimension of pain was gain emphasis. Comprehensive documentation of this component is paramount for the development of treatments for chronic pain[296,536]. Often in clinical trials and studies, questionnaires are used and may be completed by clients, attending veterinarian, or both[537–539]. They usually include a semi-objective rating of disease parameters such as "lameness" and "pain", on either a discontinuous ordinal scale or a visual analog scale[540]. While extremely useful in a clinical setting, subjective scales are more susceptible to bias, as is the case of the caregiver placebo effect, from both owners and assisting veterinarians, associated with the variability in emotional and cognitive components of pain

perception[249,541]. It is still important to measure the affective component of the pain experience, not just its intensity[296].

Several clinical metrology instruments (CMI) have been developed to measure outcome assessments usually completed by a proxy. The owner is the most common proxy with dogs, since they can identify degrees and changes of their pets' subjective states, and can interpret their signals. For these reasons, dogs are good candidates for the use of CMI to evaluate pain[542,543]. A CMI comprises a sequence of questions or items, scored based on the person's observations or experiences completing it. The individual item-scores are then used to calculate an overall instrument score[544]. They show discriminatory, responsiveness, and criterion validity as pain and impairment measures in performing daily activities. As a whole, CMIs represent a patient-centred approach that, similar to what happens in human medicine, has been incorporated in veterinary assessments for different species[111,545,546]. In human medicine, they are a standard, validated, and accepted method for measuring chronic pain and have formed an essential part of the patient's assessment for over 30 years[349,547]. They also try to represent an alternative to force platforms, as this equipment is often confined to research facilities. Also, changes in PVF only detect a treatment effect on load-bearing through an individual limb and may not detect a change in demeanor or activity in the animal's everyday environment and activities [544,548]. Whereas it is certainly a positive result to have an increased ROM in a joint after an intervention, if the animals show no improvement in its ability to perform daily activities, the owner may not perceive a clinically significant benefit from the procedure[390]. Owners may often be more focused on the dogs as a whole and its ability to perform daily activities, rather than an increase or decrease use of a single limb at a walk or trot[549]. Working dogs, in particular, will often only display their lameness when involved in activities[550]. To conduct clinical research outside these centers, the need for standardized clinical outcome measures that are cost-effective and widely available has led to the development of several CMI. The best ones developed for dogs and that have been reported to have criterion validity are the Canine Brief Pain Inventory (CBPI) and the Liverpool Osteoarthritis in Dogs (LOAD)[253,349,540,544].

The Canine Brief Pain Inventory survey is often the analysis of choice in the veterinary literature and has been recommended to compare the overall mean or median differences in pain scores between groups [551,552]. It is based on the human-equivalent brief pain inventory and reported as able to differentiate sound from OA dogs, to detect a measurable effect for individual animal assessment, with results correlating with PVF[249,544,549,551–554]. It has been used alongside objective lameness measurements, and results showed to correlate, making the CBPI a reliable evaluation tool, used in several reports[235,445,555]. The CBPI has been demonstrated as not being associated with response bias and may be used as a clinical outcome measure of chronic pain in dogs with OA treated

with pharmacological and non-pharmacological pain interventions[556]. It was also shown to be able to evaluate pain related to other conditions, as bone cancer[557]. For its data to be appropriately powered, a minimum of 29 subjects per group is recommended[549,552]. It is divided into two sections, a pain severity score (PSS) that assesses the magnitude of an animal's pain and a pain interference score (PIS) that assesses the degree to which pain affects daily activities[445]. Individual treatment success in OA dogs has been set as a decrease in PSS≥1 and PIS≥2[548,549]. These two components have high correlation coefficients, and it is reasonable to assume that "pain", as captured by this CMI, might correlate with lameness[544]. PIS was suggested as being more sensitive than PSS in assessing treatment results, maybe because the interference questions are more specific[548]. Bodyweight has a significant impact on PIS, with larger dogs showing smaller improvements. This effect may be possibly due to the fact that smaller dogs may respond more with an improvement in pain severity rather than an improvement in function[552].

LOAD was initially developed to assess dogs with elbow OA. It has shown good reliability, just lower than PVF generated by a force platform, although both results correlate[540,544]. Later, its broader use has been tested, is deemed reliable to assess canine OA in general[544]. With LOAD, a placebo effect has been detected[540]. The CBPI and LOAD results have a moderate correlation, likely due to different components of OA that are capture by each CMI, based on a reasonable approximation of what constitutes canine OA in the clinical sense[544]. A good agreement between CBPI and LOAD baseline scores exists, however[558].

The Canine Orthopaedic Index (COI) is a more recent CMI, developed to assess 4 domains in dogs with OA, stiffness, gait, function, and quality of life. It is a 4 factor, 16 item questionnaire designed to measure owner assessment of those four domains, shown to have excellent reliability and validity, and of being able to differentiate between OA and healthy subjects[559,560]. COI contains four questions that sum up to deliver a stiffness score, five questions that deliver a gait score, four questions that add up to a function score, and three questions that compose a quality of life score[561]. It can detect changes in all of them when an NSAID is compared to a placebo[561] and has been used to assess police working dogs[562].

Visual Analogue Scales are used to score pain and assess its severity, allowing a comparison of analgesic regimens. They rely on a continuous scale, meaning that data can be modeled as a continuous variable[455]. All pain scores that include modifications to a VAS are subjective and, therefore, may lack validity when performed by individuals unfamiliar with signs of pain, and not all show concordance when compared with quantitative force platform gait analysis[433,563,564]. They seem to be more accurate, with force plate gait analysis as a reference, when lameness is severe[455]. It has also been described as of particular interest to assess the need for rescue analgesia rather than a

primary evaluation[303]. The Hudson Visual Analogue Scale (HVAS) was deemed repeatable and valid to assess the degree of mild to moderate lameness in dogs, compared with force plate analysis as a criterion-referenced standard[565].

When administering these questionnaires, it is still unknown if respondents should be permitted to see previous answers (dependent and independent interviewing, respectively). However, little difference has been observed between independent and dependent interviewing, but dependent interviewing resulted in increased treatment effect sizes. Therefore, by using dependent interviewing, increased clinical trial power may be observed[558].

3.6 Ultrasound;

Ultrasound is a safe, non-ionizing radiation diagnostic method and is a mainstay of musculoskeletal imaging in horses and humans[344]. It is an accepted standard, while relatively versatile, convenient, and cost-effective method, complementary to radiography for examining musculoskeletal structures[396]. When radiography does not elucidate the diagnosis, ultrasonography should be considered before more expensive imaging modalities, such as computed tomography and magnetic resonance imaging[566]. It also enables dynamic assessment of joints, ligaments, and tendons, with structures typically scanned in longitudinal and transverse planes to assess fiber alignment in muscles or tendons[567]. Most commonly, linear, high-frequency probes (9-14MHz) are used, giving better resolution but decreased tissue penetration. As a general principle, all examinations should be conducted with a higher frequency that allows sufficient penetration and best resolution[344].

Many musculoskeletal ultrasound studies can be completed without anaesthesia or sedation, although a still, relaxed patient facilitates a detailed and thorough examination, including scanning structures during passive movement. In working and sporting dogs, clipping hair is often frowned upon, limiting ultrasound use. Comparison scanning of contralateral structures may be necessary when a suitable anatomic reference is lacking[566]. The lateral and medial portions of the coxofemoral joint can be assessed with the dog in lateral recumbence and dorsal recumbence in a frog position, respectively. Regular joint capsule thickening with preserved hyperechoic layering is a non-specific US sign found in chronic OA. The inner surface of the synovium may show irregular thickening representing hyperplasia. Osteophytes are irregular convex hyperechoic formations at joint margins. With chronic joint disorders, the defective cartilage layer shows an irregular outline and increased echogenicity[496,568]. Joint effusion is also a common radiographic change seen in early OA, but there is usually little effusion in the hip joints, making radiographic detection difficult. Ultrasound may be a more sensitive approach for this assessment, and, in the canine stifle, the presence of osteophytes has demonstrated a good correlation with joint effusion[113,569,570]. It has been studied as an early

indicator of joint laxity in young puppies and is a common screening tool in human medicine. In veterinary medicine, it is useful for depicting the joint anatomy of puppies younger than 8 weeks. Although there is a significant association between the US measured hip distraction indices in puppies 6-8weeks old and later radiographic findings, it has not been proven useful as an early HD screening tool[361,571].

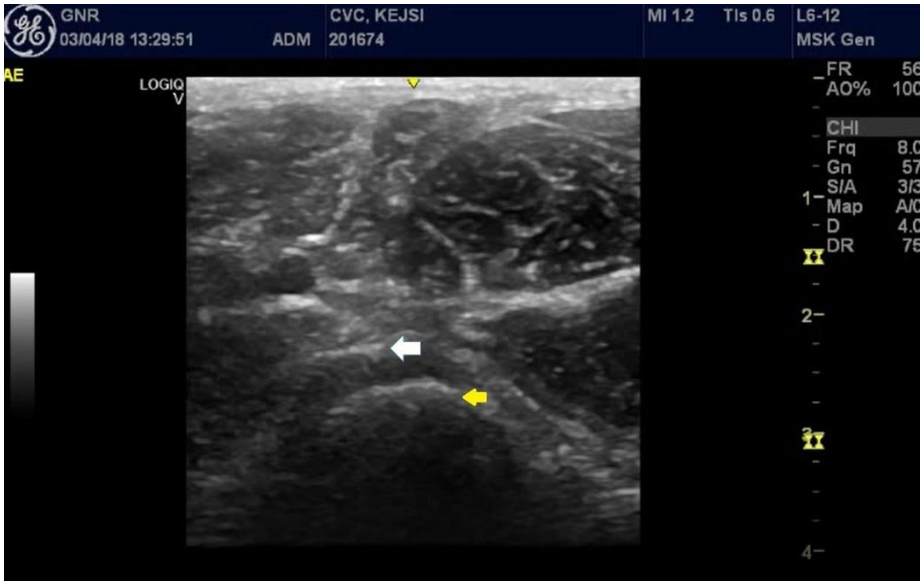

Figure 21 – Medial hip ultrasound, showing the femoral head (yellow arrow) and joint capsule (white arrow).

3.7 Arthrocentesis;

Analysis of SF permits classifying the sample as normal, traumatic, degenerative, or inflammatory (septic or immune-mediated) in nature, but has been characterized as of limited value in OA[12,45,313]. For other conditions, total and differential cell estimates can be extremely informative[572]. SF is known for its sensitive and rapid response to joint insult and injury, and changes are primarily joint specific[2]. Still, SF analysis is an underused diagnostic test in clinical practice. Joint fluid samples should be assessed for colour, turbidity, viscosity, and volume and submitted to a laboratory for cytology[346]. Arthrocentesis of normal joints usually yields only small volumes of SF (0.5ml or less), and it usually is colourless or light yellow, clear. It should form a strand of at least 2.5cm between fingers before breaking (Figure 22). It forms a gel when placed on a tube or syringe, reversible when the recipient is agitated[45]. Typical total and differential cell counts for canine SF in normal joints, and different joint diseases can be observed in Table 5.

Condition	Total cell count	Percentage of mononuclear cells	Percentage of neutrophils
Normal	$<2 \times 10^9$ /L	94-100	0-6
Osteoarthritis	$2\text{-}5 \times 10^9$ /L	88-100	0-12
Rheumatoid arthritis	$8\text{-}38 \times 10^9$ /L	20-80	20-80
Infective arthritis	$40\text{-}267 \times 10^9$ /L	1-10	90-100

Table 5 - Total and differential cell counts for canine synovial fluid in normal joints and different joint diseases (adapted from Innes, 2012)**.**

Figure 22 – Evaluation of a synovial fluid strand.

In OA, early changes occur in the SF, namely increased fluid volume, cell counts (predominantly monocytes, usually low or within normal limits), and the colour remains clear to pale yellow. In the early stages of the disease, viscosity is not reduced, as the hyaluronic acid concentration diminishes over time[573]. In a study characterizing inflammatory cytokines in dogs with OA and rheumatoid arthritis, SF analysis of dogs with either disease showed that IL-1 and TNF-α bioactivity was not readily detectable at increased levels, but IL-6 was increased in both diseases[189].

The most common complication of arthrocentesis is the failure to obtain an SF sample. With experience, the clinician's ability to successfully perform the technique successfully improves, but, on some occasions, such as markedly arthritic joints, a negative tap may be obtained. Joint sepsis, although perceived as a significant risk, is extremely rare[45]. Other authors, however, have reported difficulties in obtaining SF samples consistently[12]. The sampling method may be important and influence measured marker activities. Samples obtained by serial dilution of SF, as opposed to those collected by aseptic percutaneous arthrocentesis, may exhibit a concentration dilution[155].

4. TREATMENT

OA is a common, chronic and clinical incurable condition that, despite extensive research, still has limited treatment options available[13,111,335,574]. Its management is mostly palliative, focusing on alleviating symptoms, mainly pain, and slowing down the progression of the disease[246,575]. It is a highly heterogenic disease characterized by varying degrees of clinical and functional impairments, with functional status correlating with the severity of pain rather than radiographic grading. Therefore, treatment should be planned according to the patient's clinical features and functional status instead of radiological findings[286,576,577]. Management of OA is a lifelong commitment, and the standard approach often involves a multimodal perspective, from activity control, rehabilitation, weight loss, nutritional support, NSAIDs, and nutraceuticals[578–581]. There is evidence that, when targeting inflammatory mediators for OA treatment, more than one needs to be addressed[582–585]. This multi-targeted anti-inflammatory approach also has the potential to prevent cartilage loss associated with OA. An ideal treatment would be focused on blocking the catabolic activity of cartilage while enhancing the regeneration of normal cartilage[586]. Early intervention has the most significant potential for providing the most effective OA management since it provides the opportunity to start long-term care and disrupt the progressive vicious cycle of deterioration. However, over 50% of OA cases are diagnosed in dogs over 8 years[339]. The treatment goals are to improve joint health, overall function, and quality of life of affected patients. This implies relieving pain and associated muscle spasms, maintaining and regaining joint ROM, strengthen support muscles, address proprioceptive deficits, and advise on lifestyle modifications[338]. For sporting and working patients, an optimal treatment should aim to restore them to their pre-injury status in a safe, cost-effective way and as quickly as possible[587]. It is essential to realize that, in contrast to humans, dogs support themselves more with muscular effort and less with bony segments, making it essential to address all involved tissues of the joint organ during treatment[588].

Many interventions are directed at the symptoms of the disease rather than the disease process itself. This approach is not misguided since pain is usually the dominant symptom, and its control represents a fundamental goal in the management of the disease [13]. In humans, relieving pain is, by itself, sufficient to produce an improvement in gait function but not in joint loading[589]. Still, a growing interest in understanding the mechanisms that govern cartilage turnover has emerged, intending to develop treatments that preserve or restore this tissue by promoting its synthesis, inhibiting its breakdown, or both[590]. Given the limited capacity of adult articular cartilage for functional repair, cartilage preservation based on maintaining a balance between anabolism and degradation is paramount to joint function[272] and a target of many treatment modalities.

Pharmacological and nutraceutical agents used in OA treatment are usually divided between two categories, those aimed at modifying clinical signs and those aimed at modifying joint structure[579,591]. Disease-modifying agents are defined as agents capable of delaying, stabilizing, or even repairing OA lesions[592]. Symptom-modifying agents can be described as agents that do not affect the disease's progression but help to alleviate clinical signs, with pain being the most targeted one [579]. Frequently, the first-line of treatment is mild analgesia. The next escalation of treatment consists of the lowest dose of non-steroids anti-inflammatory drugs (NSAIDs) for the shortest period, and weak opioids can also be considered. In refractory cases, full dose NSAIDs, along with intra-articular glucocorticoids or hyaluronan, may be used. Chondroitin sulfate and Glucosamine may provide symptomatic benefit but, if no response is apparent in 6 months, they should be discontinued[575]. There is a real need for effective, safe, disease-modifying therapies that treat established disease and prevent or delay progression[593]. The development of such treatments is constrained by the slow progression of the disease, heterogeneous clinical manifestations, and the need for long-term follow-up to detect structural changes[16]. Also, since joint health and pain status are affected by a wide range of factors, it is difficult to provide a specific recommendation applicable in all situations[594]. Due to the diversity of treatments, it is also difficult for practitioners to choose the most appropriate for their patients. Systematic reviews of the efficacy of OA treatments in dogs highlight the poor quality of study design and reporting, limiting their authors' ability to make strong recommendations[18].

Studies in humans have shown wide discrepancies in results can be observed between reports for the same treatment. These variations may be due to an enormous placebo effect, and the fact that prospective, randomized, double-blind, placebo-controlled studies to treat analysis are rare[13]. Most of the available data rely on symptomatic outcome measures, as the ability to identify disease-modifying outcomes has often been challenging[250]. An alternative approach would be the use of serum or SF biomarkers [250,595]. A placebo effect has also been reported in dogs, of higher degree on subjective evaluation tools, usually not accompanied by the same result in objective measurements[235]. It has been assigned to the caregiver placebo effect, reported to occur both in owners and assessing veterinarians. In some instances, it was described to be as high as 41%[541,596,597]. It may be attributed to the wish of the person making the assessment for the dog to get better, and may therefore perceive an improvement that is not real. Another component of the placebo effect, particularly in OA cases, is attributed to the fact that some animals do improve during the evaluation despite not receiving any treatment. This improvement is attributed to the disease's natural evolution and a regression to the mean effect[539,552,561,598]. An additional consideration to keep is the degree of initial impairment, as it has a significant effect on several outcome measurements, with greater initial impairment being associated with more extensive positive changes in activity[599].

Veterinary randomized controlled trials in dogs with OA and chronic pain might reliably predict treatment efficacy in humans due to the rigor of the functional outcome measures used[552,600,601]. These trials are free from the ethical objections associated with the use of experimental dog models, are aligned with the three R's agenda, and are less expensive than using experimental dog models[602]. Considering veterinary randomized controlled trials as an alternative intermediary for the development of new therapies is reinforced by the similar clinical benefits achieved when novel treatments are trialed in dogs and humans, suggesting that good clinical responses in dogs with OA undergoing treatment might also be seen in humans[111].

4.1 Intra-articular modalities;

Since OA is symptomatic only in the affected joint while lacking obvious extra-articular manifestations, it is well suited to have a local therapy administered by intra-articular (IA) injection, reducing the total amount of drug required to produce an effect and possible side effects[13–15]. IA therapies several advantages over systemic medications. These can be physiological and practical, and include safety, especially when certain comorbidities are present, and bioavailability[603]. It can also be of interest in patients that present other co-morbidities, which have an increased risk of drug interaction and adverse effects[604]. Despite all this, intra-articular injections are less routinely used in the management of canine OA compared to their use in horses and humans. Intra-articular treatments are applied in the intended place of action, providing a higher concentration of the medication in the joint space, minimizing systemic exposure, thus avoiding several systemic side effects. It also addresses the difficulty that systemically administered drugs have to reach the articular cartilage, due to its avascular nature, raising the need for high doses[15–17]. However, many drugs are rapidly cleared from the synovial space, phenomena further accelerated by synovitis, raising the need for more stable presentations, such as depot formulations[14,15]. Novel IA approaches are being developed, include the creation of biomaterials encapsulating corticosteroids, designed to sustain their release locally for prolonged periods of time[605]. This directed route of treatment requires a precise diagnosis and correct identification of the joint to be treated. There are cases in which joint pain is attributed to peri-articular soft tissues and adjacent trabecular bone, that are inconsistently reached by drugs administered IA[606]. As an example, levels of pain in OA are affected by synovitis, but also osteochondral pathology and sensitization[403].

Frequently and true for many of the currently used IA treatments, it is uncertain if the treatment is having its effect noted merely by relieving the symptomatic pain associated with joint disease or through a positive effect on the joint environment[250,607]. The IA injection of any solution,

even saline, can produce a positive short-term effect because, since it favourably alters the abnormal joint environment, by diluting cytokines and cartilage degrading enzymes[608]. There are accounts of improvements in the symptoms of human knee OA for up to three months following saline injections[609–611]. A recent review of published effects of placebo saline injections shows that functional improvements last even longer than those reported for pain perception, up to a 6-month follow-up[612].

Intra-articular administrations of an anaesthetic, US-guided or not, may also be used as a diagnostic tool[613]. Using an imaging guide is not necessary for infiltrative procedures, but it can add extra value and provide safer and accurate treatments[614,615]. When done, sterile gel and probe covers should be used[616]. Failure to inject the medication in the intra-articular space is one of the possible causes of treatment failure[617]. Interestingly, a recent study concluded that accurate IA injections of corticosteroids do not produce a superior outcome in terms of pain when compared to inaccurate injection in symptomatic knee OA[615]. For intra-articular administration to the canine hip (Figure 23), animals are placed in lateral recumbency, with the side of the affected joint uppermost. The puncture site is clipped and aseptically prepared. With the limb parallel to the table surface and in a neutral position, a 22-gauge, 55 to 75mm spinal needle is then inserted closely dorsal to the greater trochanter and perpendicular to the long axis of the limb[618]. In a human study, no detrimental effect has been observed with variations of total injection volumes when treating OA of the hip, with practitioners using from 3 to 9 ml[619].

Figure 23 – Access to the hip joint, confirmed through the collection of synovial fluid.

Side effects of IA administrations are rare but include infection, post-injection-flare, crystal-induced synovitis, cutaneous atrophy, and steroid arthropathy[14,620]. In humans, the risk of joint

infections when a stringent aseptic technique is used, ranges from 1/14000, 1/50000 to 1/80000 injections[621,622]. In a retrospective study, previous use of IA corticosteroids increases the risk of postoperative sepsis following elective arthroscopy[623], although no reference to repeated IA injections was made. Other factors may compromise IA treatment efficacy, as is the case of the acute synovitis present in OA, resulting in distension of the joint and increased synovial permeability. Inflammation increases synovial blood flow, and capillary permeability[217], which these facts combined may lead to the fast removal of the applied medication from the joint space. Some authors, therefore, recommend preceding the administration of medication with a fluid aspiration, or even a joint lavage, to decrease *in situ* inflammation and limit the dilution effect of the eventual present effusion[624–626]. Joint lavage, by itself, contributes to the amelioration of the symptoms, as it washes out loose cartilage, inflammatory mediators, and collagen debris, which cause synovitis and effusion[627].

a. Corticosteroids (CS);

IA CS has been used for several decades in horses and humans to successfully palliate pain and control inflammation associated with OA and surrounding tissues[595,628]. Amongst the medications available for the treatment of OA, these are the ones with the most potent anti-inflammatory activity and have been the mainstay in the treatment of joint disease[606]. It is still the most commonly prescribed IA therapy in the horse, despite intense debate either concerning their beneficial or and potentially deleterious effects[250]. Proponents argue that such therapy is needed to decrease inflammation and musculoskeletal pain, while opponents feel that it only masks pains and leads to joint deterioration[629].

CS act through cytoplasmic receptors, thus reducing capillary dilation and margination[629]. They reduce the number of inflammatory cells such as lymphocytes, macrophages, and mast cells, and also slow down the synthesis of inflammatory mediators such as IL-1β, TNF-α, and COX-2 in the SF[227,606,630,631]. In humans, CS was also described as being able to reduce macrophage infiltration into the synovial lining, but not of some MMPs and tissue inhibitors of matrix metalloproteinase in the synovial membrane[632]. The pain relief they provide is attributed to the inhibition of prostaglandin synthesis, specifically by inhibiting phospholipase A2 and COX-2 expression[606]. With time, pharmaceutical companies selectively modified the basic glucocorticoid molecule to develop analogues with more anti-inflammatory and less mineralocorticoid activity[629]. These molecules are effective for the management of chronic pain, preventing the progression of cartilage degeneration in the early stages of OA, particularly in patients who did not have success with NSAIDs or cannot take them due to side effects[633].

Many OA animal studies have demonstrated a decrease in OA progression or a protective role of CS injections, based on histological and biochemical findings[268,634–638]. Not only the incidence and severity of lesions are decreased following IA CS, but also lesion size is reduced by confinement of cartilage erosion, reduction of fissure formation and cell clones, and preservation of matrix PG[638]. A double-blind, placebo-controlled, *in vivo* study, has shown that IA CS do not influence the expression of some essential mediators of cartilage destruction in OA[632]. A chondroprotective effect has been described in canine models, alongside some undesired effects, such as chondrocyte apoptosis and decreases proliferation[639,640]. Several animal model studies have demonstrated a cartilage-sparing effect of low-dose CS without marked effects on chondrocyte health while reducing the severity of cartilage lesions and the size of osteophytes[638,641–643]. An additional limitation is that their effects have been described as of limited duration, due to a relatively fast clearance from the synovial space[605]. Early evaluations of their use in equine joints do not point out any direct toxic effects of methylprednisolone on articular tissues, even at high doses, but decreased staining for GAG content in the histological examination was observed[644,645]. Additional reports described no deleterious side-effects to the articular cartilage, based on histology and histochemistry, and exercise also did not have any harmful effect in the presence of IA CS administration[646]. Reports presenting deleterious effects, mainly of methylprednisolone, usually point out the low quantity and high viscosity of SF, often are based on multiple injections, while a single dose does not seem to cause long-term detrimental effects[647,648].

The debate regarding the use of IA CS is a long-standing one. There is a concern that over-use of a diseased but pain-free joint would result in accelerated cartilage degeneration, an impression compounded by some report of harmful effects of CS on chondrocyte metabolism[606]. Changes in articular cartilage and collagen metabolism have been described, but with one caveat, the results were based on the effect on normal joints[595]. Interestingly, several studies in humans that reported adverse effects of corticoids (decreased ECM synthesis or degradation after treatment) were based on formulations of which the vehicle excipients were shown to be toxic[605]. Some CS, by themselves, may have a deleterious effect, even when combined with hyaluronan, as is the case of dexamethasone and prednisolone[633]. High dosages of CS appear to have the most significant negative effects on GAG synthesis but, in the presence of inflammatory cytokines, they may limit cartilage degradation, which supports the potential for observing differing effects of CS in normal and disease cartilage[635,649]. Some authors measure cartilage degradation through the determination of GAG in SF. This procedure has been debated, as SF GAG content may be due to increased GAG release from cartilage degradation but also to increase synthesis[635,650,651].

On the other hand, a long-term prospective study in humans has described no significant deleterious effects on follow-up radiographic evaluation following repeated IA triamcinolone acetonide (40mg) injections over a 2-year period[652]. Different animal studies have also presented interesting data regarding the effects of CS. In rabbits, structural and biochemical deleterious effects have been described, although associated with frequent administration and high doses. In dogs, significant beneficial effects have been described, both with methylprednisolone and triamcinolone hexacetonide. In horses, methylprednisolone shows detrimental effects, while triamcinolone acetonide was classified as having positive effects in a highly rated study[653].

There are some recommendations available for the use of IA CS in human medicine, which are thought to apply to canine medicine as well, providing varying strength of recommendation for the use of intra-articular CS, from weak to strong recommendation[654–658]. On the other hand, other guidelines state an inability to recommend for or against the use of intra-articular corticosteroids, in this case, specifically for patients with symptomatic knee OA[659]. The dose to be used in a specific joint depends on joint volume, the severity of inflammation, and the number of other joints requiring treatment[606]. In general, a period of at least 6 to 12 weeks should be respected between administrations, without exceeding 2 to 4 injections of the same joint within a year[4,660,661]. The recommendations for hip and knee OA stated that IA CS can be used and should be especially considered in patients with moderate to severe pain, non-responding to oral analgesic/NSAIDs, with triamcinolone hexacetonide proving pain relief and improved mobility for prolonged periods[580,654,655]. Variations in the duration of treatment may be due to disease, treatment, and patient-related factors. The characterization of such factors is poor, making it difficult to select patients who are the most likely to benefit from this approach[662,663]. Still, CS efficacy may be greater in early disease stages and when there is more significant inflammation, for example, in the presence of hip synovitis[663,664]. The individual preference of the clinician often determines the choice of CS[653,665]. Among the most used are methylprednisolone, triamcinolone acetonide, and triamcinolone hexacetonide[614]. A survey of the American College of Rheumatologists to its members found that 34.6% preferred methylprednisolone, 31.2% preferred triamcinolone hexacetonide, and 21.7% preferred TA[666]. A systematic review recommended that practitioners refine and individually tailor their selection of agent and dosing regimen to individual patient's needs and clinical response[660]. Some human reports have compared different CS.

A systematic review has deemed triamcinolone more effective than betamethasone and methylprednisolone[667]. In humans, methylprednisolone has shown similar clinical improvements, and for as long as hyaluronan, both administrated IA to patients with knee OA[664]. It also showed the same

effect as triamcinolone hexacetonide in another report, with improvements in pain and function being sustained for up to 24 weeks, for even longer, up to 8 months[617,668]. When compared, triamcinolone hexacetonide and methylprednisolone had similar functional effects at eight weeks, with triamcinolone hexacetonide being more effective at week 3[669]. A different report comparing methylprednisolone (40 mg/1ml) and triamcinolone hexacetonide (40 mg/2ml) in the management of knee OA, concluded that both have similar efficacy in relieving pain and improving function, with efficacy peaking at two weeks after injection and lasting until the 24th week post-treatment[670]. In an *in vitro* model, methylprednisolone produced a dose and time-dependent decrease in chondrocyte viability after exposure to clinically relevant doses. The combination of methylprednisolone with lidocaine also did not mitigate the inflammatory effects of IL-1β, further potentiating chondrotoxicity[671].

Triamcinolone is presented as having an extended duration of action[672], and the administration of triamcinolone acetonide in an abnormal joint does not alter SF GAG levels as dramatically as IA triamcinolone acetonide in a non-fragment containing joint[635]. It is addition to a culture of synoviocytes inhibits cytokine production and release and MMP activity, in both cartilage and synovial tissues. It was also able to counteract the inhibition of GAG production by synoviocytes but reduced GAG production in a human cartilage monoculture[194]. In dogs, 120h after IA injection, serum triamcinolone acetonide levels are diminished to undetectable levels (<0.5ng/l)[605], while in horses, its SF levels are detectable up to 14 days post injection[673]. Early treatment with IA triamcinolone acetonide after joint injury may entirely mitigate the injury-induced increase in synovial fluid collagen fragments[268]. It has also been described as being able to stimulate joints contralateral to a treated one to increase GAG production. This effect was attributed to low triamcinolone acetonide concentration in all joints secondary to systemic absorption, which stimulated GAG synthesis[635]. In a human report, triamcinolone acetonide was able to provide similar improvements in knee pain, function, and ROM when compared to a single administration of high molecular weight hyaluronan (G-F 20) at a 6-month follow-up. Both relieved pain and showed similar functional improvements, with triamcinolone acetonide having the advantage of providing better pain control in the first week and better knee functional improvements in the second week[674]. Although it provides excellent results concerning chronic pain, the mechanisms by which it exerts its effects are not fully elucidated. It can significantly suppress the expression of pain mediators induced by IL-1β in the spinal cord[675]. Some reports suggest that it may decrease chondrocyte viability and increase their oxidative stress in a dose-dependent manner[672]. Several human reports have evaluated the effects of IA triamcinolone acetonide. A 2-year follow-up study showed that triamcinolone acetonide has long-term safety, with no deleterious effects being observed deriving from long-term IA administration. Patients treated had

significantly increases in ROM and pain[652]. High doses (80mg) do not have any additional benefit when compared to lower doses (40mg) for the treatment of knee OA[676]. An extended-release formulation of triamcinolone acetonide, in which the active principle is incorporated in poly(latic-co-glycolic acid) microsphere, is able to provide a clinically relevant improvement in pain relief in patients with knee OA, compared with immediate-release triamcinolone acetonide. These effects did also remain relevant for a longer period of time[634]. Comparing triamcinolone acetonide and a different triamcinolone presentation, triamcinolone hexacetonide, and their elimination profiles in patients with knee OA, triamcinolone acetonide metabolites were detected in urine up to 48h post administrations, while plasma concentrations were detectable for up to 10 days post-treatment. Triamcinolone hexacetonide metabolites, on the other hand, were not detected in urine and while being detected in plasma, it was at lower concentrations[677]. It produces a decrease in serum cortisol levels after administration, reaching a peak at 24-48h. Recovery can take up to 4 weeks[667]. In the assessment of triamcinolone hexacetonide compared to a saline injection, triamcinolone hexacetonide (40mg) had a higher effectiveness than the placebo in the four weeks for pain in movement, pain scale, and ultra-sound measurement of synovial hypertrophy[678]. Treatment with 20mg of 40mg of triamcinolone hexacetonide produced equal relapse after six months in patients with chronic polyarthritis and when treating medium-sized joints. With that in mind, since no difference in outcome was found between compared doses, the lower one should be preferred, also reducing pharmaceutical costs and metabolic side effects[679,680]. Triamcinolone hexacetonide is more effective for managing knee pain than hyaluronan in humans up to week four post-treatment. Beyond week 8, however, hyaluronan has greater efficacy[681]. Duration of treatment efficacy also varies greatly with reports, raging between 1 to 2 months, 3 to 6 months, or up to one year[619,667,674,682,683]. A recent report indicated that the combined IA use of triamcinolone hexacetonide and an NSAID (tenoxicam) could have a synergistic effect. With the treatment group showing significantly improved CMIs scores when compared to only CS and only NSAID group at 3 and 6 months follow-up, in patients with knee OA[684]. TH has higher potency, a long duration of action, and rare flare reaction when compared with other CS[628,631]. It decreases the release of COMP, which lowers cartilage degradation[685]. In a placebo-controlled trial in humans with hip OA, triamcinolone hexacetonide was effective in the treatment of pain, with effects lasting up to 3 months in many cases. A 2-year follow up randomized trial showed no loss of joint space after IA triamcinolone hexacetonide injections at 3-month intervals[652]. The treatment group also had significant gains in the measurements of stiffness and physical function[686]. In another trial with humans with rapid destruction hip OA, the authors found no evidence that triamcinolone hexacetonide accelerated the course of the disease but did also fail to slow down the disease course[687]. Triamcinolone hexacetonide is also used to treat OA of other

joints, such as the interphalangeal, with clinical success, but not in the thumb carpometacarpal joint[688,689].

In a canine report, the authors recommended the use of triamcinolone over methylprednisolone[690]. Still, a systematic review of canine models of OA induction concludes that reports regarding IA CS use appear to be unanimously positive, with lower doses with sustained joint concentrations having a protective effect[653]. In contrast, triamcinolone acetonide has also been described as having deleterious effects on normal and OA chondrocytes and explants, with this effect not being counteracted by a concomitant administration of hyaluronan[691]. Long-action preparations are usually preferred for IA use[4]. In a Cranial Cruciate Ligament transection canine model, IA corticosteroid injection has shown to be able to reduce the progression of OA, associated with a reduction in the synthesis of stromelysin by chondrocytes. A single injection of 20mg of methylprednisolone produced favourable results in the structural changes of OA and, when given at the time of OA induction in a canine model, significantly reduces the incidence and size of osteophytes and histological severity of cartilage lesions[639,642,692,693]. This cartilage sparing effect may also be related to the down-regulation effects that CS have on urokinase plasminogen activator, one of the first enzymes in the activation cascade of MMPs[636]. A single administration of triamcinolone and lidocaine can achieve pain relief in dogs with cervical articular facet joint osteoarthritis[613]. This combination has a more prolonged action when compared with triamcinolone acetonide, being absorbed more slowly, with SF levels being maintained for over two weeks[694]. In a canine model of OA, IA triamcinolone hexacetonide was compared with oral prednisone and to a no-treatment group. Both treated groups had a significant reduction of osteophyte size, with femoral condyle cartilage erosion being observed in 25% of non-treated animals, 8% of those receiving oral prednisone, and in none of those under IA triamcinolone hexacetonide treatment. Histologically, triamcinolone hexacetonide significantly reduced the severity of OA structural changes of cartilage and had no deleterious effects on normal cartilage. No evidence of increased cell degeneration or death was associated with both CS[638].

In horses, a study comparing the difference between methylprednisolone and triamcinolone acetonide showed no difference between the two, nor between single or multiple administrations[665]. methylprednisolone may, however, have a protective effect against the degradation of newly synthesized GAGs[649]. Horses that showed the most significant response to treatment are less likely to have had radiographically moderate or severe OA[665]. Another report described that neither methylprednisolone nor triamcinolone acetonide was able to counteract the adverse effects of IL-1 and did not maintain cartilage metabolism at control levels. Lower doses of both were also not less detrimental to cartilage metabolism than higher doses[695]. Horses treated with IA triamcinolone

acetonide had lower protein and higher hyaluronan and GAG concentrations in SF. Synovial membranes from treated joints also had less inflammatory cell infiltration, intimal hyperplasia, and subintimal fibrosis. Results show beneficial clinical effects on detectable lameness and synovial fluid, synovial membrane, and articular cartilage morphological parameters, with no deleterious effects on subchondral bone. No macroscopic, microscopic or biochemical evidence of irreversible damage secondary to IA triamcinolone acetonide was observed[635,696]. These results, along with the observed chondroprotective effect of triamcinolone acetonide in vitro, have given body to the recommendation that it is an ideal corticosteroid, particularly in high motion joints[629,697,698]. A systematic review favoured triamcinolone hexacetonide over betamethasone due to a significant difference in terms of pain reduction[699].

As a whole, different CS have varied in terms of benefits and deleterious effects, generalizing that their IA is harmful inappropriate. Their IA use has prolonged effects, hypothesized as being due to its interaction with cytoplasmatic receptors, which may increase with rest[629]. Side effects are mainly related to discomfort from the procedure, localized pain post-injection, and flushing[676]. IA CS can, however, cause synovitis, in a reactive reaction called a steroid flare. This event has been described in horses and humans, with a prevalence of 2-6%[14,630,700]. Other than that, very few side effects have been reported and may include crystal-induced synovitis, calcification, steroid arthropathy, amongst others. Rarely, systemic side effects have been described in humans, as fluid retention, hyperglycemia, and hypertension[699]. It is crucial to guarantee a rest period after IA administration. Rest after an IA corticosteroid is a common practice amongst human rheumatologists, which may lead to a substantially higher duration of effect when a gradual return to use follows rest[606]. A prolonged clinical response has been described in humans that received a 24-hour period of rest after administration. This effect was attributed to a reduced clearance of the medication from the joint due to restricted joint movement, which in turn enabled better IA tissues penetration [701]. When compared to other therapeutic options, such as hyaluronan, CS may be a more cost-effective option because of its lower cost[674].

b. Hyaluronan;

Hyaluronan is a high molecular GAG composed of continuously repeating molecular sequences of glucuronic acid and N-acetyl-glucosamine, synthesized by chondrocytes and synovial fibroblasts (type B synoviocytes) in articular cartilage and synovial intima, respectively[246,624]. In the cartilage matrix, it forms the backbone of PG aggregates that are interwoven with collagen to create the unique structure of hyaline cartilage[702]. In SF, hyaluronan provides joint lubrication and helps limit inflammation, pain, and cartilage degradation, while acting as a shock absorber and allowing

the joint to move in a smooth manner[246,247]. In diarthrodial joints, it is also responsible for the viscoelastic and lubricating properties of SF, which is primarily a dialysate of plasma[703].

Currently, its wide use as a treatment for OA is still somewhat controversial because its mode of action is unclear and clinical trials have provided contradictory results[13]. In normal stifle joints of dogs, a mean SF concentration of 15.3mg/ml has been reported. During the early stages of OA, SF hyaluronan concentrations rise due to increased synthesis in response to pathological stimuli, and then gradually diminishes to severely low levels in most clinically affected animals[2,245]. Other studies reported significantly reduced levels of hyaluronan in canine OA joints, with a negative correlation between its concentrations and disease severity[704]. Dogs younger than eight months tend to exhibit higher levels while older dogs value tend not to differ significantly from the adult animal[705]. Endogenous hyaluronan is cleaved by free radicals, and its quantity and quality are affected in OA joints, more severely in clinically affected dogs. The fragmented, low-molecular-weight hyaluronan has been shown to have a pro-inflammatory effect[706]. Its concentration is also affected by dilution attributable to joint effusion, with aberrant forms also being produced[2,79,703,707,708]. Contradictory reports regarding the effects of unuse and immobilization on hyaluronan levels have been published[79]. The rapid turnover and degradation of PG aggregates components in OA may also involve turnover and loss of hyaluronan[709].

The observation of decreased hyaluronan concentrations in OA patients leads to the hypothesis the disease resulted from increased wear and tear and that lubrication was impaired due to reduced hyaluronan concentration. With this in mind, the exogenous administration of hyaluronan seemed an obvious corrective measure, but the assumption is presently known not to be correct[13]. Hyaluronan treatment aims to reduce pain and improve function by supplementing the viscosity and elasticity of SF[574]. The full mechanism of action is not entirely known, but anti-inflammatory, anti-nociceptive, and chondroprotective properties have been suggested, by enhancing cartilage synthesis, blunting response to IL-1 and TNF-α, and significantly decreasing TNF-α, prostaglandins, and IL-6 levels. It also reduces the damage of oxygen free radicals, inhibits phagocytosis, and affect leukocyte function[13,624,710–713]. Evidence towards the role of hyaluronan in chondroprotection is less prevalent[30], even though it may suppress chondrocyte apoptosis, stabilize PG structure, and help to diminish gross morphological and histological damage[714–716]. Its direct analgesic effect has also been suggested in animal models, by a proposed action over the opioid receptor[712,717,718]. An additional proposed mechanism of action is the stimulation of endogenous hyaluronan through exogenous administration, based on in vitro and in vivo studies[703,715,719]. In animal models, IA hyaluronan demonstrated similar effects inhibiting degenerative cartilage changes within chondrocytes and ECM, decreasing synovial inflammation, enhancing PG content, and inducing chondrogenic differentiation[720,721]. Even when

given intravenously, hyaluronan seemed to be able to decrease chondrocyte apoptosis[714]. Suggestions have been made that these effects are mainly due to the effect of hyaluronan on proinflammatory cytokines and degradative enzymes[722]. The injection is rapidly cleared from the joint, but maximal clinical improvement does not occur for several weeks and persists for much longer, although there are reports of improvements after only one week in humans[13,710,723]. In a normal joint, hyaluronan half-life is approximately 20h, while in an inflamed joint can be reduced to 11-12h[724].

A large number of human studies have addressed the use of hyaluronan, mainly focusing on pain and joint function. The conclusion presented by these studies varies widely from dramatic improvements in some to no beneficial effects in others[13,725–727]. High molecular weight products seem to produce better results, particularly in patients with mild radiographic disease[667,728–730]. A recent report showed that both single or 1–3 weekly injections of hylan G-F 20 at 1 year following the first injection for knee OA are efficacious and generally well tolerated for long-term use[731]. Even in active patients, the benefits of these presentations are still noted, particularly when added to usual care[732]. Still, in general, the majority of hyaluronan products seem to be superior to placebo[726]. A 2-year randomized controlled trial has suggested its beneficial effects on cartilage volume and defect scores[733]. Recommendations for the treatment of human hip and knee OA have deemed hyaluronan as useful, is characterized by a delayed onset but of prolonged duration[580,710], an unexplained characteristic. Other recommendations provide a weak recommendation for the use of IA HA [658], a conditional recommendation against [657], or that they should not be offered as an option [656]. Comparative reports also deem hyaluronan superior to IA corticosteroids[681]. A recent report in humans determined that patients with knee OA are the ones who benefit the most from the treatment, in terms of pain reduction and improved function, particularly those with high levels of pain, younger, with higher body mass index, and with less severe structural damage[725]. Concerning specifically the hip joints in humans, efficacy seems to be greater in cases of less radiographic changes, lasting up to 3 months after the first injection. Sub sequential administrations may sustain treatment benefits[734,735]. Another report showed similar clinical benefits between corticosteroids and hyaluronan, with those benefits being no different from saline at a 3-month evaluation point[736]. A meta-analysis concluded that efficacy of treatment reached its highest point at 2 months after injection, and then declined over time[737].

Many studies performed in canine experimental models of OA have failed to demonstrate clear benefits of hyaluronan supplementation[578,579,594]. The same results have been described in horses, were hyaluronan seems to be more effective in the treatment of incipient joint lesions rather than in established disease[606]. In a canine surgical model, IA hyaluronan provided clinically significant improvement in animals with stifle OA in terms of pain, function, lameness, and kinetics

when compared to pre-treatment and saline control. Maximum benefits were noted at 4-8 weeks and gradually tampered down by a 6-month evaluation time point. It was not, however, able to prevent the progression of OA based on radiographic, arthroscopic, and histologic assessment[738]. Improvement in cartilage preservation has been described when using a conjugate of HA with autologous fibrinogen in platelet-rich plasma, compared to HA alone[739]. In reports regarding dogs with naturally occurring OA, treatment groups have significantly better results than a control group by the 6th-week post-treatment, with the authors' suggestion and the effect that lasts from 6 to 12 months[740]. Other reports state that IA hyaluronan delayed the onset of OA, slowed cartilage degradation, decreased signs of pain, and improved joint function after the onset of OA[741]. In a different canine model of arthropathy, the hyaluronan treated group had significantly less ultrastructural changes when compared to a placebo group. The authors described that hyaluronan seemed to cover cartilaginous defects and diffuse through the cartilaginous matrix to re-aggregate with degraded PG and re-stabilize the matrix. Due to these effects, chondrocytes seemed to have an increased potential for recovery and became hyperthophic[742]. Reports in horses show that HA treatment significantly inhibits the digestion of cartilage proteoglycans and SF HA breakdown, while also having anti-inflammatory actions. Comparing low to high molecular weight HA, the first seemed to be most effective in reducing the release of cytokines[743]. In a study with dogs, significant score improvement when compared with control was seen from between 60 and 90 days. Larger dogs achieved an improvement of 30% or more at 12 weeks[555].

Typical protocols in humans include three injections one week apart or five injections weekly. Horses are generally treated with a single injection or weekly injections if needed[246]. As a part of post joint surgery recovery, no difference was observed between one or two IA injections, with both protocols being able to improve recovery and delay OA signs[744]. Other reports indicate that three injections weekly are more effective in reducing pain in humans when compared to a single administration, although both protocols improved joint function[745]. A popular approach in horses is the combined administration of hyaluronan with a corticosteroid, thus providing more rapid onset of action, with prolonged effect and decreasing the potential side effects of IA corticosteroids[665,745,746]. This therapeutic synergy has also been reported in human patients with OA, where viscosupplementation results can be improved by prior joint lavage followed by corticosteroid administration[625,700,747,748]. A recent recommendation for the management of knee OA in humans outlines that hyaluronan administration is best in mild to moderate OA cases and that prior or concomitant use of TH may optimize the effect of hyaluronan[749]. This addition of triamcinolone hexacetonide improves first-week symptoms and a functional score of IA hyaluronan administration, but not beyond this point [750]. Other reports, however, found no additional advantage in using this

practice or even an association with lower short-term clinical success rate, and similar medium-term outcome compared with IA triamcinolone alone[698,751]. In a 1-year follow up report, patients treated with hyaluronan with or without a combination of triamcinolone acetonide for knee OA, showed similar improvements for both pain and function, with the difference of better early pain control in the patients that also received triamcinolone acetonide. At the 1-year follow-up evaluation point, magnetic resonance imaging also showed that neither group had a significant progression of cartilage damage[752]. This approach has also been described in dogs with elbow OA, with improvements in activity, lameness, and pain, for up to 6 months[690].

IA hyaluronan administration has been described as producing mild heat, swelling, and/or erythema post-injection, which resolves spontaneously within a week[738]. These adverse effects are well tolerated and usually restricted to the injected joint[700]. In horses, the injection is usually well-tolerated, and has been used in the treatment of the clinical signs of OA but also as an adjunctive in the post-surgical period[753].

c. Platelet Rich Plasma (PRP);

PRP is an autologous biologic treatment, composed of the patient's plasma, containing growth factors released from platelets and endogenous fibrin scaffold. The rationale behind its use is to stimulate the natural healing cascade and regeneration of tissues by a supraphysiologic release of platelet-derived factors directly at the treatment site, without the risk of immune rejection or disease transmission[754–756]. All healing processes go through three phases: inflammation, proliferation, and remodelling. After an injury, which starts the inflammation phase, platelets are on the front line and have a critical role in mediating healing by releasing growth factors from their α granules[756–758]. A list of these growth factors is presented in Table 6. When activated, platelets also release a group of biologically active proteins that bind to the transmembrane receptors or their target cells, which leads to the expression of gene sequences that promote cellular recruitment, growth, and morphogenesis[759].

Growth factor	Function
IGF-1	Early inflammatory phase Anabolic effects Protein synthesis, the proliferation of myoblasts and fibroblasts Enhances collagen and matrix synthesis May modulate swelling
TGF-β	Proinflammatory Immunosuppressant during the inflammatory phase Aids in cell migration and fibronectin binding Augments production of tendon sheath fibroblasts, expression of type I and III collagen Improves tendon mechanics during the healing Control of angiogenesis and fibrosis
PDGF	Role in the early phase of tendon damage Facilitates the proliferation of other growth factors Attracts stem cells and white blood cells Stimulates angiogenesis Contributes to tissue remodelling
VEGF	Expression peaks after the inflammatory phase Promotes angiogenesis-neovascularization
b-FGF	Appears to stimulate angiogenesis Helps in the regulation of cell migration Stimulates proliferation of capillary endothelial cells Influences fibroblasts to create collagenase Enhances angiogenesis Contributes to the production of granulation tissue

Table 6 – Growth factors involved in the healing process (adapted from R. T. Nguyen et al., 2011). Legend: IGF-1 - insulin-like growth factor; TGF-β - transforming growth factor-β; PDGF - platelet-derived growth factor; VEGF - vascular endothelial growth factor; b-FGF - basic fibroblast growth factor.

The α granules have a key role in tissue regeneration and can release more than 800 different proteins, including serotonin, adenosine, dopamine, calcium, histamine, adenosine diphosphate, adenosine triphosphate, and catecholamines[758,760,761]. Growth factors also signal cells to proliferate and influence maturation, differentiation, and tissue repair[757,762]. Besides the attractive concentration of growth factors and anti-inflammatory cytokines, PRP also contains small concentrations of cytokines, such as TNF-α[584,763]. As a whole, PRP is seen as able to control the activities of different cell types that target multiple biological processes, such as apoptosis, ECM synthesis, modulation of angiogenesis, and inflammation[764]. Several studies in animal models have demonstrated the efficacy of PRP in accelerating the healing process after injuries in muscle, ligament, joints, and tendons[762,765–770]. It has a protective and regenerative effect on the degenerative cartilage[586] and can also be used as an adjunctive treatment, for example in ligament reconstruction, adding resistance to the grafts used, seen as soon as one week after reconstruction[770,771]. PRP also has fewer safety concerns than cell-based regenerative therapies[772]. In joints, platelet growth factors appear to have chondroinductive effects. transforming growth factor-β contributes to chondrocyte phenotype expression and

mesenchymal stem cell chondrogenic proliferation. Insulin-like growth factor-1 also has anabolic properties in cartilage regeneration. PDGF has mitogenic action, influences chondrocyte proliferation, and PG[614,757,773]. B-fibroblast growth factor stimulates the migration of fibroblasts and collagen synthesis[614]. They also exert autocrine and paracrine functions, promoting angiogenesis, ECM, and aggrecan production and collagen synthesis, together with influencing tissue regeneration[774]. PRP also induces a significant decrease of MMP-13, MMP-9, and MMP-2 levels, decreases the chondrocyte apoptosis cascade, while increasing hyaluronan synthase-2 expression in chondrocytes, thus enhancing hyaluronan secretion and contributing to joint homeostasis[711,775–777]. It also alters the expression of specific target genes, particularly during the early stages of remodelling[778]. Some reports also present a direct analgesic effect, through the augmentation of cannabinoid receptors CB1 and CB2[779].

Different types of PRP exist, with different compositions and properties, classified according to its profile regarding leukocyte and fibrin levels: pure platelet-rich plasma, leukocyte, and platelet-rich plasma, pure platelet-rich fibrin, and leukocyte and platelet-rich fibrin[780]. There is a belief that red blood cells and neutrophils should be reduced due to their inflammatory role, while the effect of mononuclear cells presence remains largely unknown[781]. The interest of preserving leucocytes in PRP is, as referred, a matter of an ongoing debate since some authors attribute deleterious effects due to protease and free radicals released by these cells, increasing the catabolic cascade. In contrast, others focus on their production of important cytokines and enzymes[782,783]. Nonactivated leukocytes in the formulation are also thought to contribute with antimicrobial properties[784]. Leukocyte rich PRP is produced by centrifuging whole blood and obtaining the platelet and leukocyte rich plasma.

In contrast, leukocyte poor PRP does not contain leukocytes and has been described in humans as having better results when treating knee OA than leukocyte rich-PRP[783]. Also, in humans, the concentration of inflammatory cytokines in PRP is correlated with leukocyte concentration[785]. An overlook of the classification of platelet preparations is presented in table 7.

Classification	Contents	Advantages	Disadvantages	Constituents
P-PRP	Platelets with low-density fibrin network after activation, without leukocytes	Liquid solution or as gel after activation can be injected or placed on wound	Dissolves quickly like a fibrin glue	Platelets: 500× 10^3/μL; leukocytes: 0.2× 10^3/μL
L-PRP	Platelets with low-density fibrin network and leukocytes	Liquid solution or as gel after activation can be injected or placed on wound	Dissolves quickly like a fibrin glue	Platelets: 500× 10^3/μL; leukocytes: 20× 10^3/μL
P-PRF	Platelets with high-density fibrin network and without leukocytes	Only exist as a gel after activation	Solid gel, cannot be injected	Platelets: 400× 10^3/μL; leukocytes: 100-600/μL
L-PRF	Platelets and half of the leukocytes (mainly lymphocytes), with a high-density fibrin network	Gel without anticoagulant; natural blood clot	Solid gel, cannot be injected	Platelets: 400× 10/μL; leukocytes: 60× 10^3/μL

Table 7 – Classification of platelet preparations and characteristics (adapted from Huang et al., 2017). Legend: L-PRF, leukocyte- and platelet-rich fibrin; L-PRP, leukocyte- and platelet-rich plasma; P-PRF, pure and platelet-rich fibrin; P-PRP, pure and platelet-rich plasma.

Considering clinical effects, leukocyte rich-PRP has been described as able to promote cartilage healing on the stifle, at macroscopic and histologic levels in an animal model[769,787]. In the treatment of human OA, both LR and leukocyte poor-PRP showed similar safety profiles according to some reports, while others attribute more painful reactions to leukocyte rich-PRP[782,783]. Evaluation of functional results was marginally affected by leukocyte concentration, in favour of leukocyte poor-PRP[783]. In a rabbit model, the presence of leukocytes in PRP elicited a short-lived inflammation that did not alter the therapeutic effect[788]. Detractors of leukocyte rich-PRP use point out that these preparations lead to increased inflammatory mediators, more MMP gene expression, and less COMP and decorin gene expression, effects attributed mainly to the presence of neutrophils[781]. Monocytes, in particular, are associated with increased cellular metabolism and collagen production in fibroblasts, and a decrease in the release of anti-angiogenic cytokines, as interferon-γ and IL-12[789,790]. Macrophages have the role of removing debris and also to balance pro and anti-inflammatory aspects of healing. Since it is impossible to keep or remove different types of white blood cells from PRP, the absence of macrophages may be more detrimental to the healing process rather than any damage inflicted by neutrophils[616]. Interestingly, an *in vitro* relation between biological response was found with the concentration of growth factors in PRP and not with the type, with more positive roles found with higher concentrations. This same report indicated that leukoreduced PRP might not be more indicated for the treatment of OA, as leukocytes may also have an immunomodulatory capacity and influence growth factor concentration, through their release of growth factors or by stimulating platelet release[763,791,792].

PRP is prepared from autologous, anticoagulated whole blood, with a 3-8 fold the concentration of platelets, containing a hyperphysiological amount of autologous growth factors[757]. Human studies show that the ideal PRP product should have a 4 to 7-fold increase in platelets[776,793–795]. Increasing platelet numbers beyond this point may not add any benefit or may even be detrimental, as they can be a source of inflammatory cytokines and potentially prevent tissue healing[796–798]. Good results have also been reported with the use of lower concentrations[587]. PRP at 40% volume/volume increased bone marrow-derived multipotent mesenchymal stromal cells, while PRP at 80 and 100% v/v concentrations suppressed their viability[799]. There are reports that very large volumes of PRP might have a cytotoxic effect on healing grafts[800]. Blood collection is usually performed from the jugular vein (Figure 25).

Figure 24 – Collection of blood from the jugular vein.

Commercial kits are available for the production of in-house PRP, with variable characteristics[781,785]. They vary in the amount of whole blood required, the anticoagulant used, time and speed of centrifugation, final volume, and the number of platelets in the final concentrate[756]. Some require specific equipment, such as a dedicated centrifuge (as the Companion Pure PRP® system), while others do not (V-PET, Pall Corporation, Figure 25). V-PET is a platelet concentrate as well as conditioned plasma, that contains many autologous anti-inflammatory mediators and growth factors, reported to reduce pain and lameness scores, and increase weight bearing in dogs with OA[690,801]. It can target multiple inflammatory mediators, producing up to 6.5ml of platelet concentrate with a 3x concentration of platelets and a 2x concentration of white blood cells[235,802].

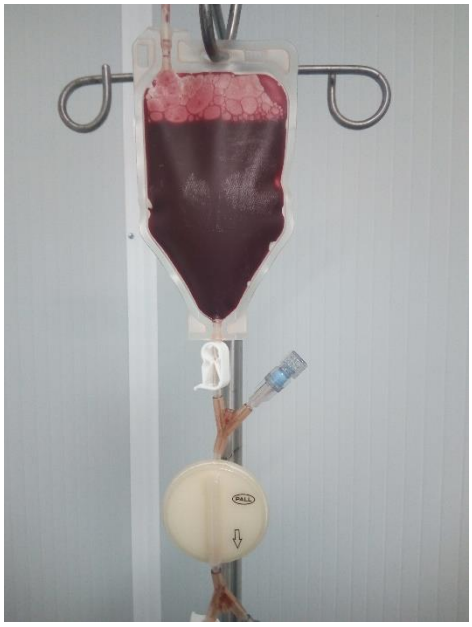

Figure 25 – The V-PET kit, ready to start blood platelet concentration.

PRP can also be produced using centrifugation, with the majority of protocols comprising a double spin procedure[780]. The first spin is usually slow, to avoid spinning down platelets, whereas the second spin is fast so that the platelets are spun down. With this procedure, after the first spin, platelets are mostly concentrated right on top of the buffy coat[772] (Figure 25). Similar efficacy of treatment has been reported with both single and double spin techniques[782], and even with double-spinning protocols, complete separation is not possible, because of the components' slightly overlapping specific gravities[803].

Figure 26 – The separation of different blood components, observed after the first spin, using the Companion Pure PRP® system.

Excessive centrifugation speed may damage platelets and produce a bad quality PRP[804]. Additional care is necessary to guarantee sterility throughout the process[805]. Closed, commercial kits, secure the procedure sterility. After preparation, the final appearance of the platelet production reflects cell content (Figure 27).

Figure 27 – Two different platelets products, V-PET® on the left, and the Companion Pure PRP® on the right.

The anti-coagulant to be used has also been an object of study. Many kits use acid dextrose (ACD), which acts by binding calcium and preventing the clotting cascade initiation by the coagulation proteins[616]. Different reports indicate that Ethylenediamine tetraacetic acid may be a better anti-coagulant, leading to larger yields of platelets in PRP, while others attribute better results to sodium citrate[772]. There is also a continuous ongoing debate regarding the need or even the adequacy of PRP activation before administration. The addition of calcium chloride is frequently used as an activation mechanism, that triggers the prompt release of 70% of growth factors contained in α granules within 10 minutes, and the remaining within the hour[757]. Other authors defend that platelets will activate at the injection site, when in contact with substances as collagen type I or through paracrine mechanisms, which may be a more normal physiological activation[614,616]. Once activated, platelets are morphologically modified, developing pseudopods that promote platelet aggregation and later degranulation[806]. Large bore needles (>22G) should be used during blood collection to prevent unintentional activation. After activation at the injection site, the release of growth factors initiates an inflammatory response that lasts approximately three days[616]. Since platelet products can present such a variety of preparation steps and characteristics, a group of experts outlined the minimum reporting requirements for clinical studies evaluating PRP[807].

There are several reports regarding the use of PRP in human OA. Three injections proved to be significantly more efficient than a single injection of hyaluronan in terms of reducing knee pain

and stiffness while improving function. Even a single injection has produced better effects than placebo, with effects being present at six months to 1-year post-injection[737,808–810]. Other studies, however, describe only mild improvements but lack a control group[810]. Specifically, regarding hip OA, PRP injections seem to provide significant clinical improvements without considerable side effects, which remain relevant and stable for 12 months, when compared to other treatments. In the same study, the combination of PRP with hyaluronan did not lead to significant improvements in pain symptoms[203]. In general, and in the long term, PRP seems to be more effective than hyaluronan or other therapeutics, as ozone[780,811,812]. Better results seem to be observed in younger, more active patients, with a lower degree of cartilage degeneration, than in those with more advanced OA[737,813]. Highly demanding patients may not see their needs fulfilled by the marked clinical improvement provided by PRP, observing a decrease in results over time[587].

In dogs, a single IA PRP injection has resulted in clinical improvements for 12 weeks, in some cases without progression of radiographic signs[777,801,814]. Through this period, radiographic scores were the same as assigned before treatment[777]. Multiple injection protocols have also been described, providing improvements in ROM, pain, lameness, and kinetics. Authors associated this response to treatment while the anti-inflammatory activity of PRP rather than any effect on tissue anabolism or catabolism[769,815]. It has also been used as a part of surgical protocols, leading to significantly better gait performance in the postoperative period[814,816,817]. Additional reports, conducted in animal models, show that IA PRP significantly suppresses morphological and histological changes of OA, increasing neochondrogenesis and PG content in the ECM[818].

Currently, no ideal number of injections or intervals are set, which is usually based on clinical experience or a desired improvement level[757,812]. Generally, patients are re-examined 2-6 weeks after the procedure to evaluate pain, function, the injection site, and also to discuss concerns and future management course[616]. As a whole, it is still unclear which PRP product is ideal for most uses, and even if one product deemed as superior for one specific treatment is ideal for all applications[785].

Post administration withhold of NSAIDs is recommended for ten days and, preferably 3-6 weeks after the procedure, due to a theoretical assumption that they may impede or delay tissue healing and may even produce fibrosis[819,820]. In humans, PRP side effects are usually local and transient, consisting of injection pain, local inflammation of short duration, and reaccumulation of effusion, taking 2-10 days to resolve[755,757,782,810,821]. Usually, no systemic effects attributable to the local administration of PRP are noted[822]. A human study, however, reported systemic rather than local effects, such as nausea and dizziness, which resolved within 30 minutes[823].

d. Stanozolol;

Stanozolol is a synthetic derivative of testosterone. Its properties include anabolic/androgenic activity, probably associated with its affinity for androgenic and, at lower doses, glucocorticoid receptors[824]. Due to its potent anabolic effects, studies regarding its use in human OA are not performed[825,826]. Despite this concern, long-term, high-dose treatment with stanozolol has failed to produce significant changes in activity patterns and aggressiveness in treated mice[827]. It can induce fibroblasts to increase collagen production in a dose-dependent pathway, through transforming growth factor-1β synthesis, while decreasing NO production and stimulation of autocrine secretion of insulin-like growth factor-1, which induces osteoblast proliferation and collagen synthesis[828–831]. In humans, an increase of transforming growth factor-1β synthesis is related to a decrease in articular pain[832]. It also demonstrated chondroprotective effects through the downregulation of genes for pro-inflammatory/catabolic cytokines and enzymes associated with OA, in an equine *in vitro* chondrocytes[833]. An additional possible mechanism of action may be related to its induction in aromatase expression[834]. It has been demonstrated that the human articular cartilage expresses aromatase and that reduced expression of aromatase could facilitate the development of OA[835,836]. The use of aromatase inhibitor therapy in humans to address other medical conditions might be associated with common musculoskeletal symptoms and with substantial functional disability[837].

In an ovine surgical OA model, IA stanozolol was able to preserve the gross anatomy of the stifle joint, reducing osteophyte formation, subchondral bone reaction, and promoting articular cartilage regeneration, evaluated at 3 and 9 months post-surgery. These results were assessed via digital radiography, lameness data, and histological examination. No gain weight was attributed to the anabolic effect of stanozolol[829]. In horses, a positive response in the treatment of OA was observed in 82.5% of patients, with acute cases presenting higher treatment success. In the majority of cases, this meant a complete lameness resolution. An improvement in the physical characteristics of the SF was also observed and overall results persisted after the end of the treatment period. In some animals, transient post-injection swelling was observed in the treated joint, which disappeared after a few days without intervention[830].

In humans, the dose demonstrated to produce anabolic effects is 10mg twice a week through intramuscular administration[838]. In dogs, a 0.3mg/kg dose has been described for IA and oral use[839,840]. In horses, after bilateral IA administration of 5mg of stanozolol, the maximal plasma concentration registered was 1.7ng/ml at 6 hours post-administration. Therefore, stanozolol passes rapidly from the joint space to systemic circulation, being eliminated rapidly and detected in plasm for no more than 36h post local administration[841].

e. Stem Cells;

Stem cells are defined as potential and undifferentiated cells, with the ability to convert into differentiated cells and to regenerate tissues[842,843]. Considerable clinical attention is currently devoted to mesenchymal stem cells (MSC), due to their ability to differentiate into chondrocytes, osteoblasts, adipocytes, and fibroblasts[842,844]. Pre-clinical and clinical studies have demonstrated their efficacy in muscle, tendon, bone, and cartilage regeneration[845]. They have also been described as having a protective and regenerative effect on degenerative cartilage[586]. MSC have been identified in normal joints, originated from the synovium[846]. They can be obtained from bone marrow or adipose tissue, and then go through a culture-expansion process[847]. Their mechanism of action is both contact-mediated and contact independent, modulated by immune-modifying chemicals[445]. They inhibit activated B-cell, T-cell, and natural killer cell proliferation and downregulate the expression of major histocompatibility complex II on inflammatory cells[848,849]. They have an affinity for damaged joint tissue, with recent *in vivo* studies showing that they localize and participate in the repair of damaged joint structures, including cartilage lesions[850,851]. Still, it is not fully known if they differentiate into a tissue-specific cell if they improve tissue repair or a combination of both[847].

Stem cells are most commonly used in clinical veterinary medicine in therapeutic applications for the treatment of musculoskeletal injuries, both in horses and dogs[847]. IA MSC administration can decrease cartilage destruction, osteophyte formation, subchondral bone sclerosis, and even lead to regeneration of meniscal and articular cartilage[852–855]. The use of MSC as a treatment strategy for OA is increasing considerably, thanks to their capacity to improve joint function, reduce pain, improve mobility, increase daily activity and, therefore, the patients' quality of life[856,857]. In dogs, it produces statistically significant improvements in lameness, pain, and ROM when compared to control animals[858,859] or placebo[860]. In the treatment of hip OA, specifically, MSC improved average lameness score and reduced pain scores, with varying amounts of cell numbers[861]. The synovium may be a promising source for MSC for canine cartilage regeneration, with a higher chondrogenic potential than those from the infrapatellar fat pad, adipose tissue, and bone marrow[862].

Some concerns arise from the use of MSC, the first being the need for *in vitro* expansion of cells to obtain a sufficient number. Another major concern is the possibility of neoplastic transformation of cells, increased patient susceptibility to infection, embolism of cells, and acute or chronic immune reaction to the cells themselves[445]. Other challenges include the determination of the adequate number of cells in the tissues undergoing repair, long-term safety, and the durability of the benefit[593,856]. While the majority of reports as described as safe and elicited beneficial patient responses, they were also open-label, uncontrolled studies[863].

The use of PRP, along with stem MSC, has been described and presents itself as an interesting approach. PRP provides a three-dimensional scaffold for stem cells to differentiate, may prevent the depletion of MSC, enhance their effectiveness through the activity of GF and guide them to properly differentiate into chondrocytes, improving joint function and cartilage regeneration[586,760,777,864]. It has been used as an approach to hip OA in dogs, that showed improvements in kinetic outcome variables, reducing dog's pain, and improving physical function[850,865]. The use of alternative scaffolds along with MSC is an interesting approach, which leads to the development of additional options, such as urinary bladder ECM scaffold[866].

f. Autologous Conditioned Serum;

The use of Autologous Conditioned Serum, as part of the use of platelet concentrate, is based on its content of supra-physiological concentrations of autologous anti-inflammatory mediators and growth factors. Specifically, high concentrations of autologous IL-1ra and sTNF-RI, which inhibit the binding of IL-1β and TNF-α, leading to a reduction in inflammatory mediators such as MMP-13, IL-6, IL-8, NOS, and PGE_2[582,583,661,867]. It can reduce pain and lameness scores and increase weight bearing when injected into OA joints[690,801,868]. Current cytokine-modulating therapies for OA yield variable clinical results and compelling in vivo mechanistic evidence is sparsely available[57]. In particular, the presence of IL-1ra is of great importance, due to its effectiveness in counteracting the role of IL-1. Local natural IL-1ra concentrations may be too low in degenerative diseases to inhibit the destruction of cartilage, muscle, and other joint structures[869]. Several reports of IA IL-1ra have been published in both animals and humans, and even by gene transfer[212,260,870]. The effects of Autologous Conditioned Serum seem to be due to the involvement of other factors rather than IL-1ra alone, mainly since some questions are recently being raised regarding the effectiveness of targeting IL-1 in the treatment of OA[871,872].

The production of Autologous Conditioned Serum consists of the incubation of 50-60ml of blood in syringes that contain medical-grade glass beads, incubated at 37°C with 5% CO_2 for 24h, and then centrifuged for ten minutes[661]. It has been deemed a safe and effective procedure, reducing pain and with sustained effect that lasts of up to 2 years, possibly through a re-establishment of a healthy joint homeostasis[873,874]. Additional reports of at least one year of improvements have been published, with mean improvements of 78% compared to 7% in the control group[875]. Autologous Conditioned Serum has shown to be superior to both placebo and hyaluronan in several clinical outcome measures[13,873]. In dogs, studies have shown that it can produce improvements up to 12 weeks, both in subjective scores (CBPI and HVAS) but also in weight-bearing (PVF and VI)[235]. It has also been described in dogs with elbow OA, with improvements in activity levels, decreased

lameness, and pain[690]. No side-effects have been related to its use, other than the possible ones derived from the IA route of administration[875].

4.2 Other therapeutic modalities;

Many of the pharmaceutical agents included in this section act via the anti-inflammatory activity. They do not, however, target most of the pro-inflammatory mediators, being unable to stop the catabolic state found in OA joints[445]. For that reason, they often provide insufficient symptomatic relief.

a. Non-steroidal anti-inflammatory drugs;

The term non-steroidal anti-inflammatory drugs (NSAIDs) refers to those drugs that inhibit one or more steps in the metabolism of arachidonic acid cascade into prostaglandins (particularly those in the PGE series) and thromboxane, but are not classified as steroids[876,877]. NSAIDs are frequently the first-line of treatment in OA and are the mainstay of treatment of hip OA. Their popularity is probably due to a rapid efficacy in palliating pain in animals with OA and relative ease of administration[575,594,876,878]. In the USA, the cost of treating dogs with NSAIDs was estimated to exceed US$130 million in 2005, growing at 13% a year[544].

NSAIDs tend to be well absorbed after oral administration, and hepatic elimination is the primary route of elimination via biliary secretion, conjugation reactions, and metabolic reactions such as cytochrome P450 metabolism[879]. They work by inhibiting COX and, in consequence, PGE_2, and this action relieves pain but is not considered able to ameliorate cartilage loss in persons with OA[53,879]. COX has two main isoforms, 1 and 2, with COX-1 being generally identified as involved in regulatory processes, while COX-2 is more linked with the inflammatory processes of OA, even though this division has been proven not to be so formal[877,879]. COX-2 is constitutively expressed in the dorsal horn of the spinal cord and contributes to the propagation of nociceptive stimuli, and its inhibition can also produce central analgesic effects[880]. Both COX-1 and -2 are up-regulated in the synovium of dogs with naturally occurring hip OA[881]. A third isoform, COX-3, has been identified primarily in the canine cerebral cortex, with minimal amounts found peripherally[882]. COX selectivity has been reported as not associated with the efficacy of any given NSAID[879], although this claim is a matter of an ongoing debate. Different NSAIDs inhibit different isoforms of COX. Even though joint inflammation alone does not suffice for the diagnosis of OA, it has a predominant role in clinical presentation, leading to pain and effusion, making it a relevant target for symptomatic treatment[272]. NSAIDs, through the inhibition of prostaglandin production, can return the nociceptive threshold to

a normal level[883]. The plasticity of pain transmission pathways that leads to central sensitization may be reversed through COX inhibition[884,885]. Inhibition of COX-2 has proven to reduce OA clinical signs, and also to have a chondroprotective action[886,887]. In humans, however, COX-2 inhibitors can produce a higher rate of cardiovascular adverse events[888]. Dogs, on the other hand, have higher basal levels of COX-2 expression in the kidney compared with humans. For that reason, dogs with chronic kidney disease show increase COX-2 expression, and the synthesis of prostaglandins shifts to the COX-2 pathway. For those reasons, NSAIDs that target COX-2 may be expected to adversely affect renal function in dogs[889].

Recommendations for the prescription of NSAIDs for the management of OA vary greatly, from intermittent ("as needed") therapy to a continuous modality (long-term treatment being defined as 28 days or more of continuous therapy. The latter has the benefits of providing better pain control, better improvements in mobility, and potentially slowing down of the disease process through improved joint usage[317]. When prescribed for long-term use, the dose should be titrated to the lowest effective dose possible[580]. The continuous use of NSAIDs leads to a decrease in central sensitization and reduction of disease progression, through the prevention of nitric oxide-induced cell death[317,890,891]. There is evidence that response to NSAIDs is significantly lower in dogs with lameness for six months or longer, compared with those with lameness lasting for shorter periods[892].

Carprofen is the NSAID with a higher number of published studies regarding its effectiveness in reducing OA signs in dogs. It is considered a preferencial COX-2 selective and can reduce pain in the post-surgical period, but may not provide the same amount of relief in all dogs[433]. In a systematic review, it has been attributed to a moderate level of confidence in its ability to reduce clinical signs of OA[578]. When used in working dogs with hip OA, carprofen was not able to significantly reduce signs of pain[893], nor in companion dogs with HD, when compared to a placebo[894]. Carprofen has also been shown as able to reduce histologically graded cartilage lesions and significantly decrease biomarkers related to the progression of OA, as C-terminal telopeptide of collagen type II, and delay osteophyte progression, as observed in radiographic findings, though the reduction of osteoblasts' activity[895,896]. Also, it has *in vitro* ability to stimulate the synthesis rate of GAGs[897].

Other reviews have pointed to strong evidence of the efficacy of carprofen, meloxicam, firocoxib, and mavacoxib[579,898]. In a large scale study, firocoxib was described as effective in over 90% of animals, and with a low percentage of side effects[899,900]. Meloxicam is also a COX-2 selective, with potent anti-inflammatory activity and low gastrointestinal and renal toxicity[876]. Additional reports suggest that both mavacoxib and carprofen are remarkably effective in the treatment of canine OA, with 93.4% of mavacoxib-treated dogs and 89.1% of carprofen-treated dogs demonstrating overall improvement[901]. Both carprofen and meloxicam improve GRF in treated animals, but only

meloxicam can bring some of them to normal values, suggesting that meloxicam could improve joints suffering from a more severe inflammatory process[537]. The same systematic review has pointed to four studies on the use of meloxicam, which received a high level of comfort that it can reduce clinical signs of OA[578]. As a whole, NSAIDs are all usually considered superior to placebo for pain relief, presenting no significant differences between them. It is also reasonable to presume that all dogs have a similar response to them[594,902]. While there are no studies that indicate that any given NSAID is superior to any other, it is important to keep in mind that an individual patient may have a better response to one NSAID than to another. A specific patient may also develop adverse side effects with one NSAID but not with another, while some may not tolerate them at all[879]. It is also established that NSAIDs provide only modest control over the signs and symptoms of OA. Some patients may also become refractory to therapy. Neuropathic pain, a component of chronic OA, is recognized as non-responsive to NSAIDs[866,903–905]. Since NSAIDs block COX, an increase in the production of leukotrienes from arachidonic acid that would otherwise be metabolized to prostaglandin products occurs. Leukotriene activity, associated with hyperalgesia, may partially explain the incomplete relief provided by NSAIDs[876]. In a human study, the authors found that only 15% of patients with knee OA for whom an NSAID was prescribed were still taking the same drug 12 months latter[906]. A novel class of pharmaceuticals, the pripants, has been developed due to their role as prostaglandin receptor antagonists. By inhibiting just the EP4 receptor, primarily responsible for the pain and inflammation associated with OA, the homeostatic function of PGE2 is better maintained[907]. Gapripant, a drug of this group, as shown to be safe for long-term oral administration and effective for decreasing OA signs, when compared to a placebo[908–910]. Different approaches aim at neutralization of key factors involved with pain, as is the case of nerve growth factor. A recent report has presented a vaccine, which was able to generate anti- nerve growth factor antibodies in a mice surgical model, thus reversing pain behaviours[334].

Particularly when given for long periods, NSAIDs have well-documented side-effects, with the most common being gastrointestinal tract disturbances[578]. Gastrointestinal complications may occur in some individuals with their use, and it is most likely that is this perception of this risk that restricts their long-term use, even though there are no accurate and controlled estimates for the incidence of adverse effects with long-term NSAID use in dogs[317]. Newer veterinary-approved NSAIDs have a lower incidence of gastrointestinal side effects, which may be attributed to differential effects on the COX isoforms[879]. Renal and hepatic adverse effects are reported at a lower frequency, while inhibition of coagulation, lethargy, and polydipsia are infrequently reported in clinical studies[513,892,911,912].

b. Other analgesic drugs/modalities;

The combination of other analgesic drugs with NSAIDs is a common practice. It has the goal of helping control refractory pain but also to reduce total NSAID dose and, consequently, the risk of potential side effects[913]. Examples of these drugs include, but are not restricted to, tramadol, amantadine, or gabapentin[879,913]. In humans, the efficacy of treatment with NSAIDs or pure analgesics does not provide enough pain relief for patients with OA to be considered a *solo* adequate treatment[589].

Tramadol is an opiate-like agonist with μ-receptor activity, used in the management of mild to moderate acute pain, and as an adjunctive analgesic in the management of chronic pain resulting from OA or neoplasia. Its mechanism of action is through weak inhibition of opioid receptors, along with the interference of the release and reuptake of noradrenaline and serotonin, in the descending inhibitory pathways[594,914]. Other opioids can also be used, normally pure μ agonists. Morphine is one of the available possibilities, which has inclusively been described in IA administrations. Its effects were comparable with triamcinolone[915]. Opioids have also been administered IA as a form of analgesia, both as a treatment of OA and for postoperative analgesia. This route is effective due to the high density of opioid binding sites found in the inflamed canine joint tissues[916]. In a recent study with 25 dogs, ten days of treatment with tramadol, as administered at 5 mg/kg, given three times a day *per os*, provided no clinical benefit for dogs with osteoarthritis of the elbow or stifle joint[917]. On the other hand, the combination of tramadol with low dose ketoprofen showed interesting results[918].

Amantadine inhibits the N-methyl-D-aspartate receptors that, found in the dorsal spinal horn, and whose activation is associated with chronic pain[594,919]). It is not likely to be effective when administered as the only analgesic, but together with an NSAID as meloxicam, for example, and in cases refractory to opioids, it might be beneficial[914,920]. Gabapentin is beneficial in the treatment of neurogenic pain, although the mechanism by which gabapentin exerts its analgesic action is not entirely understood. It appears to bind to a specific modulating protein of the voltage-gated calcium channels, resulting in a decreased release of excitatory neurotransmitters[594,914]. It can be administered along with an NSAID or not.

Biophosphonates are commonly used to inhibit bone resorption, osteophyte formation, cartilage degeneration, and reduction in bone turnover. In OA, subchondral bone sclerosis is preceded by its resorption[921–923]. Tiludronate has demonstrated the ability to decrease structural changes and also an anti-inflammatory effect in the canine Cranial Cruciate Ligament pain model[924,925]. It has a positive effect on gait disability and joint symptoms, when compared to a placebo, most likely due to its ability to improve some of the structural changes that occur with OA, and also by reducing the synthesis of catabolic and inflammatory mediators[925]. The effect on pain and function is likely due to

a reduction of synovial effusion size, synovitis, and level of inflammatory mediators (PGE_2, NO, and also MMPs)[921]. It also reduces pronociceptive substance P and concurrently increases transthyretin, a specific spinal cord peptide compared to placebo, keeping treated animals without peripheral or central sensitization even at 56 days post-surgery[926]. An *in vitro* study suggested that a 30-day course of treatment lowers PGE_2 release from tissues, with neutral effects on tissue chondrocyte content and matrix composition[927]. While being able to reduce pain, it is not efficient against central sensitisation[926]. A long-term study evaluating the effect of risedronate in a rabbit model of OA showed no positive effect on the reduction of cartilage damage, and a failure to prevent subchondral bone changes and osteophytogenesis[928]. Zoledonate, on the other hand, was able to reduce histological joint degradation in a rodent model, and its inhibition of subchondral bone lesions alleviates both joint pain and central nociceptive activation[921,929,930].

Mesotherapy (from the greek mesos, referring to the mesoderm of the early embryo that develops into tissues such as muscle and cartilage) is a drug administration technique developed by the French physician Michel Pastor in the 1950s [931,932]. It consists of local intradermal therapy, with pharmaceuticals being given in small amounts through multi-punctures in the skin over the area of the pathological condition to be treated. This process creates a deposit of the drug in the skin, which is released over time into overlying tissues (Figure 28).

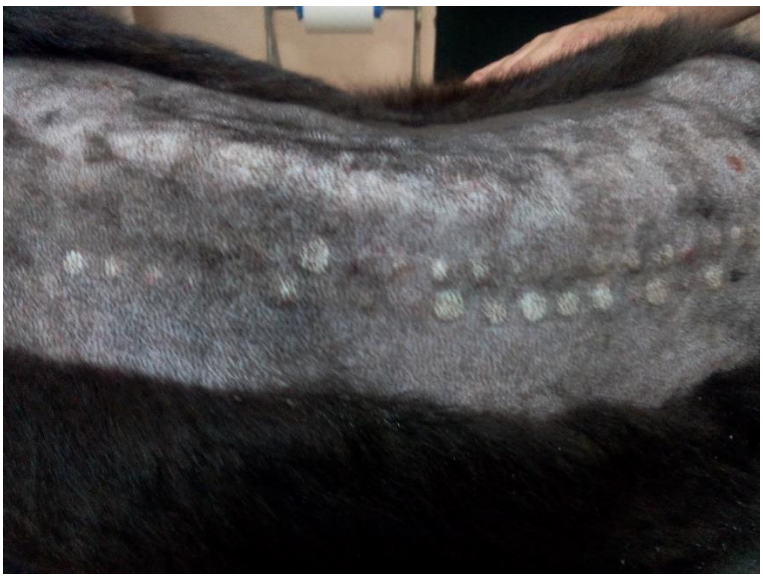

Figure 28 – The microdeposits created following a mesotherapy session, in a dog being treated for back pain.

It has a rapid onset of action, since a short time to reach the intended site is necessary, a prolonged local action and a drug-sparing effect[931–934]. It has been described in humans, horses, and dogs [935–939]. A human review study described that mesotherapy shows an excellent effect to reduce acute and chronic musculoskeletal pain while being a well-tolerated treatment[940]. In dogs, a

combination of an NSAID or CS with lidocaine and thyocolchicoside was able to significantly reduce pain when compared to carprofen, with effects lasting through several months with a single session[935,936].

Prolotherapy is a form of regenerative therapy with "proliferants" injected into diseased joints or periarticular area, intending to provoke an inflammatory response and increase proliferation of tissues during repair[941,942]. It consists of injecting a hyperosmolar dextrose solution into the area to be treated[943]. Dextrose is an irritant causing, inflammation with the release of growth factors, or act as a sclerosing vascular tissue factor, creating a regeneration process[944]. Practitioners that started using PRP for tendinopathies in the early 1990s were primarily trained in the use of prolotherapy[616]. In dogs, it has been described for the treatment of animals with elbow or stifle OA, with improvements in lameness scores and ROM, while lowering pain levels, compared to a placebo[942].

Medical ozone is a mixture of ozone and oxygen, administered at low concentrations, and has been used for knee, hip, and shoulder OA in humans, and also for other painful conditions such as disc herniation[945,946]. It can reestablish cellular redox balance, increase adenosine availability through an ozone oxidative conditioning mechanism, and activation of enzymes responsible for protecting against the overproduction of superoxides[947,948]. It acts by reducing inflammation, IL-1β, TNF-α levels, and ROS (NO and H_2O_2), through the production of interferon and interleukins[948–950]. The analgesic action is thought to be based on stimulation of the antinociceptive apparatus mediated by endogenous opioids and serotonin, raising the pain threshold[946]. As it has a strong analgesic effect and almost no side effects, it has been suggested as a good alternative to CS injections, but not for hyaluronan[812].

Other therapeutic options have been described, such as botulinum toxin Type A. The botulin toxin has a marked analgesic activity independent of its neuromuscular activity, contributing to block peripheral sensitization which, indirectly, reduces central sensitization[951]. Its IA use is hypothesized as having antinociceptive and possibly anti-inflammatory action[593]. Glycosylated undenatured type-II collagen, given daily to dogs with OA, is also able to ameliorate signs and symptoms of OA similarly to an NSAID, but signs return upon withdrawal[952,953]. Agmantine, a ubiquitous compound formed during the process of arginine decarboxylation, is also able to significantly improve GRF in dogs with hip OA, compared with carprofen and placebo[954]. Oral cannabidiol is gaining interest in the management of pain in humans and animals, particularly in dogs with OA. Even at low doses, cannabidiol can increase comfort and activity in dogs with OA, without observable side effects, while significantly attenuating the production of proinflammatory cytokines IL-6 and TNF-α and elevating levels of anti-inflammatory IL-10[955–957]. The use of these substances is based on the findings that changes in the endocannabinoid system are pivotal in joint functioning and pathological processes[958].

Targetting the inhibition of endocannabinoid degradative enzymes or receptor agonisms are possible pathways to reduce inflammation and pain[959,960]. A recent study showed that the addition of cannabidiol to a standard multimodal pharmacological approach to OA led to a significant reduction n pain scores, in addition to an improvement in the quality of life scores[961].

The existence of such a wide variety of therapeutic options or approaches for the treatment of OA reflects the complexity of the disease, the impact it has on a patient's life, and how complex it is to manage, points to an impossible task for a single therapeutic option.

c. Nutraceuticals;

Nutraceuticals (a term derived from the combination of the words nutrition and pharmaceutical) have enjoyed growing popularity over the last years in veterinary medicine[962]. They are defined as "a substance produced in purified or extracted from which, when administered orally to patients, aims to provide them with the necessary elements for their structure and normal function to better their health and wellbeing"[963].

Prevention of cartilage degradation in OA is an important treatment objective, which requires long-term use of safe modalities. Amongst performance or working dog owners, there is also an increasing interest in alternative therapeutic modalities for the medical management of OA and slowing the process of cartilage breakdown and promotion of cartilage turnover. Such prevention and alternative solutions could come from nutrition and more particularly from dietary supplements[310,581]. Specific nutrients used in the management of OA may provide a reduction in inflammation and pain, enhance cartilage repair, slow the degenerative process, complement prescribed medications, and provide tangible improvements in clinical signs[347]. Despite this growing interest, there is still a lack of good-quality clinical trials to help determine if nutraceuticals, and glucosamine/ chondroitin supplements specifically, are better then an NSAID such as carprofen in reducing clinical signs of OA[964]. Unlike steroids and NSAIDs, these treatments do not exert an instantaneous effect but are widely used due to their ease of administration, high level of safety, and the possibility of administration with few side effects, mainly related to gastrointestinal upsets[171,965]. There is a need for additional well-designed studies to conclude about the safety and efficacy of these compounds as a whole, as the evidence for their efficacy is still poor[581,962]. There are, however, some evidence that the symptomatic relief they provide is often insufficient to address the demands of sporting or working animal[606,893], as dogs with moderate to severe OA, which may be too seriously affected to benefit optimally from some of this components[966].

Chondroitin sulfate and glucosamine hydrochloride are the major components of many oral joint supplements on the market. They act as a preferred substrate for the biosynthesis of GAG chains,

and subsequently for the production of aggrecan, with reported tropism for articular cartilage. They also exert anti-inflammatory and anticatabolic effects, in addition to prophylactic prevention against synovitis, even though several studies have reported no significant differences, compared to placebo[537,578,581,967]. Despite limited and conflicting evidence, the natural products glucosamine hydrochloride and chondroitin sulfate are commonly recommended by veterinarians for treating osteoarthritis in dogs[968]. Some studies presented statistically significant improvements in pain scores, weight-bearing, and lameness severity[965], while others described no significant reduction of pain was observed or increase in activity counts when compared to NSAIDs[893,969]. *In vitro* reports have presented chondroprotective effects of both chondroitin sulfate and glucosamine[695,970], without this effect being observed *in vivo* studies[971,972]. A protective effect against synovitis and associated bone remodelling has been reported to a combination of glucosamine and chondroitin sulfate, attributed to an enhanced synthesis and turnover of proteoglycan [973,974]. In a Pond-Nuki model, IA and oral administered glucosamine sulfate significantly reduced histological signs of OA, with the IA application being more effective compared to oral administration[975]. Chondroitin sulfate has been presented as able to inhibit the action of IL-1β on osteoblast, reducing the release of PGE_2, MMP-3, and MMP-13[976], and polysulfated GAGs alone have produced improvements in orthopaedic scores in dogs with HD[977]. In humans, some studies have shown that glucosamine is as efficient as a placebo, and is not adequate for the treatment of chronic, severe, OA related pain[813,978]. Other reports concluded that chondroitin sulfate has several beneficial effects, reducing pain, improving articular function, reducing joint swelling and effusion while preventing joint space narrowing[979–981]. The recommendations for human hip and knee OA stipulate that treatment with glucosamine and/or chondroitin sulfate may provide symptomatic benefit, but should be discontinued if no apparent response is observed within six months. Structure-modifying effects have also been attributed to the compounds, although a 2-year study showed no significant reduction in joint space nor a clinically relevant improvement in pain and joint function[580,888,972].

New Zealand green-lipped mussels have a quantitative composition of GAGs, omega-3 fatty acids, vitamins, and minerals, which have been suggested to act synergistically in reducing inflammation, limiting cartilage breakdown, and supporting cartilage regeneration[982,983]. The evidence for the efficacy of green-lipped mussel is moderate[578]. It has been described as able to alleviate chronic orthopaedic pain in dogs, although it is not as effective as carprofen[966]. At a cellular level, it can inhibit COX activity, and also decrease IL-1, IL-2, and TNF-α levels[984]. Other reports indicate contradictory findings, with green-lipped mussels being unable to reduce IL-1β but able to downregulate the expression of MMPs[985]. Long term administration (8 weeks or longer) has been described as able to alleviate clinical signs of dogs presumptively diagnosed with mild to moderate

OA, in terms of veterinary-assessed mobility, owner-evaluated pain index, pain, and locomotion[596,986,987].

Polyunsaturated fatty acids are classified as omega-3, omega-6, and omega-9, depending on the position of the last bond along the fatty acid chain[581]. COX can metabolize the ω -3 and ω-6 into distinct eicosanoids, and ω-6-derived eicosanoids tend to be pro-inflammatory while the ω-3-derived eicosanoids tend to be anti-inflammatory[988]. In humans, both eicosapentaenoic acid and docosahexaenoic acid ω-3 polyunsaturated fatty acids can reduce pain, improve clinical signs of joint diseases. Also, eicosapentaenoic acid and arachidonic acid can decrease several inflammatory markers. To maintain an appropriate balance of both ω-3 and ω -6 fatty acids in dietary interventions is a point to keep in mind[985,989,990]. Fish oil and corn oil supplementation decrease the formation of pro-inflammatory prostanoids which, in excess, increase inflammation, and also monocytes, basophils, and ROS associated with cytokines and prostaglandins[991]. Supplementation with fish oil has also demonstrated the ability the beneficially change heart rate at rest and after exercise, while also improving muscle mass gain in response to exercise[992]. Several studies reported the ability of polyunsaturated fatty acids to decrease the degree of lameness, increased PVF, and ability to rise from a resting position[310,993–995]. At a molecular level, polyunsaturated fatty acids s can reduce inflammatory markers concentration, such as PGE_2, IL-1, and IL-6[996,997]. Different sources of ω-3 have different cartilage-protecting properties, with krill oil having greater/equal potential than fish oil, which, in turn, has a greater effect than green-lipped mussel[985]. eicosapentaenoic acid in dogs can alter the expression of genes responsible for the progression of cartilage degradation and can be applied in the design of pet foods[347]. In juvenile growing dogs with HD, a less severe grade of osteoarthritis at 12 months was observed in supplemented dogs, compared with a control group[998]. The combined used of fatty acids and NSAIDs seems to be more effective than their isolated use[999].

Avocado-soybean unsaponifiable are plant extracts derived from unsaponifiable residues of avocado and soya bean oils, which contain fat-soluble vitamins, sterols, triterpene alcohols, and possibly furan fatty acids[1000]. In vitro, these compounds have been shown to reduce several pro-inflammatory mediators, such as TNF-α, IL-1β, COX and PGE_2 synthesis, and anticatabolic cytokine expression, mainly MMP-3 and MMP-13[1001,1002]. They also stimulate the synthesis of matrix components by chondrocytes by increasing TGF production and decreasing MMP production[693]. In a canine model of OA, patients treated with avocado-soybean unsaponifiables had smaller and less severe macroscopic cartilage lesions, with lower scores of histological parameters, as decreased loss of subchondral bone[1003].

Other substances, such as curcumin have also been described as having antioxidant, anti-inflammatory, and antiseptic properties, with similar ability as NSAIDs to reduce IL-1β and TNF-α

levels, both *in vitro* and *in vivo*[1004,1005]. Green tea extract, in conjunction with curcuminoids extract, did not also produce significant changes in GRF, although a slight reduction of pain may have been registered[1006]. There are no controlled studies in dogs assessing the efficacy of dietary antioxidants in OA, but there is a growing scientific rationale for their use as adjuncts in the treatment of OA[347]. Dietary vitamin E, in dogs with surgically induced OA, produced consistent lower visual analogue scale and numerical rating scale scores, due to the vitamin's ability to affect the synthesis of PGE_2 and NO in SF, by inhibiting the activation of transcription nuclear factor kappa B. Even though it does not reduce IL-1β concentration levels, it was able to reduce histological lesions in the articular cartilage[1007]. Vitamin C and selenium also have powerful antioxidant effects, quenching free radicals and defending tissues against oxidative stress. Homoeopathic preparations have also been studied, with some showing beneficial effects in the management of OA related pain, such as Traumeel® and Zell®[1008–1010].

The majority of commercially available formulations are multicomponent, with the idea that several compounds may interact with multiple targets to trigger interdependent activities to achieve optimal effects[1011]. In human trials, nutraceuticals have recently been shown to have potential in relieving OA pain. Emerging evidence indicates that they may represent interesting alternatives for the relief of OA pain. However, future studies should prioritize elucidating the mechanisms of action of nutraceuticals in OA and developing nutraceuticals that not only relieve OA pain but also mitigate OA pathology and may decrease radiographic sign progression[998,1012].

d. Rehabilitation;

Rehabilitation is the treatment of diseases and injuries with physical agents, such as heat, cold, US, electricity, massage, and exercises. It involves, as athletic training, the application of controlled forces to musculoskeletal system structures, to stimulate and facilitate adaptions suited to a specific therapeutic objective. In many cases, it helps to reduce the dose of analgesics necessary to maintain a patient comfortable[29,594]. Even though rehabilitation for animals is a growing field, a limited number of studies have been conducted to address the effectiveness of these methods and their effect on the biomechanics of joints and muscles[464,1013]. There are many nonpharmacologic treatment options available for the conservative management of OA, which are often recommended by veterinarians, with client education being an important component of the management protocol[878,1014,1015]. The recommendations for non-pharmacological modalities of treatment for OA in humans stress the importance of lifestyle changes, exercise, the pacing of activities, weight reduction, and other measures to unload damaged joints[580]. It is through movement that fluid goes from the ECM to the synovial fluid and *vice versa*, the mechanism by which nutrients are delivered to chondrocytes.

For this reason, early mobilization and controlled loading are one of the focus during rehabilitation following joint trauma and/or surgery[29]. Management of body weight is one the most important of medical therapies, with dogs of appropriate body weight living an average of 22 months longer, with the later onset and less chronic disease, with fewer signs of OA[1016,1017]. Adipose tissue in obesity secretes pro-inflammatory cytokines (TNF-α and IL-1), which results in increased inflammation in OA[1018]. This reduction of body weight should be obtained through a combination of caloric restriction and an exercise program[1019]. The inclusion of a controlled exercise plan, in combination with a dietary weight loss program in these animals, helps prevent the loss of lean body mass[1020]. A therapeutic approach to dogs with hip pain due to OA usually focus on multi-modal pain relief and strengthening of the gluteal muscles.

Rehabilitation options for pain relief may include thermal modalities, laser therapy, transcutaneous electrical nerve stimulation, massage, acupuncture, and others[338,1016,1021]. Thermal modalities are routinely incorporated in the treatment of OA. Cryotherapy is used in acute inflammation, and promotes vasoconstriction, skeletal muscle relaxation, and decreases nerve conduction. It helps to reduce inflammation, minimizes oedema through vasoconstriction, decreases enzyme activity, and metabolism on tissues while providing analgesia[919,1022–1024]. Heat is mostly used after the acute inflammation phase has been resolved, and is often applied before stretching, massage, passive ROM exercises, or active exercises[594]. Heat therapy can also relieve pain, provide muscle relaxation, and increase the extensibility of articular or ligamentous collagen[1022]. The therapeutic US can be used to heat deeper tissues and to help control pain and improve tissue extensibility. While superficial heating agents only penetrate soft tissues to a depth of approximately 1 cm, deep heating agents can elevate tissue temperatures at depths of 2 cm or more. The therapeutic US also promotes a non-thermal phenomenon shown to accelerate the inflammatory phase of wound healing, promote ion transport, to increase cellular permeability, and promote healing by stimulating fibroblastic activity, increasing cellular metabolism and circulation[594,1025]. It is considered an effective treatment modality for rehabilitating musculoskeletal conditions such as a restricted range of motion (ROM) resulting from joint contracture, pain and muscle spasm, and wound healing[1026,1027].

Laser therapy, or photobiomodulation, has been increasingly incorporated into rehabilitation programs for a variety of conditions, like muscle, tendon, and ligament injuries, OA, and pain[1028] Lasers used in rehabilitation help to modulate cellular functions. This process, known as photobiomodulation, is defined as the nonthermal interaction of monochromatic radiation with a target site. This photobiostimulation upregulates the production of ATP, NO, and reactive oxygen species within cells, alters gene transcription, and leads to an increase in cell proliferation, cellular motility, and growth factor production[1029]. Also, acute and chronic pain control has been reported

using this type of low-energy photon therapy. Treatment of chronic and acute oedema, neurologic conditions, to reduce pain and postoperative care are some other prevalent conditions treated with laser therapy[341,594,1030–1032]. Its affect is affected by coat color and in unshaved treatment areas, making it necessary to adjust the treatment dose to the particular animal[1033]. In dogs, its use is associated with improvement in PVFs of pelvic limbs orthopaedic surgery and shortened duration to ambulation following hemilaminectomy[1034,1035]. Its effects have been compared to a placebo, leading to significant improvements and lower NSAID doses required to managed patients with OA[1036]. It is important to use it in conjunction with other modalities, as exercise, massage, and pharmacologic options. It may give patients enough comfort to allow patients to initiate or increase specific exercise protocols, such as increasing the range of motion of a stiff joint[1037] (Figure 29).

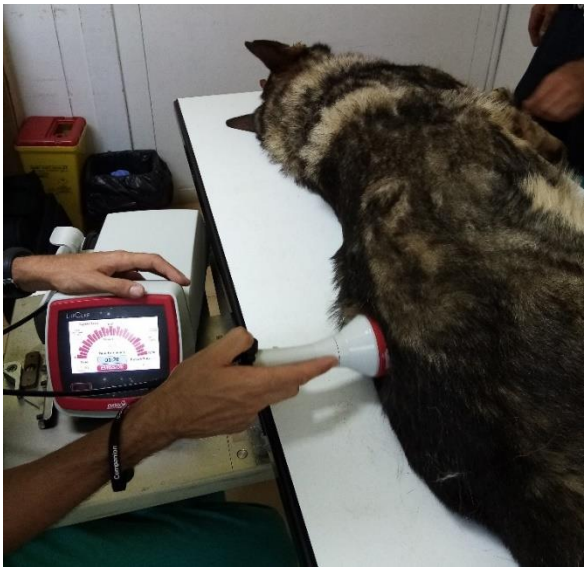

Figure 29 – A dog with hip osteoarthritis, with associated back pain, being treated with laser therapy.

Electrical stimulation is another commonly used modality in physical therapy, useful for many purposes, including increasing muscle strength, muscle re-education, increasing ROM, enhancing function, pain control, accelerating wound healing, reduce oedema, muscle spasm reduction, and enhancing transdermal administration of medication (iontophoresis). transcutaneous electrical nerve stimulation (Figure 30) is a particular type of electrical stimulation, used to increase muscle strength, improve joint ROM, re-educate muscles, and decrease oedema and pain[594,1038,1039]. Different reports show that treated dogs have increased ROM and a 'positive slope' when compared to dogs in a control group[1040,1041]. This positive effect is also true in humans, where the benefit over placebo for certain aspects of OA has been described, making it an adjunct therapy for the treatment of patients with arthritis involving the hips and/or knees[1042].

Figure 30 – A transcutaneous electrical nerve stimulation equipment.

In a systematic review, weak evidence to support the use of electrostimulated acupuncture and the extracorporeal shockwave was observed[579]. Radial shock wave therapy has also shown to increase mean PVF and VI values in treated limbs of dogs with hip OA, in contrast to no significant differences found in control limbs[1043]. In humans, it has also helped to decrease pain in patients with knee OA[1044]. Pulsed electromagnetic field therapy is a non-invasively treatment modality, used to treat a variety of conductions by delivering electric and magnetic fields to tissues via inductive coils. In OA, it assists in the reduction of pain, inflammation, and edema[1045]. The combination of acupuncture and manual therapy has shown to provide immediate short-term improvement in comfort and mobility[1046]. Differing results have been obtained when comparing acupuncture with placebo and carprofen, with neither of the therapeutic options providing significant improvements in dogs with HD[894]. Other reports describe the use of acupuncture alone or in combination with analgesics reduced pain and improved quality of life in dogs with musculoskeletal diseases[1047]. Thermal modalities, transcutaneous electrical nerve stimulation, and acupuncture have all demonstrated their ability in relieving symptoms, particularly pain[580,1048].

In human medicine, the benefit of referral to a physical therapist for evaluation and instruction in appropriate exercises to reduce pain and improve functional capacity is well established[580]. Therapeutic exercise is a cornerstone of rehabilitation and is used to improve active joint range of motion (ROM), improve weight-bearing and limb use, build strength and muscle mass, and increase conditioning (endurance, speed, and others), joint health, proprioception and overall functioning (Figure 31)[1049].

Figure 31 – A dog with hip OA during a rehabilitation session, performing balance exercises.

Hydrotherapy (swimming or using an underwater treadmill), is an increasingly popular modality for the rehabilitation of dogs and also shows benefits in the management of OA, being one the most elected modalities of the management of this condition[1050–1053]. A single hydrotherapy session increases ROM in both sound and animals with OA, to a greater degree in OA animals[1054]. This beneficial effect was also observed after joint surgery[1055]. Aquatic exercises, in particular, have been praised for OA patients due to the buoyance effect of water, which allows exercise to be conducted without significant joint impact[338,580,1056,1057]. In the post-surgical period, exercise leads to ticker repair tissue, but not necessarily of better histological quality[1058]. Aerobic exercise, in conjunction with a range of motion exercises, can effectively enhance the function of joints, reduce pain, and also improve joint function in patients[1022,1059]. Massage therapy can also be used to relieve pain and in orthopaedic rehabilitation, but the techniques described are initially intended for use in humans, and scientific data supporting anecdotal. The description of beneficial effects in domestic animals is still lacking[1060]. Stretching exercises are commonly used in companion animal rehabilitation programs and a critical aspect of the management of joint contractures[1061].

Animals with hip OA should undertake a low impact exercise program to help strengthen the hips, like swimming or hill walking, to build muscle mass, improve comfort, and increase ROM. Rehabilitation can also help reduce the progression of osteoarthritis in the post-surgical period. Targeted exercise therapy aids in the management or prevention of many of the consequences of immobility and decreased joint use and loading, including atrophy of soft and bony tissues, stiffening or fibrosis, while maintaining proper proteoglycan matrix and stimulating the synovium to replenish joint fluid[997,1062]. There are reports that physiotherapy improves kinetic parameters in different dogs

treated with the same surgical technique for ACL ruptures at four months postoperatively[814]. Moderate exercise has been shown to improve cartilage GAG content in humans at risk of developing OA[338,1063]. In dogs, regular walks or light jogs in soft surfaces, and hilly terrains should be encouraged, as it builds different muscle groups and may help decrease the risk of developing radiographically detectable hip dysplasia[338,573,1064]. Moving uphill, in particular, increases extension of the hip joint, to a level similar to non-lame dogs (Figure 32). Moving downhill, on the other hand, significantly decreases ROM[464]. Passive ROM exercises can be effective in restoring a more normal joint motion in patients, to advance to a more comfortable ROM, improve blood and lymphatic circulation, and stimulate sensory awareness[594]. Used in combination with other therapeutic options, such as PRP, rehabilitation enhances obtained results for more extended time periods[1065].

Figure 32 – A dog with hip OA on a treadmill, with an increased inclination, to simulate an uphill walk.

e. Surgery;

Juvenile pubic symphysiodesis is a preventative, minimally invasive surgical intervention for dogs between 12 and 16 weeks of age, which are at risk of development of hip dysplasia and, consequently, OA. It consists of the premature surgical closure of the pubic symphysis induced by thermal destruction of the symphyseal growth plate. This procedure results in an increase in the ventral rotation of the acetabulum during growth, providing better congruity, and preventing secondary OA[1066]. A two-year follow-up report indicated that juvenile pubic symphysiodesis surgery at 12–24 weeks of age significantly improved hip conformation and decreased laxity in at-risk HD dogs. At this timeframe, juvenile pubic symphysiodesis offers greater benefits than surgery performed at 19- to 24-weeks-old[1067]. Long-term reports indicate that the procedure does not eliminate the hip joint laxity, characteristic of hip dysplasia, or the progression of degenerative changes. Still, it increases the odds of arresting or limiting the progression of HD in mild to moderate grades[1068,1069].

Total hip replacement and femoral head and neck osteotomy are the key surgical options for managing chronic coxofemoral joint pain[313]. Femoral head and neck osteotomy is a salvage procedure, intended to eliminate most of the pain caused by OA in the mature dog. It can be performed in dogs of any age, but likely produces better results in dogs under 20kg. The goal is to eliminate bone-on-bone contact of the diseased acetabulum and the femoral head and to permit the formation of a pseudo-arthrosis[1066,1070,1071]. With femoral head and neck osteotomy, the outcome is variable and unpredictable, depending on surgical skill, length of time that OA has been present, and severity of the pathology. However, if removal of the entire femoral neck is achieved, in conjunction with appropriate postoperative rehabilitation and analgesia, many dogs achieve satisfactory limb function[313,1071].

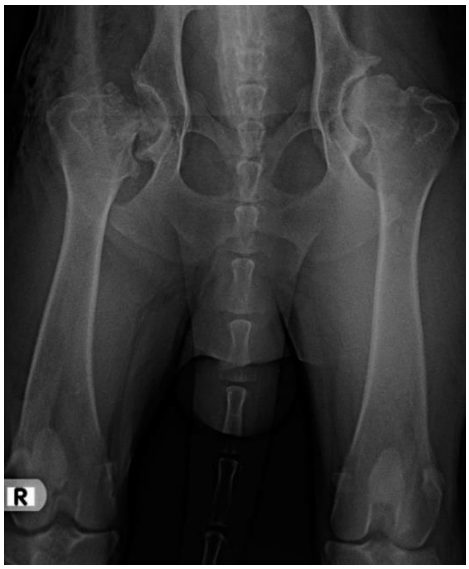

Figure 33 – Ventrodorsal extended view of a dog following femoral head and neck osteotomy, with incomplete removal of the femoral neck.

Total hip replacement is a salvage procedure, involving the replacement of the diseased acetabulum and femoral head with implants. The goal is to return a chronically lame dog, that is nonresponsive to medical treatment, to near-normal or normal function, including athletic, sporting, and working activities[1066,1071,1072]. It is a costly surgical option, with no guarantee of success, but most dogs return to full function by eight weeks after surgery. A 6-year follow-up described a high survival rate for the procedure and with an excellent clinical function[866,1073,1074].

II. MATERIALS AND METHODS

Study design and patient selection

In this experimental, randomized, double-blind study, we aimed to evaluate the effectiveness of 4 different intra-articular treatments in the management of osteoarthritis, using the species *Canis familiaris* as an OA naturally occurring canine model. The major goals of the study were:

1. Determine the effect of 4 substances delivered by IA, in patients with hip joint osteoarthritis;
2. Evaluate the variation in the synovial fluid of patients with hip joint OA of inflammatory markers levels such as CRP and IL-1 through time after the IA administration of the different substances used for the OA treatment;
3. Assess the relationship between laboratory and imaging medicine data, and the patient's objective medical examination;
4. Validate the use of digital thermography and weight bearing evaluation in OA assessement;
5. Outline a therapeutic for IA treatment protocol for patients with hip OA using the dog as an animal model, under the general concept of One Health.

The study protocol was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-Estar dos Animais da Universidade de Évora, approval nº GD/32055/2018/P1, September 25th, 2018). Written, informed consent was obtained from the Institution responsible for the animals (Guarda Nacional Republicana, Portuguese Gendarmerie) through dispatch of the Doctrine and Training Commander nº327/16, dated September 16th, 2016. Animals included in this study were recruited from the police working dog population of the Grupo de Intervenção Cinotécnico, Guarda Nacional Republicana (Portuguese Gendarmerie Canine Unit). They constituted a convenience sample, similar in size to previous reports concerning this subject, comprising 100 joints (n=1 joint)[586,686,738,898,969]. Animals selected presented clinical signs of osteoarthritis of the coxo-femoral joint, of natural progression, with clear identifiable clinical signs, namely: loss of performance, pain during joint manipulation, decrease amplitude during the swing phase of the movement, and muscle atrophy, confirmed through digital radiography and without any other illness. Animals without clinical signs were not included in the study, and those selected for the study presented mild to severe OA. The control group was also composed of animals with naturally occurring hip OA.

Inclusion criteria comprised the following items:

- Mobility impairment, as described by the trainer and detect by the assisting veterinarian;
- Bodyweight ≥20kg;
- Age ≥2 year;
- Radiographic evidence of bilateral hip OA;
- Not to be on any medication or nutritional supplements for the previous six weeks or more.

Exclusion criteria comprised the following items:

- Suspected or diagnosed neurological/musculoskeletal disorder other than hip OA;
- Documented or suspected presence of concomitant disease;
- Being on any other treatment or drugs;
- Results of routine blood testing outside normal limits.

Study design protocol

After the selection, sample animals were randomly distributed into the 5 following groups:

- Control group (CG): IA administration of NaCl 0.9% (n=20);
- Treatment with triamcinolone hexacetonide group (THG): IA administration of TH (Bluxam, Laboratórios Farmacéuticos ROVI) (n=20);
- Treatment with stanozolol group (SG): IA administration of stanozolol (Estrombol, Laboratório Fundacion) (n=20);
- Treatment with Hylan GF-20 group (HG): IA administration of high-density hyaluronan (Synvisc, Sanofi) (n=20);
- Treatment with platelet concentrate (PCG): IA administration of platelet concentrate (V-PET, PALL) (n=20);

Animals were evaluated on 6 different time-points (ranging from day 0 until day 180) using a multiple types of modalities, as described in Table 8:

Modality	Evaluation moment					
	T0 treatment day	T1 8th day	T2 15th day	T3 30th day	T4 90th day	T5 180th day
Treatment	X					
Goniometry	X	X	X	X	X	X
Thigh girth measurement	X	X	X	X	X	X
HVAS	X	X	X	X	X	X
CBPI	X	X	X	X	X	X
COI	X	X	X	X	X	X
LOAD	X	X	X	X	X	X
Digital Thermography	X	X	X	X	X	X
Pedometer	X	X	X	X	X	X
Stance analysis	X	X	X	X	X	X
Digital radiography	X			X	X	X
SF CRP	X	X		X	X	X
SF IL-1	X	X		X	X	X
Routine blood testing	X			X	X	X

Table 8 – Evaluation modalities used in each evaluation moment. Days are counted from treatment day. Legend: CBPI – Canine Brief Pain Inventory; COI – Canine Orthopedic Index; CRP – C-Reactive Protein; HVAS – Hudson Visual Analogue Scale; IL-1 – Interleukin 1; LOAD – Liverpool Osteoarthritis in Dogs; SF – Synovial fluid.

Radiographic studies, IA administrations, and SF collections were conducted under light sedation, using a combination of medetomidine (0.01mg/kg) and buthorphanol (0.1mg/kg), given intravenously. Radiographic studies were based on the ventrodorsal extended legs and frog views, as described previously. For intra-articular administration to the hip and SF collection, animals were placed in lateral recumbency, with the affected joint uppermost. The area of interest, a 4x4cm window surrounding the greater trochanter was clipped and aseptically prepared, using a chlorhexidine solution followed by the application of 70% alcohol. For these procedures, the operator used sterile gloves and 10x10cm gauzes, and the area of interest was covered with a surgical drape. With the limb parallel to the table surface and in a neutral position, a 22-gauge, 55 to 75mm spinal needle was then inserted closely dorsal to the greater trochanter and perpendicular to the long axis of the limb[618].

For each considered study group, the specific treatment dose was determined based on the manufacturer's recommendation or previous reports. For dogs, a 0.3mg/kg dose of stanozolol has been described for IA use[839,840]. Hylan GF-20 was administered at the dose of 1 pre-loaded 2ml syringe/joint. TH was administered at a dose of 1 vial/joint (20mg). V-PET was prepared following the manufacturer's instructions. Briefly, 55 ml of whole blood was collected from the jugular vein of the patient and then introduced into the provided closed system. The blood then flowed by the action of gravity through the filter, where platelets were concentrated. The final product, 6ml of platelet concentrate, was obtained, with 3ml/joint being administered within 10 minutes without activation.

Dog's handlers were blinded for the group in which their dogs were allocated. Following treatment, all animals were prescribed a 3-day rest period[606]. Due to their nature and specific mission, all animals are followed daily by their trainers and veterinarians. Any need for additional treatment for symptomatic disease control, namely pain, was determined and registered by the assisting veterinarian.

At each evaluation moment, an online copy of the HVAS, CBPI, COI, and LOAD was completed by the trainers. Before completion, handlers received the published instructions for each of them. The CMIs were completed in sequence by the same handler in each of the follow-up assessments, without knowledge of their previous answer, in a quiet room with as much time as needed to answer all items.

For the collection of digital thermography images, the procedure followed the protocol described by M. H. Vainionpää et al., 2013. Dogs were allowed to walk around and calmly adjust to room temperature for approximately 30 min before imaging, in a room with a steady temperature, set at 21°C. They were then positioned standing in an upright position, as symmetrically as possible, without the trainer or veterinarian touching the dog's torso. If needed, the trainer could help position the dog by holding it under the abdomen. Each thermographic image included the area from the last lumbar vertebra to the first coccygeal vertebra at a minimum. All images were taken with a FLIR ThermaCAM E25 at a distance of 60 cm, to simulate a clinical setting where the space around the patient could be limited.

The weight distribution platform (Companion Stance Analyzer; LiteCure LLC, Newark, Delaware, United States) was placed in the centre of a room, at least 1-meter from the walls. According to the manufacturer's guidelines, the platform was calibrated at the beginning of each day and zeroed before each data collection. Animals were then encouraged to stand on to the weight distribution platform, and allow to acclimate, and its trainer helped to ensure the patients placed one foot on each quadrant of the platform and maintained a natural stance with their centre of gravity and stability (measured by the platform) near the middle of the platform[475]. Gentle restraint was used to maintain the patient's head in a natural, forward-facing position For all animals, at least 20 measurements were performed, and the mean value determined. A left-right symmetry index (SI) was calculated with the following formula: $SI=[(WB_R-WB_L)/((WB_R+WB_L)x0.5)]x100$[468,544], where WB_R is the value of weight-bearing for the right pelvic limb, and WB_L is the value of weight-bearing for the left pelvic limb. Negative values were made positive. Weight-bearing for a pelvic limb is 20%[475],

so deviation from this value was also considered, calculated by subtracting WB to 20. Determination of thigh girth was made with a Gullick II measuring tape, and obtained at a distance of 70% thigh length, as measured from the tip of the greater trochanter, with the leg in an extended position while in lateral recumbency, and the dog relaxed[353]. ROM of the hip joints was obtained at extension and flexion with a flexed stifle[1075]. Both measurements were made in triplicate, and the mean value was calculated. Pedometers were worn around the dog's neck, attached to an adjustable lightweight collar so that they detected and counted forelimb steps only[523]. They were placed one week before the first evaluation moment, to determine a baseline value, and then maintained up to the 30th-day post-treatment. For the 90th and 180th post-treatment days evaluation, the animals worn the pedometer for a week before that evaluation moment.

Determination of SF CRP and IL-1β concentrations were made using the DuoSet Ancillary Canine IL-1β Reagent kit (R&D Systems, United Kingdom), read with a FLUOstar OPTIMA (BMG Labtech), and Fuji Dri-Chem Slides VC-CRP PS (FUJIFILM Europe GmbH), read with a DRIChem NX500i (FUJIFILM Europe GmbH).

Statistical analysis

Multiple variables were considered for data collection, for posterior use in statistical analysis work. The variable operational plan is described in the following table 9:

Name	Description	Type	Definition	Source
Breed	Breed of the animal	Categorical Nominal	0 = German Shepherd Dog 1 = Belgian Malinois Shepherd Dog 2 = Dutch Shepherd Dog 3 = Labrador Retriever	Clinical software
Age	Age of the animal	Numerical	Years	Clinical software
Sex	Sex of the animal	Categorical Nominal	0 = male 1 = female	Clinical software
Treatment	Treatment to which the animal was submitted	Categorical Nominal	0 = control 1 = triamcinolone hexacetonide 2 = Hylan G-F 20 3 = Stanozolol 4 = V-PET	Clinical software
Bodyweight	Weight of the animal	Numerical	Kilograms	Scale

Pedometer	Number of steps registered by the pedometer	Numerical	Steps	Pedometer
LTL	Percentage of body weight exerted on the left thoracic limb	Numerical	Percentage	Stance Analyzer
RTL	Percentage of body weight exerted on the right thoracic limb	Numerical	Percentage	Stance Analyzer
TLs	Percentage of body weight exerted on both thoracic limbs	Numerical	Percentage	Stance Analyzer
LPL	Percentage of body weight exerted on the left pelvic limb	Numerical	Percentage	Stance Analyzer
RPL	Percentage of body weight exerted on the right pelvic limb	Numerical	Percentage	Stance Analyzer
PLs	Percentage of body weight exerted on both pelvic limbs	Numerical	Percentage	Stance Analyzer
TermoDV-L	Skin temperature at the level of the left greater trochanter, registered with a thermographic camera, on a dorsoventral view	Numerical	Degrees Celsius	Digital thermographic camera
TermoDV-R	Skin temperature at the level of the right greater trochanter, registered with a thermographic camera, on a dorsoventral view	Numerical	Degrees Celsius	Digital thermographic camera
TermoLat-L	Skin temperature at the level of the left greater trochanter, registered with a thermographic camera, on a lateral view	Numerical	Degrees Celsius	Digital thermographic camera
TermoLat-R	Skin temperature at the level of the right greater trochanter, registered with a thermographic camera, on a lateral view	Numerical	Degrees Celsius	Digital thermographic camera
MGirth	Thigh muscle girth	Numerical	Centimetres	Gullick II tape measure
GonioFlex	Flexion angle of the hip joint	Numerical	Degrees	Goniometer
GonioExt	Extension angle of the hip joint	Numerical	Degrees	Goniometer
XRayVD-a	Presence of a misshapen femoral head, with a, lose of its rounded appearance on a radiograph ventrodorsal view	Categorical Nominal	0 = no 1= yes	Digital X-ray
XRayVD-b	Presence of a flattened or shallow acetabulum, with an irregular outline on a radiograph ventrodorsal view	Categorical Nominal	0 = no 1= yes	Digital X-ray
XRayVD-c	Presence of caudolateral curvilinear osteophyte (CCO) on a radiograph ventrodorsal view	Categorical Nominal	0 = no 1= yes	Digital X-ray
XRayVD-d	Presence of new bone formation on the acetabulum and femoral head and neck on a radiograph ventrodorsal view	Categorical Nominal	0 = no 1= yes	Digital X-ray
XRayVD-e	Presence of a worn away angle, formed at the cranial effective acetabular rim on a radiograph ventrodorsal view	Categorical Nominal	0 = no 1= yes	Digital X-ray

XRayVD-f	Presence of subchondral bone sclerosis along the cranial acetabular edge on a radiograph ventrodorsal view	Categorical Nominal	0 = no 1= yes	Digital X-ray
XRayVD-g	Presence of circumferential femoral head osteophyte (CFHO) on a radiograph ventrodorsal view	Categorical Nominal	0 = no 1= yes	Digital X-ray
OFA	Hip grade, according to the Orthopedic Foundation for Animals.	Categorical Nominal	A = Excelent B = Good C = Fair D = Borderline E = Moderate F = Mild G = Severe	Digital X-ray
XRayFL-CCO	Presence of caudolateral curvilinear osteophyte (CCO) on a radiograph frog-leg view	Categorical Nominal	0 = no 1= yes	Digital X-ray
XRayFL-CFHO	Presence of circumferential femoral head osteophyte (CFHO) on a radiograph frog-leg view	Categorical Nominal	0 = no 1= yes	Digital X-ray
IL-1	The concentration of interleukin 1 in the synovial fluid	Numerical	pg/mL	ELISA test
CRP	Concentration of C-reactive protein in synovial fluid	Numerical	mg/dL	ELISA test
CRP-S	The concentration of C-reactive protein in the blood serum	Numerical	mg/dL	ELISA test
HVAS	The score obtained in the Hudson Visual Analogue Scale clinical metrology instrument	Numerical	Not applicable	Inquiry Answers
PSS	The score obtained in the Pain Severity Score section of the Canine Brief Pain Inventory clinical metrology instrument	Numerical	Not applicable	Inquiry Answers
PIS	The score obtained in the Pain Interference Score section of the Canine Brief Pain Inventory clinical metrology instrument	Numerical	Not applicable	Inquiry Answers
Stiffness	The score obtained in the Stiffness dimension of the Canine Orthopedic Index clinical metrology instrument	Numerical	Not applicable	Inquiry Answers
Function	The score obtained in the Function dimension of the Canine Orthopedic Index clinical metrology instrument	Numerical	Not applicable	Inquiry Answers
Gait	The score obtained in the Gait dimension of the Canine Orthopedic Index clinical metrology instrument	Numerical	Not applicable	Inquiry Answers
QOL	The score obtained in the Stiffness dimension of the Canine	Numerical	Not applicable	Inquiry Answers

	Orthopedic Index clinical metrology instrument			
COI	The overall score obtained in the Canine Orthopedic Index clinical metrology instrument	Numerical	Not applicable	Inquiry Answers

TABLE 9 – Evaluation modalities used in each evaluation moment. Days are counted from treatment day. Legend: CBPI – Canine Brief Pain Inventory; COI – Canine Orthopedic Index; CRP – C-Reactive Protein; HVAS – Hudson Visual Analogue Scale; IL-1 – Interleukin 1; LOAD – Liverpool Osteoarthritis in Dogs; SF – Synovial fluid.

According to its characteristics, each variable was referred to as dependent and independent. Thus:

Dependent variables:

- Evidence of OA signs;
- Evolution of hip grade;
- Changes in thigh muscle girth and joint range of motion;
- Scores obtained with different clinical metrology instruments;
- Variations in IL-1 and CRP concentration levels.

Independent variables:

- Age;
- Breed;
- Sex;
- Bodyweight;
- Treatment Group.

All results were analyzed with IBM SPSS Statistics version 20. Several statiscal tests were conducted, according to the intended analysis: Paired Samples T-Test, Repeated Measures ANOVA, with a Huynh-Feldt correction, or Wilcoxon Signed Ranks Test. Kaplan-Meier estimators were conducted and compared with the log-rank test. Cox proportional hazard regression analysis was performed to determine treatment survival. A significance level of $P<0.05$ was set.

III. RESEARCH & DISCUSSION

For the study results presentation, we decided to use the manuscripts already published and submitted to different international index journals. This chapter consists of six sections, one for preliminary research and each main objective.

1. PRELIMINARY RESEARCH

Police working dogs with hip naturally occurring osteoarthritis used as animal model to study the efficacy of a single intra-articular administration of two drugs: methylprednisolone acetate and triamcinolone acetonide - Submitted to Topics in Companion Animal Medicine, Impact factor 0.410, Quartile 2.

A Pilot Study on the Efficacy of a Single Intra-Articular Administration of Triamcinolone Acetonide, Hyaluronan, and a Combination of Both for Clinical Management of Osteoarthritis in Police Working Dogs - Published in Frontiers in Veterinary Science – Impact factor 2.140, Quartile 1.

A report on the use of a single intra-articular administration of autologous platelet therapy in a naturally occurring canine osteoarthritis model - a preliminary study - Published in BMC Musculoskeletal Disorders – Impact factor 2.050, Quartile 2.

Manuscript submitted to Topics in Companion Animal Medicine.

Impact factor 0.410

Quartile 2

Police working dogs with hip naturally occurring osteoarthritis used as animal model to study the efficacy of a single intra-articular administration of two drugs: methylprednisolone acetate and triamcinolone acetonide

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Abstract

We aimed to compare the effectiveness of intra-articular (IA) methylprednisolone-acetate (MPA) and triamcinolone acetonide (TA) in the management of naturally occurring hip osteoarthritis (OA) in police working dogs. Twenty police working dogs (N=20) were divided into two groups according to the drug injected per joint: GT (20mg of triamcinolone acetonide, TA) and GMPA (40mg of methylprednisolone, MPA). Animals were treated at T0 (treatment day), and further evaluations conducted at T1 (15 days after treatment), T2, T3, T4, T5, T6, and T7 (1, 2, 3, 4, 5 and 6 months after treatment respectively). Response to treatment was measured using the Canine Brief Pain Inventory (CBPI) and Hudson Visual Analogue Scale (HVAS). Significant was set at $p<0.05$.

Treatment was successful in reducing pain severity score (PSS) in two animals of GT at T1 (20%), three at T2-T3 (37.5%) and two at T4-T7 (28.6%). For GMPA, treatment was successful in two animals at T1 (20%), four at T2 (40%), three at T3 (30%) and two at T4-T5 (20%). When considering pain interference score (PIS), treatment was a success in two animals in both GT and GMPA from T1-T7. No significant differences were registered with CBPI and HVAS when comparing each moment with T0 nor between groups.

Intra-articular TA and MPA injection may be a treatment option for some patients. While some patients may benefit from IA with TA and MPA, further studies, aimed at determining better candidates, are required.

Keywords: Osteoarthritis, Pain, Dog, Animal Model.

Introduction

Osteoarthritis (OA) is a species transversal degenerative joint disease. It is difficult to treat, causing pain and dramatic changes in patient activity and overall performance [1–4]. In the dog, it presents an estimated prevalence of 20% with a trend for rising in the future [5–8].

Different types of animal models have been used to study OA, in it all its modalities and stages [9,10]. Considering the dog´s tame nature, the fact that their cartilage thickness is less than half the size of humans, and the occurrence of slowly progressing OA [10–12], it is considered a nearly ideal species for translation research of human OA and, for those reasons, the most used model for research. It has the advantages of being anatomically, biochemically, genomically and molecularly similar to humans, with clinical progression and treatment similarities [12–15]. This specie has been one of the preferred animal model to study, in particular, the progression of natural occurring OA and the efficacy of drugs in its treatment [11,12,16].

For the management of OA, intra-articular (IA) steroids (CS) have mainly been used in humans and horses (despite the doubts of their beneficial or potentially deleterious effects [17]),

aiming to control and decrease pain and inflammation levels present in cartilage, bone and soft-tissues surrounding the affected joint [18,19,28,20–27]. Amongst the most used CS, methylprednisolone acetate (MPA) and triamcinolone acetonide (TA) have been pointed out by the clinicians of the American College of Rheumatologist as their preference for OA IA treatment (34.6% and 21.7%, respectively) [29,30]. The primary concern associated with these drugs is the limited duration of their effects as a result of their fast clearance from the synovial space [31].

In animal models, gait and lameness analysis are used items to access pain levels. Multiple pain scales are already validated and routinely used in dogs [32]. The Canine Brief Pain Inventory (CBPI) is one of those validated tools, developed to access owner's opinion regarding their perception of the impact and level that chronic pain assumes in their pet [33]. It is divided into two sections, a pain severity score (PSS), that evaluates the magnitude of the pain of an animal, and a pain interference score (PIS), that measures the degree in which pain affects daily activities [34]. The Hudson Visual Analogue Scale (HVAS) is repeatable and a valid tool in the assessment of mild to moderate pain level in dogs, using force plate analysis as a criterion-reference standard [35].

We hypothesize that a single IA administration of MPA and TA can reduce pain scores in police working dogs with naturally occurring hip OA. We also aimed to describe and compare the use and effectiveness of intra-articular injection of TA or MPA.

Materials and Methods

This doubled blinded, prospective study, is a part of a project approved by the ethical review committee of the University of Évora (Órgão para o Bem Estar Animal da Universidade de Évora, approval nº GD/32055/2018/P1, September 25th, 2018). Written, informed consent was obtained from the Institution responsible for the animals (Guarda Nacional Republicana) through the dispatch nº327/16, dated September 16th, 2016.

A sample of 20 police working dogs (N=20) was selected from the population of police working dogs. It constituted a convenience sample, with patients signalled based on trainer complaints, physical exam and pelvic radiographic evaluation consistent with bilateral hip OA. Animals with other diseases were ruled out through physical examination, complete blood count and basic serum chemistry profile (BUN, Creat, ALT, AST, Gluc), and/or under any treatment, were not included in the study. Signed informed consent was obtained for all animals participating in the study. Dogs were randomly divided into two groups using the statistical analysis software, according to the type of drug used for hip joint intra-articular administration, namely: GT (treated with 20mg of TA per hip joint - Trigon depot, Bristol-Myers Squibb®, Spain) and GMPA (treated with 40mg of methylprednisolone acetate per hip joint - Depo-medrol, Pfizer®, Portugal).

All intra-articular procedures were performed by the same researcher and conducted under light sedation using medetomidine (0.01mg/kg) and butorphanol (0.1mg/kg), both given intravenously. Animals were placed in lateral recumbency, with the affected joint uppermost. A small window of 4x4cm area surrounding the greater trochanter was clipped and aseptically prepared, using a chlorhexidine solution 0,2% followed by 70% alcohol scrub, with sterile gloves and 10x10cm gauzes. With the limb parallel to the table surface and in a neutral position, the operator inserted a 22-gauge 75mm length spinal needle, closely dorsal to the greater trochanter and perpendicular to the long axis of the limb [36]. Confirmation of correct needle placement was obtained through the collection of synovial fluid. After the treatment session, animals were rested for three consecutive days and resumed their regular activity over five days.

Signs of exacerbated pain, persistent stiffness of gait and changes in posture exhibited by the dogs, were evaluated by the veterinarian on the days 1 and 3 after the IA procedure. If no complaints were reported, the animal could resume regular activity [37,38].

To evaluate response to treatment and compare it with an initial clinical condition, two validated tools for dog pain assessment were used: the CBPI (Appendix A) and the HVAS (Appendix B). Seven different time points were considered: T0 (before IA treatment), T1 (15 days after IA treatment), T2, T3, T4, T5, T6 and T7 (1, 2, 3, 4, 5 and 6 months after IA treatment respectively). During and after the six months, all animals remained in active service.

Collected data were analyzed with IBM SPSS Statistics version 20, and a significance level of $p<0.05$ was set. Normality was assessed with a Shapiro-Wilk test, and results of both groups by time points were compared using a Mann-Whitney Test. When comparing each instant with T0 within each group, a Paired Samples T-Test was used.

Results

The sample comprised animals of both genders (5 females and 15 males), with a mean age of 6±2.4 years and the bodyweight of 33.3±4.14 kg, and body condition score of 4/9. Four breeds were represented, German Shepherd Dogs (n=15), Belgian Malinois Shepherd Dogs (n=3) and Labrador Retriever (n=2), all with naturally occurring bilateral hip OA. GT included animals of both genders (2 females and 8 males), with a mean age of 6.2±2.3 years old and the bodyweight of 32.8±3.8 kg, 8 German Shepherd Dogs, 1 Belgian Malinois Shepherd Dogs and 1 Labrador Retriever. They were graded with mild (n=3) and moderate (n=7) hip OA. GMPA also included animals of both genders (3 females and 7 males), with a mean age of 6.1±0.7 years old and a bodyweight of 33.8±3.4 kg, 7 German Shepherd Dogs, 2 Belgian Malinois Shepherd Dogs and 1 Labrador Retriever. They were graded with mild (n=2) and moderate (n=7) hip OA. Of all animals enrolled in the study, three from

GT were excluded - two after T2 due to the development of unrelated medical conditions, and one after T3 due to inability to keep it medical follow-up. No side effects were detected in both GT and GMPA. No significant changes in body weight were recorded throughout the study.

A reduction of ≥1 in PSS and ≥2 in PIS has been defined as individual treatment success achieved, as measured by the CBPI [39]. Treatment was successful in reducing PSS in two animals treated with TA at T1 (20%, n=10), three at T2 and T3 (37.5%, n=8), and two at T4-T7 (28.6%, n=7). Improvements were registered in four animals at T1 (50%, n=10), three at T2 (30%, n=10), four at T3 (50%, n=9) and three at T4-T7. For the GMPA, results showed that treatment was successful in two animals at T1 (20%, n=10), four at T2 (40%, n=10), three at T3 (30%, n=10) and two at T4-T5 (20%, n=10). Scores improved in six animals at T1-T2 (60%, n=10), seven at T3 (70%, n=10), and four at T4-T5 (40%, n=10).

Considering PIS, treatment was a success in two animals in GT and two in GMPA; in GT from T1-T7 and in GMPA from T1-T5. Also, scores increased in the same proportion that PSS. When comparing results for each time moment with T0 or between groups, no significant differences were found. Overall CBPI score evolution can be observed in figure 1.

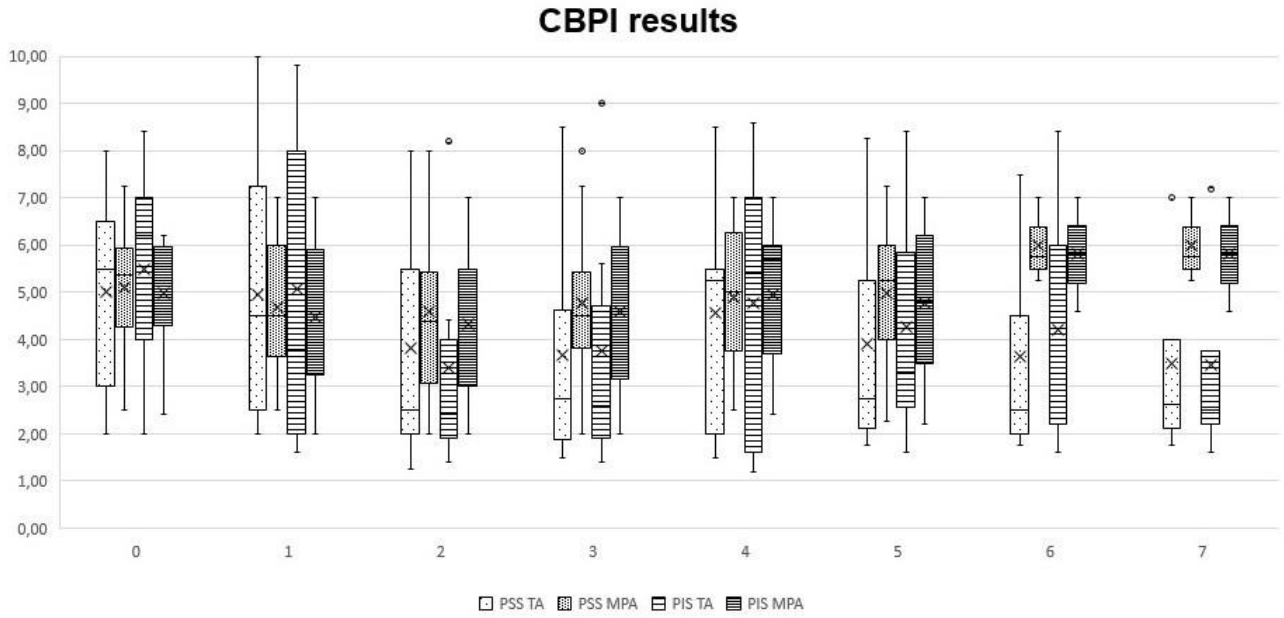

Figure 1 – Overall Canine Brief Pain Inventory scores, by section and instant for methylprednisolone acetate (MPA) and triamcinolone acetonide (TA). Box plots represent the median, 25th and 75th percentiles, and whiskers represent 10th and 90th percentiles.

When comparing each moment with T0 or between groups, no significant differences were registered in the results of the HVAS. However, individual results showed an improvement in results observed in two animals of the GT at T1 (20%), eight at T2-T3 (62.5%) and three at T4-T7 (57.1%).

In GMPA, an improvement in scores was observed in seven animals at T1 (70%), six at T2 (60%), five at T3-T4 (50%) and four at T5 (40%).

Discussion

The main focus of OA treatment is to control and decrease pain levels [40,41]. This study results show that intra-articular CS could be an effective therapeutic option for some animals since a majority of treated animals showed improved results, which may last for months. The recommendations for human hip and knee OA state that intra-articular CS can and should be used, especially in patients with moderate to severe pain, non-responding to oral analgesic/NSAIDs [42,43]. These recommendations may also be adequate for dogs.

The PSS assesses the magnitude of pain of an animal, and the PIS the level in which pain affects a dog´s daily activities, are the body of the CBPI, used for comparisons of overall mean or median differences in pain scores between groups [33,34,44]. It has the advantage of quantifying the dog's activity in its environment and over a more extensive period using the owner's assessment. Treatment success in OA dogs has been set as a decrease in PSS≥1 and PIS≥2 [39,45]. Both TA and MPA were able to reduce significantly scores of some individuals, particularly PSS. In some cases of GT, beneficial results spread up to the last evaluation point, while the majority of improvements in both groups declined around T4-T5, especially in GMPA.

Triamcinolone is presented as having an extended duration of action. In some canine reports, the authors recommended the use of TA over MPA [46,47]. In horses, a study comparing the difference between TA and MPA registered no difference between both drugs used [48]. According to our results, significant individual improvements lasted longer in GT, even though more animals showed significant improvements in the GMPA. This difference may be associated with the total amount of drug administrated in each joint.

Both drugs – TA and MPA, are approved for IA use and, since both hip joints were to be treated in each animal, we divided the content of one vial equally between joints. Therefore, 20mg of TA and 40mg of MPA were administered per joint in each animal sample. Further studies, involving different total doses, are required to determine if different doses would provide different results.

Individual results for PIS registered less marked improvements, similar for both treatment groups. Some factors may influence this. Considering that OA pain results not only from the joint tissues and structures, but also from adjacent tissues like muscles, tendons, and ligaments, it is possible that IA therapies do not completely address all of these pain sources. Besides, the fact that animals enrolled in the study are active police working dogs, their musculoskeletal structures are under great physical stress and may require a more comprehensive multi-modal approach to OA. It

may be reasonable to assume that a companion animal will need a less marked and continued pain control, thus achieving better results that can also remain significant for more prolonged periods post-treatment.

To compare different analgesic protocols, visual analogue scales are usually used to address pain scores and severity. As they rely on a continuous scale, data can be modelled as a continuous variable [49]. The HVAS is beneficial to assess lameness in dogs varying from mild to moderate, having force plate analysis as a criterion-referenced standard [35]. According to our results, no significant variations were observed in HVAS scores, even though individual results seemed to improve in almost all animals. This can be due to one of the limitations pointed out to visual analogue scales, the fact that they are more sensitive in detecting and recording changes in more obvious cases of lameness. Since the majority of animals included in the study showed only mild signs, HVAS might not be able to record subtle changes resulting from TA and MPA treatments.

Described side effects for IA CS use are mainly related to discomfort from the procedure, specifically localized pain and flushing [49]. However, no side effects were observed during the study.

Although no significant variations were observed when comparing the results of the groups, several animals showed improvements in both GT and GMPA. Future studies should include a larger number of animals since the sample size is a limitation of this study. ON the other side, animals included had similar conformations and sizes, where kept in identical housing conditions, fed the same food and submitted to similar workloads. As such, the information present may be of interest in the treatment of human and canine OA.

The lack of a control group is a further limitation and, even though both the CBPI and HVAS are validated tools for pain and lameness assessment in dogs, further studies should include other evaluation methods, such as Force Plait Gait or Stance Analysis. The determination of individual characteristics of the animals that improve with this treatment may help elect the most suitable candidates for IA CS. The effect of different treatment frequencies has also to be addressed.

Conclusions

Intra-articular CS may be a treatment option for some dogs with naturally occurring OA, being particularly useful in terms of reducing pain and return to function, with an added cost-effectiveness when compared to other therapeutic options. Further studies are required, aimed at determining which are the better candidates for the treatment, and to evaluate alternative drugs and drug dosages.

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ORIGINAL RESEARCH
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A Pilot Study on the Efficacy of a Single Intra-Articular Administration of Triamcinolone Acetonide, Hyaluronan, and a Combination of Both for Clinical Management of Osteoarthritis in Police Working Dogs

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Objectives: To describe and compare the use and effectiveness of a single intra-articular injection (IA) of triamcinolone acetonide (TA), hyaluronan (HA), and a combination of both (TA+HA) in police working dogs with natural occurring hip osteoarthritis (OA).

Study Design: Prospective, randomized, single-blinded study.

Sample Population: Thirty animals with naturally occurring hip OA.

Methods: Animals were randomly divided in three groups: GT, treated with 20 mg of TA per hip joint; GH, treated with treated 20 mg of HA per hip joint; and GTH, treated with a combination of 20 mg of TA and 20 mg of HA per hip joint. Response to treatment, measured by the Canine Brief Pain Inventory (divided in Pain Interference Score—PIS and Pain Severity Score—PSS) and the Hudson Visual Analog Scale (HVAS), was evaluated in seven different time points: T0 (before treatment), T1 (after 15 days), T2, T3, T4, T5, and T6 (after 1, 2, 3, 4, and 5 months, respectively). Results were compared using a Kruskal-Wallis test or a Wilcoxon signed ranks test, and $p < 0.05$ was set.

Results: Comparing results of the different time points considered with T0, significant differences were registered in GH at T1 for HVAS ($p = 0.03$) and PIS ($p = 0.04$); and in GTH at T1 ($p = 0.05$ for HVAS and $p < 0.05$ for PIS), T2 ($p < 0.04$ for PIS), T3 ($p < 0.03$ for HVAS and $p = 0.05$ for PIS), T4 ($p < 0.03$ for HVAS and $p < 0.05$), and T5 ($p < 0.05$ for HVAS). No significant differences were found

between groups when comparing scores in each time point. Individual treatment is considered successful with a reduction of ≥1 for PSS or ≥2 for PIS. In GTH, treatment was successful in four animals between T1 and T5 (40%, *n* = 10) and three at T6–T7 (30%, *n* = 10) for PSS and three animals of GTH at T1 (30%), two at T2 (20%), three between T3 and T4 (30%), and two between T5 and T7 (20%).

Conclusions and Clinical Relevance: This study provides direct information on the use of these treatment modalities in patients with hip OA. Intra-articular injection with TA and HA may be a treatment option for dogs with naturally occurring OA, particularly when simultaneously used, as they provide significant improvements of PIS and HVAS scores. Individual scores improved in some animals with PIS, PSS, and HVAS.

Keywords: animal model, osteoarthritis, pain, triamcinolone, hyaluronan

INTRODUCTION

Osteoarthritis (OA) is a complex joint disease with a high negative impact on patients quality of life and a high financial burden. Characterized by its inflammatory character and degradation of cartilage layers, it is a source of chronic pain, which affects all mammals, including humans and dogs (1-3). In adult active dogs, OA presents a prevalence around 20% (4-6). This value is expected to rise, due to a simultaneous increase in life expectancy and obesity. Both surgical and natural occurring canine models have been widely studied, and since pathologic process, clinical presentation and response to treatment are very similar in both species—humans and dog, this animal model is the closest to a gold standard (7-10). The changes that occur in slowly progressive spontaneous dog OA closely match those of human OA, in contrast with those seen in rapidly advancing experimental surgical induced OA (11). The grades of canine hip OA are similar to those in the classification of human OA (mild/minimal, moderate, and severe) (12, 13). In addition, companion animals share the same environment and suffer similar co-morbidities as humans, with OA usually being present for prolonged periods. Therefore, these naturally occurring painful disease models may better reflect the complex genetic, environmental, temporal, and physiological influences present in humans (12). Exploring spontaneous dog OA under the One Medicine concept can promote new insight on the disease, improving therefore the health and well-being of both species, humans, and dogs (12, 13).

Intra-articular (IA) corticosteroids (CS) have been used for several decades in humans and horses to successfully palliate pain and control inflammation associated with OA and surrounding tissues (14, 15). Different guidelines for the management of human OA provide varying strength of recommendation for the use of intra-articular CS, from weak to strong recommendation (16-20). On the other hand, other guidelines state an inability to recommend for or against the use of intra-articular corticosteroids, in this case specifically for patients with symptomatic knee OA (21). Corticosteroids reduce the number of inflammatory cells such as lymphocytes, macrophages and mast cells, and also slow down the synthesis of inflammatory mediators such as interleukin 1β, Tumor necrosis factor α, and Cycloxygenase 2 in the synovial fluid (22-25). The pain relief they provide is attributed to the inhibition of prostaglandin synthesis (24). Triamcinolone is recommended over other CS due to an extended duration of action (26, 27). Hyaluronan (HA), the high molecular glycosaminoglycan, occurs naturally in synovial fluid, and provides joint lubrication, helps limit inflammation, pain and cartilage degradation while acting as a shock absorber, allowing the joint to move in a smooth manner (16, 17). Its mechanism of action is not completely understood, but anti-inflammatory, anti-nociceptive, and chondroprotective properties have been suggested, through the enhancement of cartilage synthesis, blunting response to interleukin 1, protection from the damage of oxygen free radicals, and protection of chondrocytes from apoptosis (28-31). Guidelines for the management of OA provide a weak recommendation for the use of IA HA (20), a conditional recommendation against (19) or that they should not be offered as an option (18). The choice to use IA HA or a CS, or which CS to use, is often determined by individual preference of the clinician (18, 19). A popular approach is their combined administration, thus providing rapid onset of action (obtained from the CS), with prolonged effect and decreasing the potential side effects of intra-articular CS therapy (obtained from HA) (20, 21). Being an incurable chronic disease, treatment success in OA is often defined as the ability to manage its symptoms, mainly pain. The similarities in neurophysiology across mammals strongly suggest that the type of pain experienced by humans and animals is analogous (22). The Canine Brief Pain Inventory (CBPI) was developed to assess the impact of chronic pain in the patients life. It is divided in two sections, a pain severity score (PSS) that assesses the magnitude of the animal's pain, and a pain interference score (PIS) that assesses the degree in which pain affects daily activities (23). The Hudson Visual Analog Scale (HVAS) has been validated for the assessment of mild to moderate lameness in dogs, using force plate analysis as a criterion-reference standard (24).

With this study, we aimed to determine (1) if the intra-articular administration of triamcinolone acetonide (TA) or HA can reduce pain scores in a naturally occurring canine osteoarthritis model and if (2) the combined administration of both substances provides better results for longer periods of time.

METHODS

The study used a sample of 30 working dogs ($N = 30$) from the *Guarda Nacional Republicana* (Portuguese Gendarmerie Canine Unit) of both genders (6 females and 24 males), with a mean age of 6 ± 2.4 years old and body weight of 33.3 ± 6.65 kg. Breeds included German Shepherd Dogs ($n = 20$), Belgian Malinois Shepherd Dogs ($n = 5$), Labrador Retriever ($n = 4$), and Dutch Shepherd Dog ($n = 1$). All had bilateral naturally occurring mild and moderate hip OA, classified according to the Orthopedic Foundation for Animals scoring, This method was chosen due to the unavailability of other evaluation methods, such as PennHip. Radiographic evaluation was performed by one of the authors (JCA), not a board radiologist but with extensive training and experience in radiographic examination.

Patients were included based on trainer complaints, physical examination, and standard pelvis radiographic evaluation consistent with bilateral hip OA. Animals with other diseases were ruled out through physical examination, complete blood count, and basic serum chemistry profile (BUN, Creat, ALT, AST, Gluc) were not included in the study. Patients under any treatment, therapy or supplement were also excluded. Written, informed consent was obtained from the Institution responsible for the animals.

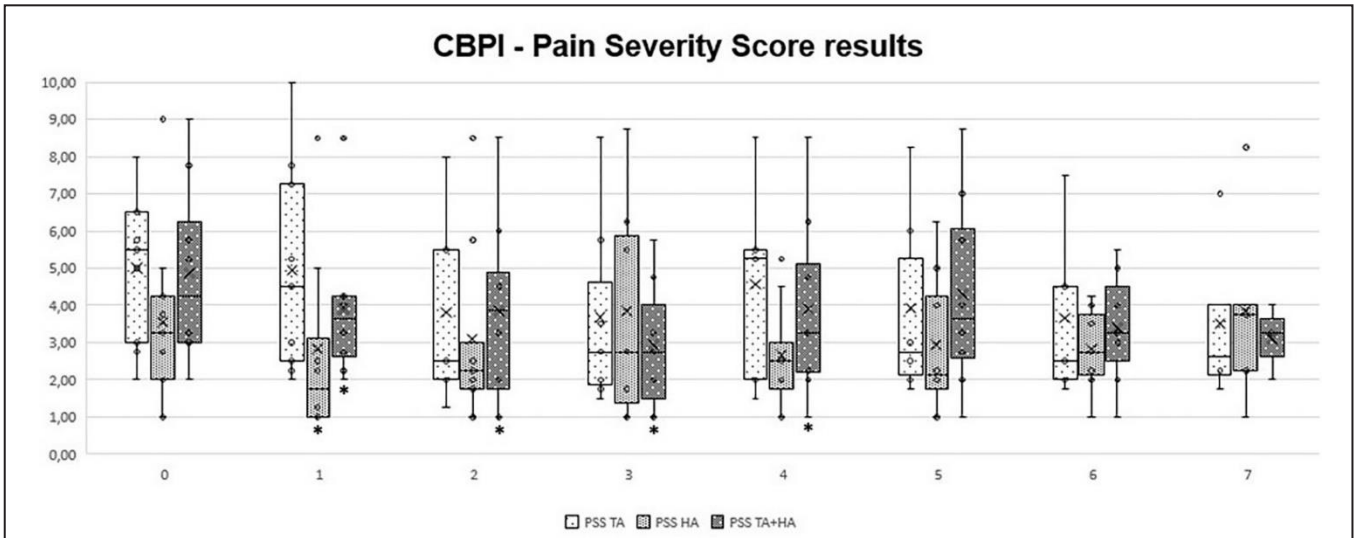

FIGURE 1 | Overall Pain Severity Scores, by section and instant for triamcinolone acetonide (TA), hyaluronan (HA), and TA+HA. Box plots represent median, 25th and 75th percentiles, and whiskers represent 10th and 90th percentiles. *indicates significant differences within the group, when compared with T0.

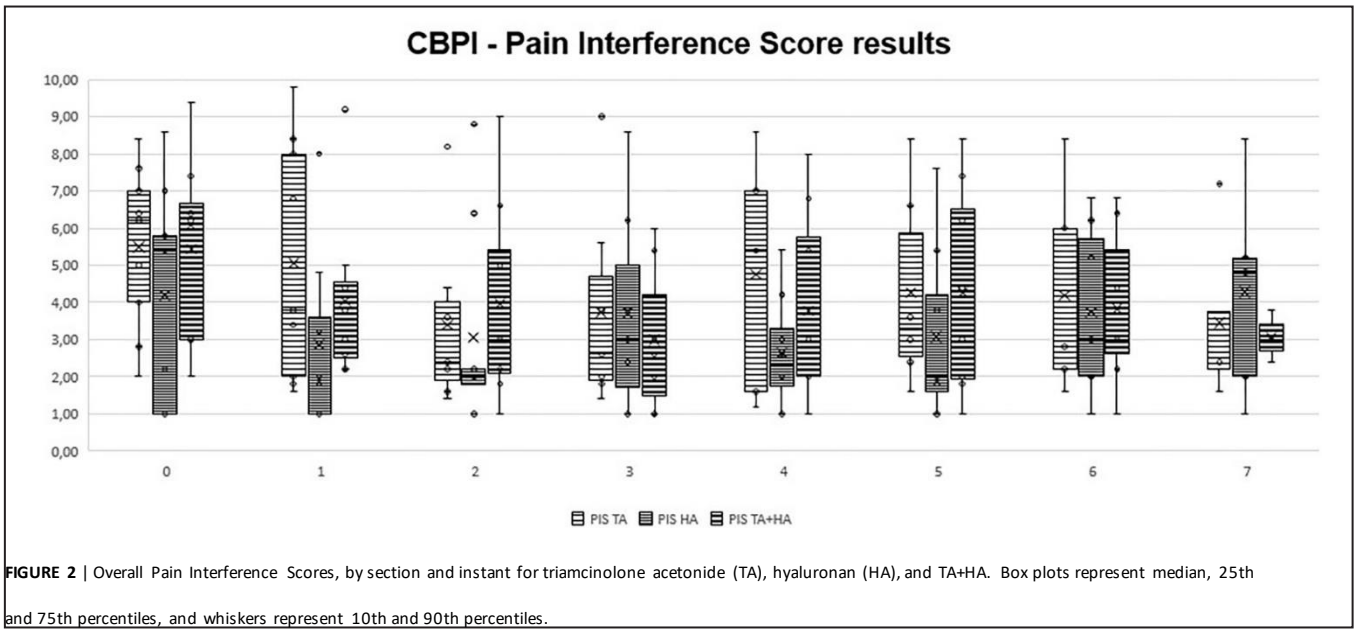

FIGURE 2 | Overall Pain Interference Scores, by section and instant for triamcinolone acetonide (TA), hyaluronan (HA), and TA+HA. Box plots represent median, 25th and 75th percentiles, and whiskers represent 10th and 90th percentiles.

Dogs were randomly divided in three groups according to the type of drug used for hip joint IA administration, namely: GT (treated with 20 mg of TA per hip joint—Trigon depot, Bristol-Myers Squibb®, Spain), GH (treated with 20mg of hyaluronanper hip joint—Hyalart, Grunenthal®, Portugal), and GTH (treated with the combination of both substances per hip joint). Breeds were similarly distributed amongst groups: GT had 7 German Shepherd Dogs, 2 Belgian Malinois Shepherd Dog and 1 Labrador Retriever; GH had 6 German Shepherd Dogs, 2 Belgian Malinois Shepherd Dogs, 1 Labrador Retriever and 1 Dutch Shepherd Dog; and GTH had 7 German Shepherd Dogs, 1 Belgian Malinois Shepherd Dogs and 2 Labrador Retrievers.

The IA administration was always made by the same clinician and conducted with dogs under light sedation using medetomidine (0.01 mg/kg) and buthorphanol (0.1 mg/kg), both administered intravenously, and with intravenous fluids of NaCl 0.9% in the dose of 2 ml/Kg/h. Animals were placed in lateral recumbency, and a small window of 4 cm × 4 cm area surrounding the greater trochanter was clipped and aseptically prepared, using a chlorhexidine solution 0.2% followed by 70% alcohol application, using sterile gloves and 10 cm × 10 cm gauzes. With the limb parallel to the table surface and in a neutral position, the operator inserted a 22-gauge with 75 mm length spinal needle, closely dorsal to the greater trochanter and perpendicular to the long axis of the limb (32). Confirmation of correct needle placement was obtained through the collection of synovial fluid. Both hips were treated with the same treatment in all animals. After treatment, animals were rested for 3 consecutive days. Signs of exacerbated pain during daily activities or physical examination (pain during joint mobilization, stiffness, and reduced range of motion), persistent stiffness of gait and changes in posture exhibited by the dogs, were evaluated by the veterinarian on the days 1 and 3 after the IA procedure. With IA treatments, some side effects were documented, and include local pain and inflammation, swelling and infection. These are usually self-limiting, and take 2 – 10 days to resolve (33, 34). If no complaints were registered, the animal could resume its normal activity over a period of 5 days (35, 36).

To evaluate the response to treatment and comparing it with the initial clinical condition, two validated tools for dog pain assessment were used: the CBPI (**Appendix A**) and the HVAS (**Appendix B**). These were completed by the trainers, who were unaware of which treatment the animal received. Evaluations were conducted at T0 (before IA treatment), T1 (15 days after IA treatment), T2, T3, T4, T5, T6, and T7 (1, 2, 3, 4, 5, and 6 months after IA treatment, respectively).

From all the sampled individuals, three dogs from the GT were excluded—two after T2 due to having developed unrelated medical conditions (one developed gastric dilatation volvulus and the other suffered a third phalanx avulsion), and one after T3 due to an inability to maintain medical follow-up. Data collected from these animals was considered up to the point of their exclusion. Data was analyzed with IBM SPSS Statistics version 20, and a significance level of $p < 0.05$ was set. Normality was accessed with a Shapiro-Wilk test and results of all groups in each time point considered were compared using a Kruskal-Wallis test. When comparing each time point with T0 within each

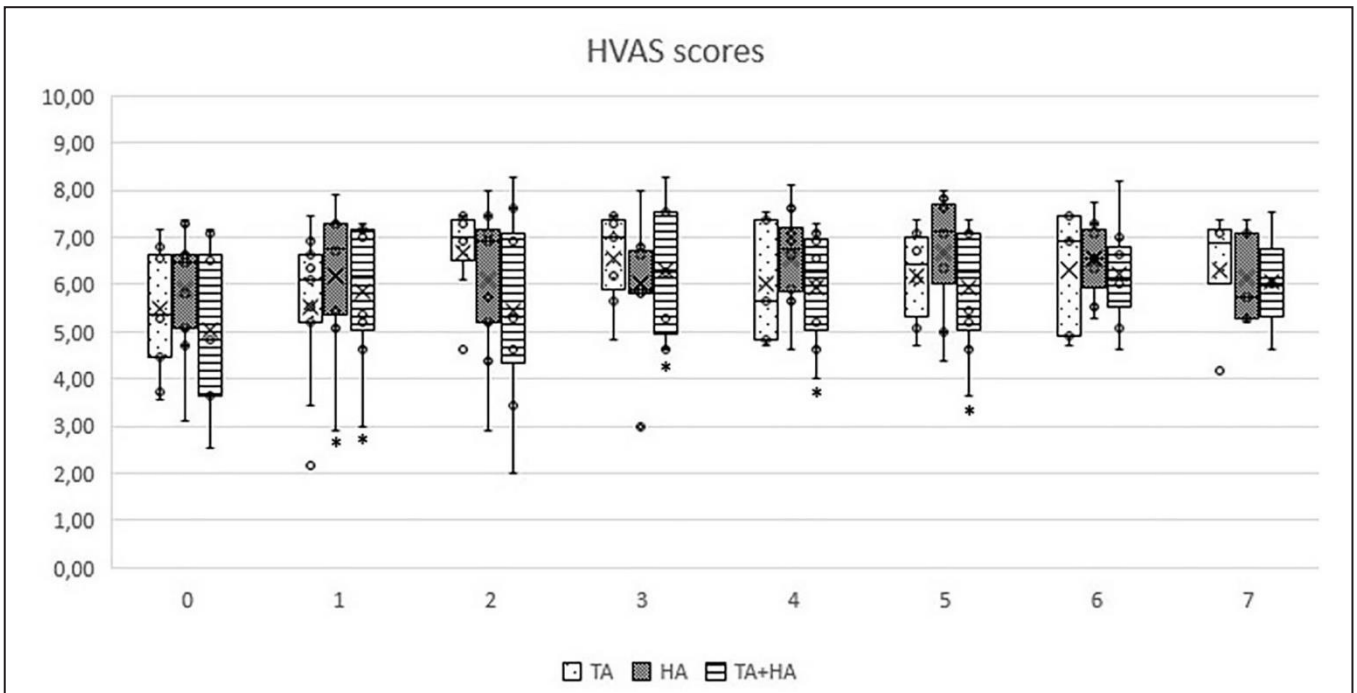

FIGURE 3 | Overall Hudson Visual Analog scores, by instant for triamcinolone acetonide (TA), hyaluronan (HA), and TA+HA. Box plots represent median, 25th and 75th percentiles, and whiskers represent 10th and 90th percentiles. *indicates significant differences within the group, when compared with T0.

group, a Kruskal-Wallis test or a Wilcoxon signed ranks test was used.

RESULTS

In GT, when comparing clinical results from T1 to T7 with patient initial condition (T0), no significant differences were recorded. In GH, significant differences were observed only at T1 ($p = 0.03$ for HVAS and $p = 0.04$ for PSS). In GTH, significant differences were observed at T1 ($p < 0.05$ for HVAS and $p < 0.05$ for PSS), T2 ($p < 0.04$ for PSS), T3 ($p < 0.03$ for HVAS and $p < 0.05$ for PSS), T4 ($p < 0.03$ for HVAS and $p < 0.05$ PSS), and T5 ($p < 0.05$ for HVAS). Comparing results of the three groups in each evaluation moment, no significant differences were found. Evolution of PSS, PIS, and HVAS scores can be observed in **Figures 1** – **3**, respectively. Evolution of mean PSS, PIS, and HVAS scores (±standard deviation), p values and percentage variations in each group, are presented in **Table 1**.

During the study, no side effects were detected in any of the animals. All patients were able to resume normal activity after treatment.

DISCUSSION

OA is a chronic disease with no cure, but with the possibility to be effectively managed in a largely palliative approach, aiming to relieve symptoms and especially pain (17, 29). Non-steroidal anti-inflammatory drugs (NSAIDs) are often considered as the first line of OA treatment. For active patients, or with more advanced OA stages, the control they provide over signs and symptoms may be insufficient (30, 31, 37). Since OA is symptomatic only in the affected joint, while lacking obvious extra-articular manifestations, it is well-suited to have a local therapy administered by intra-articular injection, reducing the total amount required to produce an effect, compared with a systemic administration (38-40). Still, this approach presents some disadvantages, as the need of a precise diagnosis, the learning curve inherent to the execution of the procedure (particularly when considering hips), better conducted with the assistance of fluoroscopy or ultrasound, and the need of placing the patient under sedation or general anesthesia. Results showed that both TA and HA, when administered through intra-articular injection, are able to reduce pain levels to some degree and up to certain time points, in a naturally occurring canine osteoarthritis model. The combination of both substances, in particular, can be an effective therapeutic option, with a majority of treated patients showing improved results in their clinical condition, lasting for several months.

Limitations of this study are associated with sample size and the lack of a control group. Additionally, even though both the CBPI and HVAS have been validated as tools for the assessment of pain, lameness, and response to treatment in dogs, further studies should include another evaluation method such as Force Plait Gait or Stance Analysis.

The CBPI is often the analysis of choice in the veterinary literature, recommended for comparisons of pain scores between

TABLE 1 | Evolution of mean pain severity score (PSS) and pain interference score (PIS) of the Canine Brief Pain Inventory (CBPI), and improvements in HVAS scores (±standard deviation), by group and moment.

Survey	Group	T0		T1			T2			T3			T4			T5			T6			T7		
		Score	SD	Score	SD	p	%	Score	SD	p	%	Score	SD	p	%	Score	SD	p	%	Score	SD	p	%	Score	SD	p	%
PIS	GT	1.90	2.33	1.00	1.1	3.82	2.15	0.42	23.6	3.68	1.97	0.49	26.4	4.55	2.24	0.46	9.0	3.92	2.14	0.42	21.7	3.65	2.15	0.19	27.0	3.50	1.75	0.27	30.0
	GH	2.32	1.97	0.17	20.9	3.08	1.80	0.23	13.3	3.86	2.55	0.75	−8.5	2.66	1.11	0.87	25.3	2.94	1.61	0.79	17.4	2.82	1.08	0.68	20.6	3.85	1.82	0.36	−8.3
	GTH	2.45	1.34	0.18	19.9	3.84	2.03	0.21	21.2	2.93	1.42	0.06	39.9	3.91	1.95	0.16	19.9	4.31	2.14	0.53	11.5	3.33	1.48	0.27	30.4	3.08	0.72	1.00	36.8
PSS	GT	2.57	2.83	0.86	7.7	3.40	1.71	0.09	38.1	3.74	2.05	0.11	31.8	4.76	2.69	0.69	13.3	4.27	2.16	0.21	22.3	4.20	2.59	0.22	23.5	3.45	1.88	0.27	37.1
	GH	2.76	1.86	0.04	32.1	3.04	2.02	0.35	27.5	3.71	2.13	0.35	11.6	2.65	1.16	0.35	36.9	3.08	1.89	0.46	26.8	3.74	2.13	0.35	10.9	4.28	0.49	0.66	43.5
	GTH	2.37	1.61	0.04	25.3	3.95	2.19	0.03	27.2	3.00	1.54	0.04	44.7	3.78	2.22	0.04	30.4	4.28	2.33	1.04	21.2	3.83	1.99	0.23	29.4	3.07	1.07	0.36	14.8
HVAS	GT										0.86	0.18	18.9														0.88	0.23	4.0
	GH																												

groups (41, 42). In this study, significant variations were observed in GH only at the first follow up, and for PSS. In GTH, improvements were also observed in PSS scores, but for a longer period. It was in GTH that the biggest improvement was observed, with a 44.7% improvement at T3. Individual treatment success has been set as a decrease in PSS ≥ 1 and in PIS ≥ 2 (28, 43). Both IA treatments with HA and TA were able to significantly reduce individual scores in naturally occurring canine OA model, particularly PSS, while improving the results for the majority of patients. In some cases of GT and in one patient of GH, beneficial results spread up to the last evaluation point, while most improvements in both groups declined around T4-T5. Results for PIS showed no significant variations, considering group results. Individual results registered some improvements, but less marked than PSS results. This may be because, for some patients, PIS scores were low to begin with, making it difficult to reach a significant reduction. In addition, these are dogs with very high prey drive and work motivation, which may lead to a good performance and low perception of pain interference during daily activities. Individual HVAS results improved in almost all animals. However, when considering group results, significant improvements were observed only in GH (at T1) and GTH, in this case up to T5. In GTH, similarly to PSS scores, improvements reached a highest of 25.5% at T3, and declined from this point, reaching an 18.2% improvement at T5. The IA use of HA for the treatment of OA is still somewhat controversial due to its mode of action being unclear and clinical trials have provided contradictory results (38). The results of our study showed improvements in some animals at T1, with the maximal number of significant improvements obtained at T2, and maintained for a couple of months. Additional administrations, compared to a single injection, may be required in order to obtain sustained results. IA CS have been used for several decades in humans to successfully palliate pain and control inflammation associated with OA and surrounding tissues (14, 15). A systematic review has deemed triamcinolone more effective in relieving pain and improving function than betamethasone and methylprednisolone acetate (44). The choice to use IA HA or CS, or which CS to use, is often determined by individual preference of the clinician (18, 19). There are reports presenting deleterious effects of IA CS, as they may induce the production of a low quantity and high viscosity synovial fluid. These results are often based on multiple injections, particularly of methylprednisolone, while a single dose does not seem to cause long-term detrimental effects (45, 46). In a canine model of OA, animals treated with IA triamcinolone showed a significant reduction of osteophyte size compared with a control group. At the histological level, it significantly reduced the severity of OA structural changes of cartilage and had no deleterious effects on normal cartilage (47). Our results show that a single IA TA is effective in reducing pain scores in some animals, but not the majority of them. For those that it was, benefits were detected for several months, in some cases up to the last evaluation point. We did not observed any clinical side-effects in the animals treated with TA, and this seems to be a safe therapeutic options in patients with hip OA. As we did not performed a follow-up radiographic evaluation of the joints, we cannot comment on the evolution of radiographic signs in the three considered groups, but it should be addressed in future studies.

Results observed in GTH support the hypothesis that combined administration of HA and TA is superior in positive effects when comparing to the individual use of each one. PSS scores show significant improvements until T3, raising from a 25.3% at T1 to a 44.7% improvement at T3. Individual PSS scores improved in several patients up to the six-month evaluation point. HVAS in GTH also improved significantly from T1 to T5, in contrast to what was observed in GT and GH. This result is in accordance with what was observed with PIS, reflecting increased mobility, presumably due to decreased pain.

According to the authors knowledge, this is the first study that presents the description of the clinical effect of IA CS, HA and the combined use of both products in a naturally occurring canine model. The study was able to establish that all therapeutic approaches are safe, since no side effects were observed after the IA procedure in all animals of the three groups considered. All treatments can be effective for the treatment of OA, particularly the combined use of both products (TA + HA). Future studies should enroll a larger sample and considered the effect of different doses and administration frequency.

CONCLUSIONS AND CLINICAL RELEVANCE

Intra-articular administration of TA and HA may be a treatment option for natural occurring OA, particularly when used simultaneously. This study provides information on the use of these treatment modalities in patients with hip OA. Further studies are required, involving a larger number of patients and the use of a more objective evaluation method.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

This study is a part of a project approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval no. GD/32055/2018/P1, September 25, 2018). Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

JA designed the protocol, conducted treatments, and prepared the manuscript. PJ and AS selected patients and conducted treatments. CL and LC revised the protocol and prepared the

manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2020.512523/full#supplementary-material

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RESEARCH ARTICLE Open Access

A report on the use of a single intra-articular administration of autologous platelet therapy in a naturally occurring canine osteoarthritis model - a preliminary study

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Abstract

Background: Osteoarthritis (OA) represents a significant burden to societies, as it affects quality of life, performance and poses a large healthcare cost. We aimed to describe the use of a single intra-articular (IA) injection of an autologous platelet therapy in the management of osteoarthritis (OA) in a naturally occurring canine model.

Methods: Fifteen police working dogs with bilateral hip OA were treated with 3 ml of platelet concentrate per hip joint, produced with the V-PET kit. Response to treatment was measured by the Canine Brief Pain Inventory (CBPI, divided in pain interference score – PIS, and Pain Severity Score - PSS), Liverpool Osteoarthritis in Dogs (LOAD), Canine Orthopedic Index (COI, divided in four dimensions: function, gait, stiffness and quality of life - QOL) and the Hudson Visual Analogue Scale (HVAS). Seven different time points were considered: T0 (before treatment), T1 (after 15 days), T2, T3, T4, T5 and T6 (after 1, 2, 3, 4 and 5 months respectively). Results from each evaluation moment were compared with T0 with a Paired Samples T-Test, and a $p < 0.05$ was set.

Results: Significant differences were observed at T1 ($p < 0.01$ for HVAS, PSS, COI, Gait and QOL; $p = 0.01$ for PIS; $p = 0.02$ for Function; and $p < 0.05$ for Stiffness), T2 ($p < 0.01$ for PSS, PIS and Gait; $p = 0.01$ for COI; $p = 0.02$ for HVAS, Function and QOL; and $p = 0.04$ for Stiffness), T3 ($p < 0.01$ for HVAS, PSS, PIS, Function and Gait; $p = 0.01$ for COI; and $p = 0.02$ for QOL), T4 ($p < 0.01$ for PSS; $p = 0.03$ for PIS and Gait), T5 ($p < 0.01$ for COI, Function and Gait; $p = 0.03$ for PSS, PIS and Stiffness), T6 ($p < 0.01$ for PSS, Function and Gait; $p = 0.04$ for PIS; $p < 0.05$ for COI) and T7 ($p < 0.01$ for PSS, Function and Gait; $p = 0.01$ for COI; and $p < 0.05$ for PIS).

Conclusions: Autologous platelet therapy was used without apparent harm in the subjects. A single administration produced significant improvements, which lasted several months, and therefore warrants further study.

Keywords: Animal model, Dog, Osteoarthritis, Pain, Autologous platelet concentrate, Clinical metrology instruments

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Background

Osteoarthritis (OA) represents a significant burden to societies, as it affects quality of life, performance and poses a large healthcare cost [1]. It is also the most prevalent musculoskeletal disease in dogs, with an ex- pected increase, due to a simultaneous increase in life expectancy and obesity [2]. For these reasons, it raises major welfare challenges and concern [3]. Translational research is a critical step towards understanding the long-term effects of OA, and animal models provide relevant ways to study the natural history and response to treatment [4]. Canine OA models have the advantages of being anatomically, biochemically, genomically and molecularly similar to humans, with close clinical pro- gression and response to treatment. These naturally oc- curring models may better reflect the complex genetic, environmental, temporal and physiological influences present in humans, being the closest to a gold standard [4–9]. Therefore, exploring spontaneous OA in dogs under the One Medicine initiative can help improve the health and well-being of both humans and dogs [9, 10].

Pain and functional ability are important parameters in the evaluation of OA treatment efficacy [11]. The gold standard for the evaluation of lameness is through gait analysis [12] but this equipment is often confined to re- search facilities [9]. Several clinical metrology instru- ments (CMI) have been developed in order to measure outcome assessments, which for dogs are normally com- pleted by a proxy. In human medicine, they are a stand- ard, validated and accepted method for measuring chronic pain, and have formed an important part of the patient clinical assessment for over 30 years [13, 14]. The best ones developed for dogs, and that have been reported to have criterion validity, are the Canine Brief Pain Inventory (CBPI) and the Liverpool Osteoarthritis in Dogs (LOAD) [9, 14–16]. The CBPI is divided in two sections, a pain severity score (PSS), that assesses the magnitude of the animal pain, and a pain interference score (PIS), that assesses the degree in which pain affects daily activities [17]. The Canine Orthopaedic Index (COI) was developed to assess four domains in dogs with OA: stiffness, gait, function and quality of life (QOL). It has been shown to have excellent reliability and validity, and has been used to evaluate working dogs [18, 19]. Visual Analogue Scales are one of the tech- niques used to score pain and assess its severity, allow- ing the comparison of analgesic protocols. The Hudson Visual Analogue Scale (HVAS) has been deemed as re- peatable and valid to assess the degree of mild to moder- ate lameness in dogs, compared with force plate analysis as a criterion-referenced standard [20].

Autologous platelets are a regenerative treatment modality for OA, used with the aim to stimulate the natural healing cascade and regeneration of tissues by a supraphysiologic release of platelet derived factors directly at the treat- ment site, without the risk of immune rejection or dis- ease transmission [21–23]. Growth factors affect nearly every biological process [24] and, in platelets concen- trates, insulin-like growth factor (IGF-1), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF), signal cells to proliferate and influence their maturation, differenti- ation and tissue repair [25, 26]. Growth factors can be obtained from other sources, such as autologous condi- tioned plasma, and are able to reduce pain and lame- ness scores, and increase weight bearing when injected into OA joints [27–29]. In dogs, a single intra-articular (IA) PRP injection has resulted in clinical improve- ments for 12 weeks, in some cases without progression of radiographic signs [27, 30, 31]. Through this period, radiographic scores were the same as assigned before treatment [30]. Multiple injections protocols have also been described, providing improvements in ROM, pain, lameness and kinetics. Authors associated this response to treatment to an anti-inflammatory activity of PRP ra- ther than any effect on tissue anabolism or catabolism [32]. It has also been used as a part of surgical proto- cols, leading to a significant improvement in gait per- formance in the postoperative period [31, 33]. V-PET is a platelet concentrate as well as conditioned plasma, which contain many autologous anti-inflammatory me- diators and growth factors, reported to reduce pain and lameness scores and increase weight bearing in dogs with OA [27, 28].

The objective of this report is to describe the use of the platelet concentrate V-PET in the management of OA in a naturally occurring canine model. We hypothesize that a single IA administration of platelet concentrate can reduce pain scores in police working dogs with naturally occur- ring hip OA for a long period of time.

Methods

The sample comprised animals selected from the popu- lation of police working dogs of the Guarda Nacional Republicana (Portuguese Gendarmerie Canine Unit). Se- lection was made by the assisting veterinarian, based on the dog's history, trainer complaints, physical and radio- graphic findings consistent with bilateral naturally oc- curring mild and moderate hip OA, classified according to the Orthopedic Foundation for Animals scoring. Ani- mals with other illnesses or under any other treatment were not included in the study, and were ruled out through physical examination, complete blood count and serum chemistry profile. Written, informed consent was obtained for all animals.

The animals were placed under light sedation using medetomidine (0.01 mg/kg) and buthorphanol (0.1 mg/

kg), both given intravenously, and then positioned in lat- eral recumbency with the affected joint uppermost. A small window of 4x4cm area surrounding the greater trochanter was clipped and aseptically prepared. The limb was then placed parallel to the table surface and in a neutral position by an assistant, and the clinician (the same in all procedures) inserted a 21-gauge with 2.5″ length needle, just dorsal to the greater trochanter and perpendicular to the long axis of the limb [34]. Confirm- ation of correct needle placement was obtained through the collection of synovial fluid. All animals received 3 ml of platelet concentrate *per* hip joint, prepared with the commercially available V-PET kit (PALL Corporation), according to the manufacturer's instructions. Fifty-five milliliters of whole blood were collected from the jugular vein of the patient, and then introduced into the pro- vided closed system. The blood then flowed by action of gravity through the filter, where platelets where concen- trated. The final product was collected using the pro- vided syringe. After treatment, animals were rested for 3 consecutive days and resumed their normal activity over a period of 5 days. Signs of exacerbated pain, persistent stiffness of gait and changes in posture exhibited by the dogs, were evaluated by the veterinarian on the days 1 and 3 after the IA procedure. If no complaints were reg- istered, the animal could resume its normal activity [35, 36].

Response to treatment, as measured by the CBPI (Additional file 1), COI (Additional file 2), LOAD (Add- itional file 3) and HVAS (Additional file 4) was evaluated before treatment (T0), after 15 days (T1) and 1 (T2), 2 (T3), 3 (T4), 4 (T5), 5 (T6) and 6 (T7) months after ini- tial treatment. These were completed by the trainers, who were unaware of which treatment the animal re- ceived, and after receiving the published instructions for each for them. Normality was assessed with a Shapiro- Wilk test and each instant was compared with T0 with a Paired Samples T-Test. All results were analyzed with IBM SPSS Statistics version 20 and a significance level of $p < 0.05$ was set.

Results

All animals enrolled were followed during a 6-month evaluation period. The sample included 15 working dogs ($N = 15$) of both genders (8 females and 7 males), with a mean age of 7 ± 2.4 years old and body weight of 31.1 ± 4.57 kg. Four breeds were represented: German Shep- herd Dogs ($n = 10$), Labrador Retriever ($n = 3$), Belgian Malinois Shepherd Dogs ($n = 1$) and Catch Dog of São Miguel ($n = 1$). These were use of force, product and hu- man scent dogs in active work at the time of treatment and during the follow up period, and where in similar kennels of the Portuguese Gendarmerie Canine Unit. Each animal received an average total solution volume of 6 ml of platelet was produced with V-PET, divided in 3 ml per hip joint. Increased lameness after the IA ad- ministration was observed in four dogs, which was spon- taneously resolved within 48 h.

When comparing results between each time moment and T0, significant differences were observed in all mo- ments and with different CMIs. With HVAS, significant improvements were observed at T1 ($p < 0.01$), T2 ($p = 0.02$) and T3 ($p < 0.01$). When considering individual re- sults, improved results were observed in 12 animals at T1 (80%), 11 at T2 (73.3%), 14 at T3 (93.3%), 9 at T4-T6 (60%) and 8 at T7 (53.3%).

With CBPI, significant differences were observed at T1 ($p < 0.01$ for PSS and $p = 0.01$ for PIS), T2 ($p < 0.01$ for PSS and PIS), T3 ($p < 0.01$ for PSS and PIS), T4 ($p < 0.01$ for PSS and $p = 0.03$ for PIS), T5 ($p = 0.03$ for PSS and PIS), T6 ($p < 0.01$ for PSS and $p = 0.04$ for PIS) and T7 ($p < 0.01$ for PSS and $p < 0.05$ for PIS). Evolution of PSS and PIS scores can be observed in Fig. 1. Individual treatment success, as measured by the CBPI, has been defined as a reduction of ≥1 in PSS and ≥ 2 in PIS [37]. Treatment was successful in reducing PSS in 8 animals at T1 (53.3%), 11 at T2 (73.3%), 10 at T3 (66.7%), 9 at T4 (60%) and 8 at T5-T7 (53.4%). In addition, scores im- proved for 10 animals at T1 (66.7%), 12 at T2 (80%), 11 at T3 (73.3%), 10 at T4 (66.7%), 12 at T5 (80%) and 11 at T6-T7 (73.3%). Considering PIS, treatment was a suc- cess in 4 animals at T1 (26.7%), 5 at T2 (33.3%), 4 at T3 (26.7%), 3 at T4-T5 and T7 (20%,) and 4 at T6 (26.7%). Treatment also improved scores for 10 animals at T1 (66.7%), 11 at T2-T3 (73.3%), 9 at T4 (60%), 10 at T5 (66.7%) and 8 at T6-T7 (53.3%).

With COI, significant differences were observed at T1 ($p < 0.01$ for COI, Gait and QOL, $p = 0.02$ for Function and $p < 0.05$ for Stiffness), T2 ($p < 0.01$ for Gait, $p = 0.01$ for COI, $p = 0.02$ for Function and QOL, and $p = 0.04$ for Stiffness), T3 ($p < 0.01$ for Function and Gait, $p = 0.01$ for COI, and p = 0.02 for QOL), T4 ($p = 0.03$ for Gait), T5 (p < 0.01 for COI, Function and Gait, and $p = 0.03$ for Stiffness), T6 ($p < 0.01$ for Function and Gait and $p < 0.05$ for COI) and T7 ($p < 0.01$ for Function and Gait and $p = 0.01$ for COI). Evolution of COI scores can be observed in Fig. 2. When considering individual re- sults, an improvement was observed in all dimensions in all evaluation points. Regarding stiffness, 7 animals re- corded better scores at T1 (46.7%), 6 at T2-T4 (40%) and 7 at T5-T7 (46.7%). Function scores improved in 6 animals at T1-T2 (40%), 8 at T3-T4 (53.3%), 11 at T5 (73.3%), 9 at T6 (60%) and 8 at T7 (53.3%). Gait scores also improved in a large majority of animals, with better results when comparing to baseline being registered in 13 animals at T1 (86.7%) at T1, 11 at T2 (73.3%), 10 at T3 (66.7%), 9 at T4 (60%) and 10 at T5-T7 (66.7%). Re- garding QOL, 11 animals recorded better scores at T1

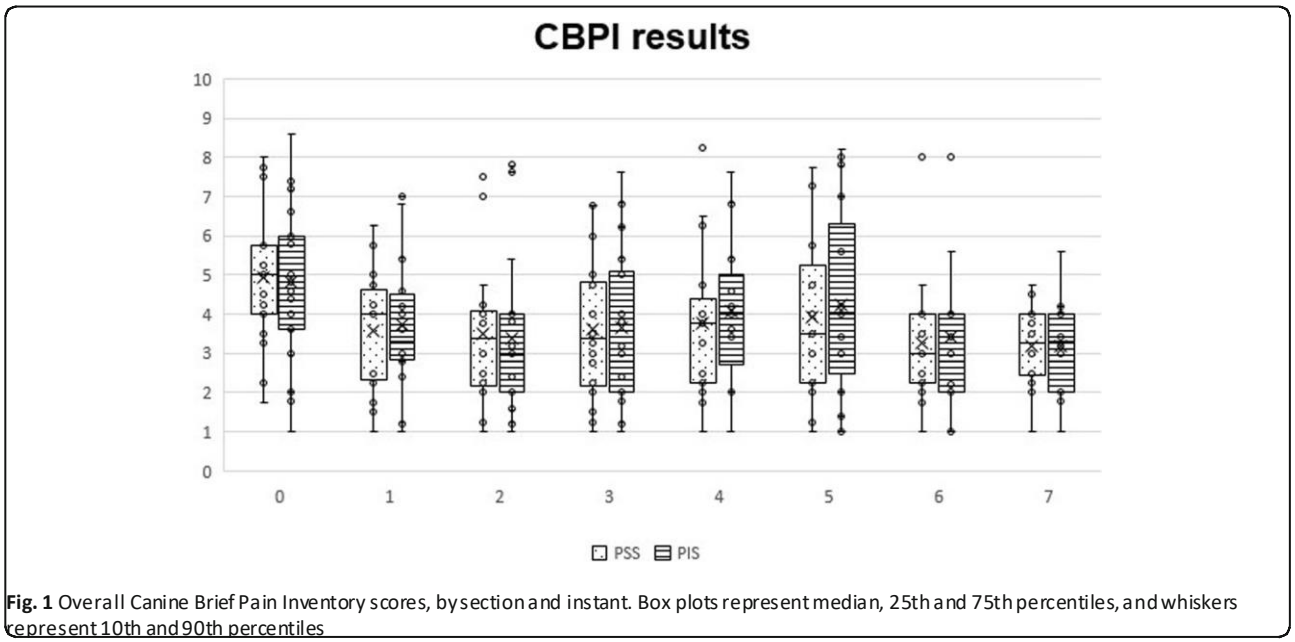

Fig. 1 Overall Canine Brief Pain Inventory scores, by section and instant. Box plots represent median, 25th and 75th percentiles, and whiskers represent 10th and 90th percentiles

(73.3%), 7 at T2 (46.7%), 9 at T3 (60%), 7 at T4-T5 (46.7%) and 6 at T6-T7 (40%). Several animals also showed better overall COI scores, namely 13 animals re- corded better scores at T1 (86.7%), 10 at T2 (66.7%), 11 at T3 (73.3%), 10 at T4-T6 (66.7%) and 11 at T7 (73.3%). No significant differences were registered in the LOAD scores when comparing each moment with T0. When considering individual results, an improvement in results was observed in 8 animals at T1 (53.3%), 10 at T2 (66.7%), 11 at T3 (73.3%), 12 at T4 (80%), 11 at T5 (73.3%) and 10 at T6-T7 (66.7%).

Discussion

OA is a common, incurable condition that, despite extensive research, still has limited treatment options available [10, 38, 39]. Its management is largely palliative, focussing on the alleviation of symptoms, mainly pain, and slowing down the progression of the disease [40, 41]. The results show that the animals included in this sample experienced significant improvements for several months, as measured with several validated CMI. Since there is a similarity in neurophysiology paths across mammals, which indicates that pain is experienced by

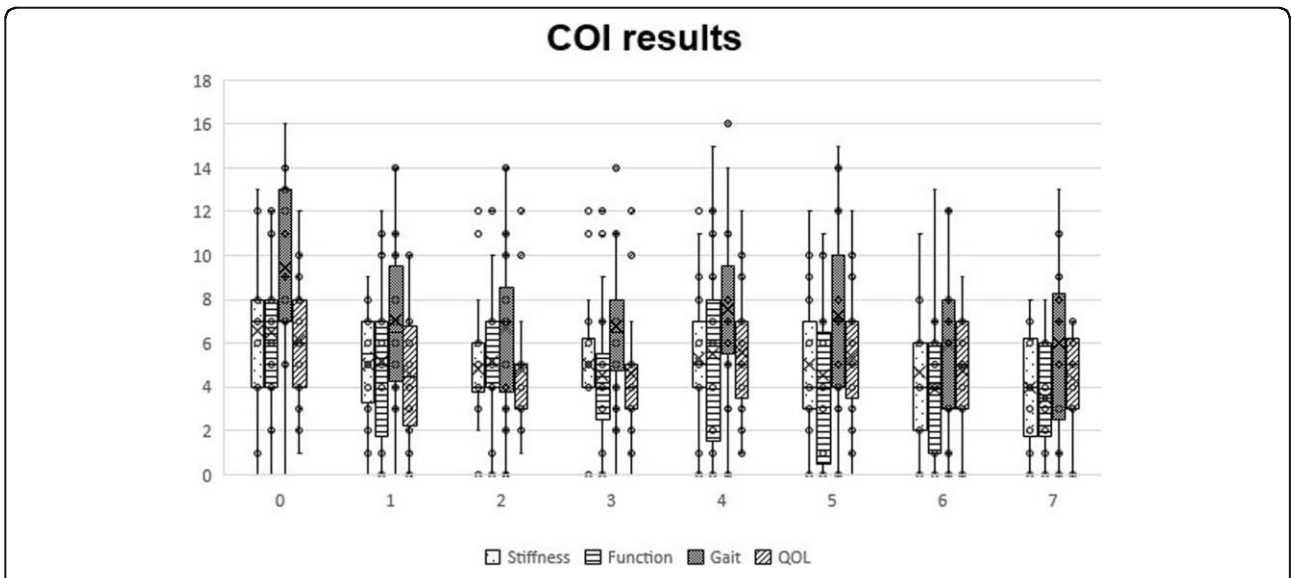

Fig. 2 Canine Orthopedic Index scores, by domain and instant. Box plots represent median, 25th and 75th percentiles, and whiskers represent 10th and 90th percentiles

humans and animals in similar ways [42], it is reasonable that these results could also be observed in humans.

Previous reports in dogs have described that a single IA autologous platelet therapy injection has resulted in clin- ical improvements for 12 weeks, in some cases without progression of radiographic signs [27, 31]. Our results show that significant improvements, when compared to baseline values, are still present at the 6-month evaluation point, a considerably longer period.

The CBPI survey is often the test of choice to evaluate chronic pain in veterinary medicine [43, 44]. Treatment success in OA dogs has been set as a decrease in PSS ≥ 1 and in PIS ≥ 2 [37, 45]. Our results show that IA autolo- gous platelet therapy was able to significantly reduce pain levels in dogs, in some cases up to 6 months. Inter- estingly, it was also able to significantly reduce pain interference scores, in contrast to other treatment mo- dalities, such as NSAIDs and nutraceuticals [46].

LOAD was initially developed to assess dog with elbow OA, but was latter deemed as reliable to asses canine OA in general [16]. It has shown good reliability, just lower than peak vertical force generated by a force platform, al- though both results correlate. CBPI and LOAD results have a moderate correlation [15, 16]. Even though im- provements in individual LOAD scores have been ob- served, no significant differences when considering the entire sample was considered. A possible explanation to this fact may be in the nature of the dogs included in the sample and of the CMI itself. Many of the LOAD items focus on the level of activity of the dog, its willingness or ability to exercise. Since the animals included in this study are working dogs with a very high work drive, it is possible that the constant willingness of these animals to exercise, even in cases of overt lameness and pain, may have led to smaller variations in LOAD scores, when compared to other CMIs. This may also be true for PIS scores, in addition to the fact that were low to begin with for many patients, making it harder to reach a significant reduction. Considering COI results, it was also interesting to ob- serve that significant improvements were observed up to the last evaluation point, specifically in overall score but also gait and function, two areas particularly affected by OA. Individual results in all dimensions improved for
most animals, in many cases up to T7.

Visual analogue scales are one of the techniques used to score pain and assess its severity, allowing to compare dif- ferent analgesic protocols. The Hudson Visual Analogue Scale (HVAS) has been deemed as repeatable and valid to assess the degree of mild to moderate lameness in dogs, compared with force plate analysis as a criterion-referenced standard [20]. In this study, significant varia- tions in HVAS scores were observed, up to T3, even though individual results improved for a majority of ani- mals during the 6-month evaluation period.

The obtained results give strength the concept that dif- ferent components of OA are captured by different CMI [16], and reinforce the advantage of using more than one of them when monitoring patients and response to treat- ment. As a whole, CMIs represent a patient-centred ap- proach, similar in human and veterinary medicine [10]. It is still unknown if respondents should be permitted or not to see previous answers. Previous reports show little dif- ference has been observed between both approaches, but allowing responders to see previous answers results in in- creased treatment effect sizes, which may increase clinical trial power [47]. In this study, in order to reduce bias, trainers were not allowed to see previous answers, as it might influence their responses, particularly with a long follow-up period.

Increased lameness was observed in four dogs, which spontaneously resolved within 48 h. This is in contrast to what is observed with NSAIDs, often the first line of treatment but with well documented side-effects, par- ticularly when for long periods [48]. It was, however, in line with what has been described in humans, were platelet concentrates can produced local and transient side-effects, such as injection pain and local inflamma- tion, that take 2–10 days to resolve [26, 49, 50]. No add- itional medication was administered to the animals during the follow up period.

This study presents some limitations, namely the lack of a control group. Even though the validity of the results is reinforced by the use of several CMIs, further studies should include other evaluation method such as Force Plait Gait Analysis or Stance Analysis. Future studies should also evaluate the effect that both different cell composition and administration frequencies have on clinical results.

Conclusions

Autologous platelet therapy showed to be a promising treatment option for the treatment of OA, as this natur- ally occurring canine model experienced significant im- provements, up to the 6-month follow up moment. Further studies are required, particularly to determine the clinical effect of different administration frequencies.

Competing interests

The V-PET kits used in this study were provided by the Pall Corporation.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12891-020-3140-9.

Additional file 1. Appendix a – the canine brief pain inventory.
Additional file 2. Appendix b – the canine orthopedic index.
Additional file 3. Appendix c – liverpool osteoarthritis in dogs.
Additional file 4. Appendix d – hudson visual analogue scale.

Abbreviations
CBPI: Canine Brief Pain Inventory; CMI: Clinical Metrology instruments; COI: Canine Orthopedic Index; HVAS: Hudson Visual Analogue Scale; LOAD: Liverpool Osteoarthritis in Dogs; OA: Osteoarthritis; PIS: Pain Interference Score; PSS: Pain Severity Score; QOL: Quality of Life; V-PET: Veterinary Platelet Enhancement Therapy

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Authors' contributions
JCA designed the protocol, conducted treatments and prepared the manuscript. PJ and AS selected patients and conducted treatments. CL and LMC revised the protocol and prepared the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials
The datasets generated and/or analysed during the current study are not publicly available since all data generated or analysed during this study are included in this published article, but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
It is a part of a project approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval n° GD/32055/2018/P1, September 25th, 2018). Written, informed consent was obtained from the Institution responsible for the animals (Guarda Nacional Republicana, Portuguese Gendarmerie) through dispatch of the Doctrine and Training Commander n°327/16, dated September 16th, 2016.

Consent for publication
Not applicable.

Competing interests
The V-PET systems used in this report were offered by the PALL Corporation.

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Publisher's Note

2. ASSESSMENT OF THE RELATIONSHIP BETWEEN LABORATORY AND IMAGING MEDICINE DATA AND THE PATIENT'S OBJECTIVE MEDICAL EXAMINATION

Clinical and diagnostic imaging findings in police working dogs referred for hip osteoarthritis - Published in BMC Veterinary Research – Impact factor 1.860, Quartile 1.

Comparison of clinical and radiographic signs of hip osteoarthritis in contralateral hip joints of fifty working dogs – Published in PLoS One – Impact factor 2.740, Quartile 1.

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BMC Veterinary Research

RESEARCH ARTICLE Open Access

Clinical and diagnostic imaging findings in police working dogs referred for hip osteoarthritis

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Abstract

Background: Osteoarthritis (OA) is the most commonly diagnosed joint disease in veterinary medicine, with at least 80% of the cases of lameness and joint diseases in companion animals being classified as OA. Sporting and working animals are more predisposed to develop OA since they are exposed to chronic fatigue injuries, leading to bone and muscular tissue damage and failure, resulting in clinical signs. To characterize the clinical signs and diagnostic findings of Police working dogs presenting with bilateral hip OA at the time of diagnosis. Fifty animals were evaluated with a bodyweight ≥ 15 kg, be older than two years, and without any medication or nutritional supplements for ≥ 6 weeks.

Results: Weight distribution, joint range of motion at flexion and extension, thigh girth, digital thermography, and radiographic signs were collected. Data from different Clinical Metrology Instruments (CMI) were collected: Canine Brief Pain Inventory, Liverpool Osteoarthritis in Dogs, Canine Orthopedic Index, and the Hudson Visual Analogue Scale. Results were compared by breed, age, sex, and Orthopaedic Foundation for Animals hip grades with the Independent Samples T-Test, ANOVA followed by a Bonferroni post hoc test, and Pearson correlation coefficient, with $p < 0.05$. The sample included 30 males and 20 females, with a mean age of 6.5 ± 2.4 years and a bodyweight of 26.7 ± 5.2 kg. Animals with weight distribution below normal levels had significant variations of joint extension and function scores. This evaluation was the only not correlated with at least one breed. Animals with caudolateral curvilinear osteophyte showed a poorer clinical presentation and worse scores in all considered CMIs. Radiographic changes correlated with age and corresponded to worse CMIs scores and weight distribution. Dutch Shepherd Dogs showed better CMI scores than the other considered breeds.

Conclusions: Police working dogs presented with complaints related to hip OA at an early stage of the disease. Hip scores influenced clinical presentation, with moderate cases showing lower thigh girth and worse pain interference and severity, and function scores than mild cases. Patients with severe OA had lower thermographic evaluations than patients with moderate OA. Age was the primary variable influencing considered CMI scores.

Keywords: Dog, Osteoarthritis, Hip, Stance Analysis, Digital Thermography Goniometry, Digital radiography, Clinical Metrology Instruments

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Background

Osteoarthritis (OA) is the most commonly diagnosed joint disease in both human and veterinary medicine, with at least 80% of the cases of lameness and joint conditions in companion animals being classified as OA [1–3]. Risk factors include breed, neutering, higher body weight, and being older than eight years [4]. Police and working animals are at increased risk of developing an orthopaedic disease than companion animals, and OA is common amongst these animals [5]. Hip OA is commonly bilateral and a consequence of canine hip dysplasia, being influenced by many genes specific for every breed [6–9].

Pelvic radiographs are frequently performed in dogs to screen hip dysplasia and OA. They have been used for over four decades in several screening mechanisms worldwide. They are also a significant determination of clinical and experimental outcome [10–12]. The most common radiographic view is the ventrodorsal hip extended view. The ventrodorsal flexed view (also called frog-legged view) enhances the visibility of the cranial and caudal aspects of the femoral head and neck. This feature helps assess the presence of circumferential femoral head osteophyte (CFHO) and caudolateral curvilinear osteophyte (CCO). These two features represent early radiographic signs that predict the development of the clinical signs of hip OA [9, 13–15].

Weight distribution and off-loading or limb favouring at stance is a commonly used subjective assessment dur- ing orthopaedic examination [16]. Animals with OA may not be overtly lame at a walk or a trot but exhibit subtle shifts in body weight distribution at a stance due to pain or instability [17, 18]. Stance analysis has been reported as sensitive for detecting lameness in dogs, with better results in large breed dogs [19]. Digital thermal imaging is a non-invasive, non-radiating, contact-free, physiologic diagnostic tool that depends on heat result- ing from physiologic functions related to skin temperature control [20–22]. It has been described as useful in several species, from humans to horses and cats, but its clinical utility has rarely been studied in small animals [21, 23, 24]. Animals with OA present a variety of clinical signs, which can vary significantly. Muscular atrophy is a consistent finding and is evident within a few weeks of OA onset [8, 25]. Restricted range of motion (ROM), including flexion and extension, is usually present [8]. The evaluation of asymmetry, assess- ment of muscle atrophy level, measurement of static weight-bearing, and ROM measurement have been described as the most valid and sensitive physiothera- peutic evaluation methods [26, 27].

Pain and functional ability are also important parameters in the evaluation of OA treatment efficacy [28]. Pain is a multi-dimensional experience with sensory, evaluative, and affective components [29]. Several clinical metrology instruments (CMI) have been developed to measure outcome assessments to approach these different dimensions. In dogs, CMIs are typically completed by a proxy. The ones developed and validated for dogs are the Canine Brief Pain Inventory (CBPI) and the Liverpool Osteoarthritis in Dogs (LOAD) [30–33]. The CBPI allows to rate a dog's pain and is divided into two sections, a pain severity score (PSS) that assesses the magnitude of the animal pain, and a pain interference score (PIS) that evaluates the degree to which pain affects daily activities [34]. The Canine Ortho- paedic Index (COI) was developed for clinical research in canine orthopedics or individual outcomes in four domains: stiffness, gait, function, and quality of life. It has been shown to have excellent reliability and validity [35]. The Hudson Visual Analogue Scale (HVAS) has been deemed repeatable and valid to assess the degree of mild to moderate lameness in dogs, compared with force plate analysis as a criterion-referenced standard [36]. By collecting information from different CMIs, it possible to characterize the disease in all dimensions, a patient's level of pain, the degree of lameness, the ability to enjoy life, and perform daily activities. It also allows characterizing the effect of a treatment in each of those dimensions.

This study aimed to characterize the clinical signs and diagnostic findings of Police working dogs presenting with bilateral hip OA. We hypothesized that differences occur when comparing breeds commonly used as Police working dogs.

Results

The sample included 50 Police working dogs, of both genders (all intact, 30 males and 20 females), with a mean age of 6.5 ± 2.4 years, bodyweight of 26.7 ± 5.2 kg, and a body condition score of 4 (70%) or 5/9 (30%). Four breeds were represented: German Shepherd Dogs (GSD, n = 17), Belgian Malinois Shepherd Dogs (BM, n = 15), Labrador Retriever (LR, n = 10), and Dutch Shepherd Dog (DSD, n = 8). Fifteen patients did not meet the inclusion criteria.

Considering OFA hip grading, 35 animals were classified as mild (70%), 10 as moderate (20%), and 5 as severe (10%). Comparing animals classified as mild and moderate, significant differences were observed in thigh girth (p = 0.01), frequency of CCO in the frog-legged view (p < 0.01), and scores of PIS (p = 0.01), PSS (p = 0.02) and Function (p = 0.01), with moderate cases presenting worse evaluations. With digital thermography, significant differences were observed comparing moderate and severe OA in the dorsoventral (p = 0.03, 25.0 ± 1.8 and 24.0 ± 1.7, respectively) and lateral views (p = 0.04, 26.1 ± 2.5 and 25.5 ± 2.4, respectively).

Measured values of overall age, body weight, weight distribution, digital thermography, thigh girth, and joint

range of motion, and divided by breed and sex, are presented in Table 1. Comparing males to females, significant differences were observed in weight and thigh girth ($p < 0.01$), with male dogs having higher values. Comparing breeds, GSD were significantly heavier than BM ($p < 0.01$) and LR ($p < 0.01$) and also had significantly higher thigh girth than BM ($p < 0.01$), LR ($p < 0.01$), and DSD ($p = 0.02$). LR were significantly older and had lower thigh girth than GSD ($p < 0.01$ for both), BM ($p < 0.01$ and $p = 0.05$, respectively), and DSD ($p < 0.01$ for both). DSD were significantly heavier than BM ($p < 0.01$). DSD also had higher measured values with digital thermography on the dorsoventral view than GSD ($p = 0.02$ for both) and on the lateral view than BM ($p = 0.04$). Thigh girth showed a correlation with breed (r=-0.34, $p < 0.01$), weight (r=-0.47, $p < 0.01$) and sex (r=-0.72, $p < 0.01$). Age correlated with joint extension (r=-0.31, $p < 0.01$), and thermographic measurement on the dorsoventral view correlated with breed (r=-0.30, $p < 0.01$). The weight distribution of both pelvic limbs correlated with joint extension (r=-0.36, $p < 0.01$), while considering the left pelvic limb, a higher value was observed (r=-0.43, $p < 0.01$). Variables considered in multiple regression statistically significantly predicted thigh girth $F(5,84) = 26.33$, $p = 0.000$, $R^2 = 0.610$, with breed ($p < 0.01$), bodyweight ($p < 0.01$), and OFA hip score ($p = 0.01$) adding statistically significantly to the prediction.

With a cut-off of weight distribution of individual limbs set at 18%, significant variations were observed on joint extension ($p = 0.02$) and the frequency of an irregular, misshapen femoral head ($p = 0.03$). At the 20% cut-off point, besides the differences in joint extension ($p < 0.01$) and on the frequency of an irregular, misshapen femoral head ($p = 0.02$), significant variations were observed in joint flexion ($p < 0.01$) and HVAS ($p = 0.03$). For both pelvic limbs with the 36 and 40% cut-offs, significant variations were observed in joint extension ($p < 0.01$), function ($p = 0.03$), presence of CCO ($p = 0.03$ at 40), and of a misshapen femoral head ($p = 0.02$).
Absolute frequencies and percentages of radiographic findings, presented by overall, by breed, and by sex, in the ventrodorsal and frog-leg views, are outlined in Table 2. Each joint was analyzed individually, for a total of 100 joints. Considering specific radiographic signs, patients with irregular wear on the femoral head were older ($p < 0.01$), with worse weight distribution ($p < 0.01$) and CMI scores ($p < 0.01$). Animals with a flattened or

Table 1 Mean values (± standard deviation) of overall weight, age, stance analysis (per pelvic limb and of the combination of both), thermography (ventrodorsal and lateral views), thigh girth and range of motion (extension and flexion) measurements, and by breed, sex and OFA score, of left and right pelvic limbs

	Weigh (kg mean ± SD)	Age (yrs, mean ± SD)	Stance Analysis (individual limb) (%, mean ± SD)	Stance Analysis (both limbs) (%, mean ± SD)	Thermography (dorsoventral) (°, mean ± SD)	Thermography (lateral) (°, mean ± SD)	Thigh Girth (cm, mean ±SD)	Joint Extension (°, mean ± SD)	Joint Flexion (°, mean ± SD)
Overall	26.7 ± 5.3	6.5 ± 2.2	18.9 ± 4.2	37.7 ± 5.7	24.9 ± 1.9	26.0 ± 2.3	30.5 ± 2.8	149.9 ± 8.4	55.9 ±4.3
German Shepherd Dog	29.9 ± 6.3	5.7 ± 1.8	19 ± 0.6	38.4 ± 4.3	24.5 ± 1.7	25.6 ± 2.5	32.2 ± 2.7	151.3 ± 6.9	56.2 ±3.6
Belgian Malinois Shepherd Dog	24.3 ± 4.1	6.5 ± 2.5	18.3 ± 5.6	37.6 ± 7.6	24.6 ± 1.5	27.6 ± 2.2	29.9 ± 2.4	148.6 ± 6.3	55.2 ±5.4
Labrador Retriever	24.3 ± 2.5	8.7 ± 2.4	19.3 ± 4.1	38.5 ± 5.6	25.1 ± 1.6	26.6 ± 2.5	28.5 ± 2.3	147.8 ± 12.4	55.1 ±3.5
Dutch Shepherd Dog	27.5 ± 3.9	5.3 ± 1.3	18.2 ± 3.5	36.4 ± 4.6	26.0 ± 2.5	26.9 ± 2.2	30.4 ± 2.0	152.0 ± 8.1	57.5 ±4.2
Male	29.0 ± 5.4	6.2 ± 2.3	19.2 ± 5.1	38.3 ± 6.4	24.8 ± 1.9	25.9 ± 2.7	31.5 ± 2.7	150.1 ± 6.4	56.1 ±4.3
Female	23.5 ± 2.8	6.9 ± 2.8	18.7 ± 3.2	37.2 ± 4.6	24.9 ± 1.7	26.1 ± 2.1	28.9 ± 2.1	149.6 ± 4.3	55.5 ±4.3
Mild	27.4 ± 5.3	6.1 ± 2.1	18.9 ± 4.2	38.1 ± 4.1	24.9 ± 1.5	26.0 ± 2.1	31.2 ± 2.9	150.9 ± 7.4	55.8 ±4.1
Moderate	25.4 ± 3.7	7.0 ± 3.4	18.4 ± 5.6	36.8 ± 6.6	25.0 ± 1.8	26.1 ± 2.5	29.7 ± 2.5	146.7 ± 11.7	56.1 ±3.1
Severe	27.1 ± 4.9	7.6 ± 1.6	18.2 ± 1.5	36.4 ± 2.3	24.0 ± 1.7	25.5 ± 2.4	29.1 ± 2.6	144.9 ± 6.2	55.0 ±4.3

Table 2 Overall, by breed and by sex, absolute frequencies and percentages within group of radiographic findings in the ventrodorsal and frog leg views, of hip joints. For each animal, both joints were considered, representing one hundred joints

Radiographic finding	Overall	GSD		BM		LR		DSD		Male		Female	
	Total/ %	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
Irregular wear on the femoral head, making it misshapen and with a loss of its rounded appearance	95	34	100,0	30	100,0	17	85,0	16	100,0	28	46,7	20	50,0
Flattened or shallow acetabulum, with irregular outline	60	23	67,6	15	50,0	13	65,0	9	56,3	35	58,3	25	62,5
Caudolateral curvilinear osteophyte (CCO)	35	18	52,9	13	43,3	4	20,0	4	25,0	24	40,0	15	37,5
New bone formation on the acetabulum and on femoral head and neck	86	31	91,2	25	83,3	19	95,0	15	93,8	51	85,0	37	92,5
The angle formed at the cranial effective acetabular rim is worn away	77	26	76,5	23	76,7	20	100,0	12	75,0	45	75,0	34	85,0
Subchondral bone sclerosis along the cranial acetabular edge	98	34	100,0	30	100,0	20	100,0	16	100,0	60	100,0	40	100,0
Circumferential femoral head osteophyte (CFHO)	28	13	38,2	10	33,3	6	30,0	1	6,3	18	30,0	14	35,0
CCO on the Frog Leg view	33	14	41,2	12	40,0	8	40,0	5	31,3	20	33,3	17	42,5
CFHO on the Frog Leg view	88	32	94,1	25	83,3	19	95,0	16	100,0	55	91,7	35	87,5

Legend: *GSD* German Shepherd Dog, *BM* Belgian Malinois Shepherd Dog, *LR* Labrador Retriever, *DSD* Dutch Shepherd Dog

shallow acetabulum, with an irregular outline, had lower weight distribution values ($p = 0.03$). Animals with CCO, on both the ventrodorsal and frog-legged views, were older ($p < 0.01$), had lower weight distribution values ($p = 0.04$), and had worse CMI scores (for all, $p < 0.01$). Those with new bone formation on the acetabulum and femoral head and neck were older ($p < 0,01$) and had worse PSS, Function, quality of life ($p < 0.01$), and PIS ($p > 0.05$) scores. Animals with a worn away angle at the cranial effective acetabular rim had lower thigh girth ($p < 0.01$) and joint flexion ($p = 0.04$). When CFHO was observable on the ventrodorsal, animals were heavier ($p = 0.04$) and had worse stiffness, function ($p = 0.02$), Gait, COI ($p < 0.01$), quality of life ($p = 0.03$) scores. The presence of CCO on the ventrodorsal was correlated with its presence on the frog-legged view ($r = 0.51$, $p < 0.01$). On the frog-legged view, the presence of CCO correlated with age ($r = 0.47$, $p < 0.01$) and joint extension ($r = -0.51$, $p < 0.01$).

Overall scores, by breed and sex, of the considered CMI, are presented in Table 3. While no significant differences were observed between male and female animals, the opposite was observed between breeds. GSD had lower function scores than LR ($p = 0.04$), while DSD had better results when compared to other breeds with HVAS ($p < 0.01$ for GSD and $p = 0.02$ for LR), LOAD ($p = 0.02$ for GSD, and $p = 0.02$ for BM and $p < 0.01$ for

Table 3 Median (range) for CBPI, HVAS, LOAD and COI, by breed, sex and OFA score, of different Clinical Metrology Instruments

	CBPI		HVAS	LOAD	COI				
	PIS	PSS			Stiffness	Function	Gait	QOL	Total
	(0–10)	(0–10)	(0–10)	(0–52)	(0–16)	(0–16)	(0–20)	(0–12)	(0–64)
Overall	2.9 (1.9–9.1)	2.8 (2.1-9.0)	6.2 (2.3–8.2)	10 (1–39)	3 (1–12)	2 (1–16)	4 (1–17)	3 (-12)	13 (1–54)
German Shepherd Dog	3.0 (1.8–9.4)	3.1 (1.3-9)	6.4 (2.1-8)	9 (1–39)	4 (1–11)	2 (1–11)	6 (1–17)	3 (0–4)	18 (3–50)
Belgian Malinois Shepherd Dog	2.5 (1.2-6.0)	2.4 (1.8–6.0)	7.0 (4.8–7.7)	8 (3–39)	3.5 (1–12)	1 (0–16)	3.5 (1–17)	4 (1–9)	9 (3–54)
Labrador Retriever	2.7 (1.0-8.2)	2.8 (1.5–7.8)	6.9 (4.1–7.9)	16 (4–36)	3.5 (1–10)	4.5 (0–10)	6 (1–15)	4 (1–12)	16.5 (2–47)
Dutch Shepherd Dog	2.2 (1.0-6.2)	2.0 (1.0-7.3)	7.3 (5.1–8.3)	5.5 (1–17)	2 (0–4)	0.5 (0–4)	1 (0–9)	2.5 (0–7)	5 (0–23)
Male	2.7 (1.2–8.6)	2.5 (1.3–7.3)	6.2 (4.3–8.3)	9.5 (1–39)	4 (0–12)	2 (0–16)	4.5 (1–17)	4 (0–9)	13.5 (4–54)
Female	2.3 (1.0-9.4)	2.9 (1.5-9.0)	6.1 (2.1–8.3)	10 (1–39)	1 (1–11)	3 (0–12)	4 (1–17)	3 (0–12)	11 (2–54)
Mild	6.5 (1.5–6.2)	2.1 (1.4–6.3)	6.3 (4.-8.3)	10.5 (1–36)	3 (0–12)	2.5 (0–16)	4.5 (0–17)	3.5 (0–12)	13 (0–47)
Moderate	5..0 (1.0-8.6)	5.0 (1.0-7.8)	5.7 (4.1–7.7)	16 (1–36)	4 (1–12)	4.5 (0–16)	6.6 (1–17)	5.0 (0–12)	20 (3–50)
Severe	5.0 (1.0-9.4)	5.0 (1.0–9.0)	5.7 (2.1–7.9)	23 (1–39)	5 (1–12)	6 (0–16)	8 (1–17)	6.0 (0–12)	25 (3–54)

Legend: *GSD* German Shepherd Dog, *BM* Belgian Malinois Shepherd Dog, *LR* Labrador Retriever, *DSD* Dutch Shepherd Dog, *CBPI* Canine Brief Pain Inventory, *PIS* Pain Interference Score, *PSS* Pain Severity Score, *HVAS* Hudson Visual Analogue Scale, *LOAD* Liverpool Osteoarthritis in Dogs, *COI* Canine Orthopedic Index, *QOL* Quality of Life

LR), stiffness ($p = 0.05$ for GSD, and $p = 0.01$ for BM and LR), function ($p < 0.01$ for GSD, BM and LR), Gait ($p < 0.01$ for GSD and LR, and $p = 0.02$ for BM) and COI scores ($p = 0.02$ for GSD, and $p < 0.02$ for BM and LR). Age was the considered variable adding statistically significance ($p < 0.01$) for the prediction of PSS $F(5,82) = 2.498$, $p = 0.04$, PIS $F(5,82) = 3.177$, $p = 0.01$, $R^2 = 0.162$, LOAD $F(5,82) = 7.873$, $p < 0.01$, $R^2 = 0.324$, stiffness $F(5,82) = 4.637$, $p < 0.01$, $R^2 = 0.220$, function $F(5,82) = 11.160$, $p < 0.01$, $R^2 = 0.405$, gait $F(5,82) = 4.074$, $p < 0.01$, $R^2 = 0.199$, QOL $F(5,82) = 3.691$, $p < 0.01$, $R^2 = 0.184$ and COI $F(5,82) = 6.046$, $p < 0.01$, $R^2 = 0.269$. Besides age, only the OFA hip score contributed to the prediction of PIS ($p = 0.03$). Correlation of age, joint extension, and CCO on a VD are presented in Table 4. Comparing animals at several cut-off points for PSS (scores of 4, 6, and 8), the same significant differences being observed consistently, with animals above the cut-off having worse joint extension ($p < 0.01$) and higher frequency of CCO on the ventrodorsal and frog-legged views ($p < 0.01$). When comparing the same cut-offs for PIS, at the 4 and 6 cut-offs, animals had to have a worse joint extension ($p < 0.01$) and higher frequency of CCO on the ventrodorsal and frog-legged views ($p < 0.01$). On the 8 cut-off point, the occurrence of all other radiographic signs was significantly higher ($p < 0.01$), and weight distribution on the left pelvic limb and both limbs was worse ($p < 0.01$).

Discussion

Hip OA is very common in large breeds such as German Shepherd Dogs and Labrador. In working dogs, it has a toll on performance and quality of life [37, 38]. To our knowledge, this is the first study to describe the clinical presentation of Police working dogs first diagnosed with hip OA. It presents a wide variety of physical examination results and several diagnostics to provide an in-depth description of affected animals.

Radiographic examination is a staple in OA evaluation. Still, it is also well established that radiographic signs develop later than the structural changes associated with OA, and clinical symptoms do not always correlate with radiographic signs [9, 39, 40]. CFHO and CCO are considered the radiographic predictors of future OA development [9]. Animals presenting with these radiographic signs had a significantly worse clinical presentation, particularly with CCO, with animals showing worse results in all considered CMIs scores, ranging from pain to lameness level and functionality. If the presence of CCO, or other radiographic findings, influences response to treatment is still to be determined. Several differences were found between OFA grades, specifically considering pain and function scores and thermographic evaluation. The sequence of these differences may occur alongside the course of OA. From mild to moderate, structural changes occur and are detected on radiographic examination, specifically CCO, one of the predictive signs of OA development [13–15]. These structural changes are then reflected in clinical signs, such as muscular atrophy and pain, which takes a toll on daily activities. With severe OA, a corresponding loss of functional tissue and muscle masses surrounding the joint occurs [21, 41]. These facts may account for the decrease in thermographic evaluation observed in severe hip grades compared to moderate hip grades. OFA hip was also one of the variables, alongside age, adding statistically significantly to the prediction of PIS scores.

Some of the differences observed during the physical examination, as the fact that GSD were significantly heavier than other breeds (such as BM and LR), also having greater thigh muscle masses, were expected. This relation also applies to male dogs being heavier than females and with higher thigh girth. Multiple regression analysis showed the effect of breed and bodyweight in predicting thigh girth, confirming these findings. It also showed that OFA hip significantly influenced thigh girth, making it a useful measure in evaluating hip OA. These variables combined may lead to a positive correlation

Table 4 Correlation of age, joint extension and presence of caudolateral curvilinear osteophyte (CCO) on a ventrodorsal view with different Clinical Metrology Instruments

Measure		Score							
		PSS	PIS	LOAD	COI	Stiffness	Function	Gait	QOL
Age	r_s	0,56	-0,32	0,5	0,48	0,43	0,59	0,38	0,40
	Sig.	0,10	< 0,01*	< 0,01*	< 0,01*	< 0,01*	< 0,01*	< 0,01*	< 0,01*
Joint extension	r_s	0,33	0,41	0,44	0,5	0,48	0,49	0,44	0,40
	Sig.	< 0,01*	< 0,01*	< 0,01*	< 0,01*	< 0,01*	< 0,01*	< 0,01*	< 0,01*
CCO	r_s	-0,45	-0,35	0,42	0,33	-0,37	0,07	-0,36	-0,31
	Sig.	< 0,01*	< 0,01*	0,23	0,56	< 0,01*	0,12	< 0,01*	< 0,01*

Legend: *PIS* Pain Interference Score, *PSS* Pain Severity Score, *LOAD* Liverpool Osteoarthritis in Dogs, *COI* Canine Orthopedic Index, *QOL* Quality of Life. * indicates significant difference

observed between thigh girth, weight, sex, and breed. The role that weight exerts in the development of hip dysplasia, and consequent hip OA, has been intensively studied, with heavier dogs showing to be more prone to develop OA earlier in life [42, 43]. This role is particularly true in dogs with higher body condition scores [4]. All of the animals included in this sample had either a 4 or 5 body condition score. Still, the fact that male dogs tend to be heavier than females (a tendency confirmed in this study) may place them under greater risk of developing OA and may account for the higher number of males observed. However, the OFA hip score was not predicted based on breed, age, sex, or bodyweight, so future studies should clarify these facts. Hip OA, when compared with OA in other joints, seems to be better tolerated by animals. This ability is mainly due to the higher amount of muscle masses surrounding this joint [8]. The quadriceps muscle group is particularly prone to atrophy secondary to decreased limb function. Therefore, measuring thigh girth helps make an initial assessment and measure patient evolution and treatment outcome [44]. In this study, we described thigh girth measurements of dogs initially diagnosed with hip OA, specifically of the breeds most commonly used as working and sporting dogs. However, it would be of interest to also have healthy subjects' values to compare both groups.

The evaluation of joint ROM is a standard measurement, with OA joints usually exhibiting ROM restrictions. In the hip joint, specifically, a ROM decrease and particularly during extension, can also be present, even though this is not a universal finding [33, 39]. It showed a correlation with age, which may be attributed to disease progression since some of the older animals had worse OFA scores. Normal ROM of the hip joint for some breeds have been described. In military working GSD, a normal ROM of 44°±6 at flexion and 155°±6 at extension, and in LR of 50°±2 at flexion and 162°±3 at extension have been reported [45–47]. Our study measured lower values in both breeds, which could be expected due to OA. Still, it would be interesting to have a group of disease-free dogs to compare these values and describe normal values in the other two considered breeds.

The mean age of animals included in this sample was 6.5 years, which is earlier than the commonly considered risk factor for OA of > 8 years [4]. GSD and DSD were even younger than 6.5 years, with only LR being beyond this point and significantly older than the other breeds. Multiple regression analysis showed that age was the primary variable adding statistically significantly to CMI scores' prediction. All of the animals included in the sample were screened before starting training and active work, so the earlier diagnosis may be attributed to the high demand and stress that these animals' musculoskeletal structures are under and the subsequent toll on performance [48]. Since these animals are active working dogs, it is possible that the disease actually develops or is simply detected earlier than in other dogs. The reason leading to a later diagnosis of LR is not clear. It may be due to breed characteristics, with LR being less explosive and less driven than BM, for example. Also, a less physically demanding mission of these dogs (most were product detection dogs) compared with the remaining animals included in the sample (mostly involved in search and rescue and use of force activities) might be an important factor to consider.

Normal weight distribution on the weight distribution plate is the same as for pressure-sensitive walkway total pressure index—30/30/20/20 (left thoracic limb/right thoracic limb/left pelvic limb/right pelvic limb) [49, 50]. For the evolution of hip OA, bodyweight distribution at a stance may even be a superior measurement to VI and PVF since dogs present different standing postures to increase acetabular coverage. Sensitivity and specificity seem to be higher with a cut-off point of 18% for pelvic limbs [8, 18, 51]. We considered both the 20% and 18% cut-off, with more differences being found at 20%. Mean values were below the 20% value but showed some dispersion. Since included animals had bilateral disease, it is quite possible that at any given point, they would be overloading one side to protect the other, leading to very different weight distribution values when comparing contralateral limbs in the same animal. Dogs presenting with pelvic limb-lameness tend to distribute weight more side-to-side than pelvic-to-thoracic compensation [52, 53]. For that reason, we also analyzed weight distribution for both pelvic limbs, with two different cut-off points. This analysis may be an interesting approach since it accounted for significant joint extension and function scores and CCO variations. It would be interesting to see the importance of these cut-off points in evaluating response to treatment. It should be the subject of further research, mainly since it did not show associated breed variations. It has been described that male dogs tend to carry more weight on the thoracic limbs naturally and may exhibit fewer improvements in response to treatment [17]. No significant variation comparing males and females in weight distribution was found, but future studies should evaluate this hypothesis.

Canine thermal imaging has been documented only recently. Still, a growing interest in this modality has led to an increase in the number of studies evaluating its use to assess the canine hip, stifle, elbow, and intervertebral disc [24, 54–58]. To our knowledge, this is the first study describing values for dogs with hip OA. The coat's type and color are variables that must be taken into account, and its influence documented [55, 56, 59, 60]. Our results seem to confirm this fact since DSD showed

significantly different values than other breeds, and this may be due to its brindle coat, in opposition to lighter coats in the other breeds. In humans OA studies, increased temperatures have been related to even slight degenerative changes and low temperatures in more severe disease cases [61]. In this study, this effect was not found, but it may be due to the coat variation effect. Still, its value in evaluating response to treatment has to be determined.

CMIs represent a patient-centred approach that, simi- lar to what happens in human medicine, has been incorporated in veterinary assessments in different species [62–64]. They may also capture a different dimension of OA since owners may often be more focused on the dog's ability to perform daily activities, rather than an increase or decrease of ROM or use of a single limb at a walk or trot [65, 66]. While no differences were observed when comparing animals by sex, several differences were observed between breeds and reported values for the same breeds' pet dogs. One of the reasons for this may be the nature of the specific mission of the animals. When involved in a more physically challenging task, it is more likely that complaints or limitations arise. Another reason may be age (which correlated with several scores), since older animals tend to be more experienced and able to manage the effort, making them less prone to injury [67]. Also, since these animals are selected based on working predisposition, they present high drive, which may mask some complaints and lead, for example, to relatively low PSS. We also aimed to see if different cut-off points of pain scores (measure with the PIS and PSS) presented significant differences. The main finding was that, as could be expected, animals with higher PIS scores had significantly lower weight distribution, but also had higher frequencies of all radiographic signs.

This study presents some limitations, namely the lack of a control group with non-lame dogs. This limitation is mainly related to the sample's convenience nature, comprised of dogs specifically presenting for treatment. Some of the previous report results of similar evaluations were conducted in the same breeds included in our sample, which is still useful. Since data was only collected in a single moment, we cannot comment on the interest of each of the findings for the prognosis or treatment monitoring of OA, which should be addressed in future studies.

Conclusions

To our knowledge, this study first describes several clinical and radiographic findings of working dogs of different breeds to hip OA. Police working dogs presented complaints related to hip OA at an early stage of the disease and a younger age than non-working dogs. LR were significantly older than other considered breeds. Hip scores influenced clinical presentation, with moderate cases showing lower thigh girth and worse PIS, PSS, and function scores than mild cases. Patients with severe OA had lower thermographic evaluations than patients with moderate OA. Age was the primary variable influencing considered CMI scores.

Methods

The sample comprised fifty (N = 50) Police working dogs with bilateral hip OA. It was a convenience sample, composed of patients presented at the Clínica Veterinária de Cães (Portuguese Gendarmerie Canine Clinic) to undergo hip OA treatment after initial diagnosis. Subsequent treatment was randomly determined, as the animals took part in a study evaluating intra-articular treatments for OA. Patients were active police working dogs of the Guarda Nacional Republicana (Portuguese Gendarmerie Canine Unit). The diagnosis was based on the dog's history, trainer complaints (difficulty rising, jumping and maintaining obedience positions, stiffness and decreased overall performance), physical examination (pain during joint mobilization, stiffness and reduced range of motion), and radiographic findings (OFA hip scores of mild, moderate or severe) consistent with bilateral hip OA. Inclusion criteria were: bodyweight ≥ 15 kg, animal older than 2 years and without any medication or nutritional supplements for 6 weeks or more before the beginning of the study. Animals suspected or with any other orthopaedic or concomitant disease (ruled out through physical examination, complete blood count, and serum chemistry profile) and not tolerant of data collection were excluded. All evaluations were performed at the same moment by the same researcher, which had extensive experience in the conduction of all procedures to reduce inter-observer variability.

Digital thermography

For the collection of digital thermography images, dogs were allowed to walk around in a large, plain wall room and adjust to room temperature (set at 21 °C) in a relaxed way for approximately 30 min before imaging. They were then positioned in an upright standing position, as symmetrically as possible, without the trainer or veterinarian touching its torso. A dorsoventral and two lateral images (one for each limb) were obtained from every animal. Every dorsoventral thermographic image included the last lumbar vertebra area to the first coccygeal vertebra at a minimum, at a distance of 60 cm (Fig. 1) [23]. Lateral views had the greater trochanter in the centre of the image, also at a distance of 60 cm. All images were captured with a FLIR ThermaCAM E25® camera model and kept when the anatomical landmarks

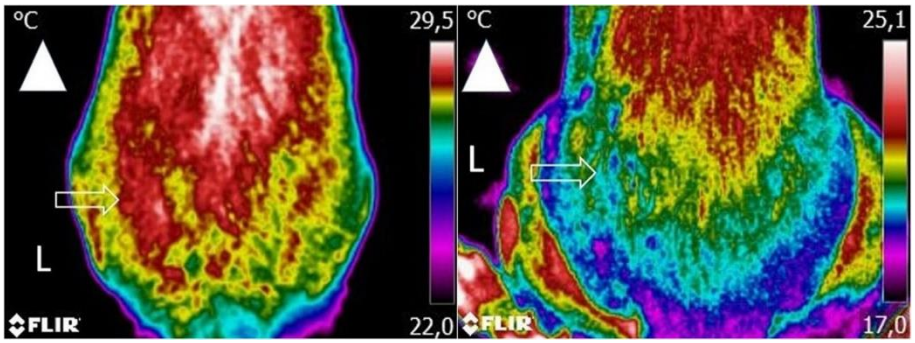

Fig. 1 A dorsoventral view of a dog with moderate osteoarthritis (left) and another with severe osteoarthritis (right), including the area from the last lumbar vertebra to the first coccygeal vertebra at a minimum, at a distance of 60 cm. Arrowhead indicates cranial direction. Arrow indicates the anatomical location of the hip joint. An area of increased temperature is observed on the patient with moderate OA and of lower temperature on the patient with severe OA

were included, and the image was steady enough to determine their location. The free software Tools (FLIR Systems, Inc) was used to analyse the images, with a rainbow color pallet. Temperature boxes of equal size were placed on the hip joint's anatomical area on both views, with mean and maximal temperatures determined.

Stance Analysis

Stance analysis was conducted with a weight distribution platform (Companion Stance Analyzer; LiteCure LLC®, Newark, Delaware, United States). According to the manufacturer's guidelines, it was placed in the centre of a room, at least 1 meter from the walls. It was calibrated at the beginning of each day, and zeroed before each data collection. Animals were encouraged to stand on to the weight distribution platform. Its trainer helped ensure the patients placed one foot on each quadrant of the platform while maintaining a natural stance with the centre of gravity and stability (measured by the platform) near the platform's middle. Gentle restraint was used to keep the patient's head in a natural, forward-facing position when needed. For all animals, at least 20 measurements were performed, and the mean value was determined. Normal weight distribution for each pelvic limb was considered 20% of the total weight [18]. Since all animals included had bilateral OA, weight distribution on both pelvic limbs was also considered and set at 40% (20% left pelvic limb+20% right pelvic limb).

Clinical Assessment

Determination of thigh girth was made with a Gullick II measuring tape at a distance of 70% thigh length, measured from the tip of the greater trochanter, with the leg in an extended position while in lateral recumbency, and the dog relaxed [44]. With the patient in the same position, hip joint ROM was obtained with a goniometer (Veterinary Instrumentation, United Kingdom) at extension and flexion, with a flexed stifle [68]. These measurements were made in triplicate, and the mean value was calculated.

Radiographic examination

Radiographic studies were conducted under light sedation, using a combination of medetomidine (0.01 mg/kg) and butorphanol (0.1 mg/kg), given intravenously. A ventrodorsal extended legs view and a frog-legged view were obtained. Hips were graded according to the OFA hip grading scoring scheme[69] by the researcher, blinded to the patient's identification. A mild score corresponded to a partially subluxated femoral head, causing an incongruent and widened joint space, with a shallow acetabulum, only partially covering the femoral head. In young dogs (24 to 36 months), OA lesions may not be present. Moderate grades were attributed when significant subluxation was present, and the femoral head was barely seated into a shallow acetabulum. Secondary remodeling along the femoral neck and head, acetabular osteophytes, and subchondral sclerosis were present. In severe cases, the femoral head was partly or completely out of a shallow acetabulum, with extensive secondary arthritic bone changes along the femoral head and neck head, acetabular rim changes, and large amounts of abnormal bone pattern changes. A full description of the OFA hip grading scheme is available online (https://www.ofa.org/diseases/hip-dysplasia/grades). The presence of specific radiographic signs was also recorded: irregular wear on the femoral head, making it misshapen and with a loss of its rounded appearance; a flattened or shallow acetabulum, with irregular outline; CCO; new bone formation on the acetabulum and femoral head and neck; a worn away angle formed at the cranial effective acetabular rim; subchondral bone

sclerosis along the cranial acetabular edge; and CFHO [9, 39, 70, 71]. In the frog-legged view, the presence of CCO and CFHO was also recorded.

Clinical metrology instruments

At the evaluation moment, an online copy prepared for the effect of the HVAS, CBPI, COI, and LOAD was completed by the trainers. The same trainer completed all CMIs for each dog.

Statistical Analysis

Normality was assessed with a Shapiro-Wilk test. Each measured parameter was compared with an Independent Samples T-Test (when two groups were considered, like sex) or ANOVA, followed by a Bonferroni post hoc test for multiple comparisons (when more than two groups were considered). CMI scores were compared with a Wilcoxon signed-rank test. Different score cut-off points (4, 6, and 8) were analyzed for PIS and PSS. 20% and 18%[18] pelvic limb percentages cut-off points were considered for weight distribution. Since hip OA is often bilateral, results for the combination of both pelvic limbs were also analyzed, at 36% (18% left pelvic limb + 18% right pelvic limb) and 40% (20% left pelvic limb + 20% right pelvic limb). The correlation between parameters was assessed with the Pearson correlation coefficient. Multiple regression was run to predict evaluated parameters from age, sex, breed, body weight, and OFA hip score. All results were analyzed with IBM SPSS Statistics version 20, and a significance level of $p < 0.05$ was set.

Abbreviations

BM: Belgian Malinois; CBPI: Canine Brief Pain Inventory; CCO: Caudolateral curvilinear osteophyte; CFHO: Circumferential femoral head osteophyte; COI: Canine Orthopeadic Index; DSD: Dutch Shepherd Dog; FL: Frog-leg view; GSD: German Shepherd Dogs; HVAS: Hudson Visual Analogue Scale; LOAD: Liverpool Osteoarthritis in Dogs; LR: Labrador Retriever; OA: Osteoarthritis; PIS: Pain Interference Score; PSS: Pain Severity Score; ROM: Range of motion; VD: Ventrodorsal view

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Authors' contributions

JCA designed the protocol, conducted treatments, and prepared the manuscript. PJ and AS selected patients and conducted treatments.CL and LMC revised the protocol and prepared the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the Guarda Nacional Republicana (Portuguese Gendarmerie) but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [the Divisão de Medicina Veterinária of the Guarda Nacional Republicana.

Ethics approval and consent to participate

This protocol was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval nº GD/32055/2018/P1, September 25th, 2018). Written, informed consent was obtained from the Institution responsible for the animals (Guarda Nacional Republicana, Portuguese Gendarmerie) through dispatch of the Doctrine and Training Commander nº327/16, dated September 16th, 2016.

Consent for publication

Not applicable.

Competing interests

Companion, LiteCure LLC provided the Stance Analyser used in this study, and Specman, Lda, provided the digital thermography camera.

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RESEARCH ARTICLE

Comparison of clinical and radiographic signs of hip osteoarthritis in contralateral hip joints of fifty working dogs

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Abstract

Objective

This study aimed to compare the symmetry of clinical and radiographic signs of right and left pelvic limbs of dogs with bilateral hip osteoarthritis (OA) and evaluate the association of physical findings and radiographic abnormalities.

Patients and methods

One hundred pelvic limbs of police working dogs with bilateral hip OA were evaluated, fol- lowing a screening program. Weight distribution, joint range of motion at flexion and exten- sion, thigh girth, and radiographic signs were recorded and compared with the results of the contralateral limb and by breed, age, and sex with the Paired Samples T-Test and Pearson correlation coefficient, with $p<0.05$.

Results

The sample mean age was 6.5±2.2 years, and the bodyweight of 26.7±5.3kg. No significant differences were observed when comparing weight distribution, joint range of motion, and thigh girth of left and right limbs. Weight distribution and age showed a statistically significant correlation with joint extension. The right limbs showed a significantly higher frequency of circumferential femoral head osteophyte (CFHO) regarding radiographic signs. Limbs with CFHO or caudolateral curvilinear osteophyte had significantly larger joint flexion angle ($p = 0.02$) and smaller extension angle ($p<0.01$), respectively, compared to those that did not. Age showed a significant correlation with the presence of several radiographic findings, as did different breeds.

Conclusion

Clinical and radiographic signs occur symmetrically in naturally occurring hip OA in police working dogs. Several correlations were observed between the evaluations performed and

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differences between breeds, which can be useful in assessing and early diagnosis of hip OA.

Introduction

Osteoarthritis (OA) poses significant welfare challenges and concerns, as it affects the quality of life, performance and implies a considerable cost in terms of healthcare [1, 2]. It is the most prevalent musculoskeletal disease in the dog and is estimated to affect around 200 000 dogs annually in the United Kingdom [3]. At least 80% of lameness cases and joint disease in companion animals are classified as OA, with 20% of middle-aged and 90% of older dogs having OA in one or more joints [4–7]. Risk factors include breed, neutered, higher bodyweight, and older than eight years [3]. Sporting and working animals are at increased risk, being exposed to repetitive loading and chronic fatigue injuries, leading to tissue damage, wear, tear, and ultimate tissue failure, resulting in clinical signs [8]. Chronic fatigue injuries are a critical predisposing condition for hip OA development, a disease commonly diagnosed in dogs, with various degrees of severity [9, 10].

Imaging plays a key role alongside the clinical review of patients with joint disease and can be done repeatedly and safely within recognized limits, which is important for the follow up of chronic conditions [11, 12]. The most common radiographic view for evaluating the hip is the ventrodorsal (VD) hip extended view, for which sedation is required for most dogs [13–15]. Main radiographic changes include femoral periarticular osteophyte formation, subchondral sclerosis of the craniodorsal acetabulum, osteophytes on the cranial/caudal acetabular margin, remodeling of the cranial and caudal acetabulum, flattening of the femoral head, and irregular widening of the femoral neck [16, 17]. The features that have been deemed of significant importance are the circumferential femoral head osteophyte (CFHO), caudolateral curvilinear osteophyte (CCO), and subchondral bone sclerosis, early radiographic signs that predict the development of the clinical signs of hip OA [18–21]. The ventrodorsal flexed view (also called frog-legged view, FL) enhances the visibility of the cranial and caudal aspects of the femoral head and neck, helping in the assessment of CFHO and CCO [21].

Weight distribution and off-loading, or limb favoring at the stance, is a commonly used subjective assessment during the orthopedic examination, but the subtle changes in posture or weight-bearing may occur in the early stage of the disease process can be easily missed with visual assessment only [22–25]. Stance analysis and weight-bearing distribution have been reported as sensitive for detecting lameness in dogs, with better results in large breed dogs [26]. Muscular atrophy is a consistent finding in OA patients and may be evident within a few weeks [10, 14, 16, 27]. The evaluation of the joint range of motion (ROM) can also be per- formed, including flexion and extension [10]. The evaluation of asymmetry, assessment of muscle atrophy, measurement of static weight-bearing, and ROM measurement have been described as the most valid and sensitive physiotherapeutic evaluation methods [28, 29].

This study aimed to compare the symmetry of clinical and radiographic signs of right and left pelvic limbs of police working dogs with bilateral hip osteoarthritis, and evaluate the asso-ciation of physical findings and radiographic abnormalities, at the time of diagnosis. We hypothesize that multiple asymmetries are present in several of the evaluation parameters.

Materials and methods

The study protocol was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval n˚ GD/

32055/2018/P1, September 25th, 2018) and comply with ARRIVE guidelines. Written, informed consent was obtained from the Institution responsible for the animals.

This study's sample constituted a convenience sample, similar in size to previously published reports on this topic [30–32]. The sample comprised 100 hips of 50 police working dogs with bilateral hip OA, from the population of police working dogs of the Guarda Nacional Republicana (Portuguese Gendarmerie Canine Unit), scheduled to undergo treatment of hip OA. Patients were active police working dogs, selected after screening of the Portuguese Gendarmerie Canine Unit, based on history (difficulty rising, jumping and maintaining obedience positions, stiffness and decreased overall performance), physical (pain during joint mobilization, stiffness, and reduced range of motion), orthopedic, neurological and radiographic (OFA hip scores of mild, moderate or severe) examinations compatible with bilateral hip OA.

All patients underwent medical evaluation before acquisition from multiple breeders and trainers, and starting active training to become police working dogs. Additional inclusion criteria comprised a bodyweight ≯15kg, age over 1 year should not have received any medication nor nutritional supplements for six weeks or more. Animals that did not tolerate the data collection procedures, which had any other suspected or diagnosed neurological/musculoskeletal disorder, had a diagnosis of suspected concomitant disease (ruled out through physical examination, complete blood count, and serum chemistry profile), were excluded. Subsequent treatment was randomly determined, as the animals took part in a study evaluating intra-articular therapies for OA. Two-hundred and eighty-one dogs were screened, and 231 were excluded. Sixty-three due to suspected or documented orthopedic, neurological, or concomitant disease, 50 due to having a bodyweight <20kg, 25 for having received medication in <6 weeks, and 13 for not being tolerant of data collection, and 80 due to an inability to maintain medical follow-up throughout the study, for work-related reasons.

Radiographic studies were conducted under light sedation, using a combination of medetomidine (0.01mg/kg) and buthorphanol (0.1mg/kg), given intravenously. A VD extended legs view and an FL view were obtained. In the VD view, the presence of the following radiographic hip OA findings was recorded [21]: irregular wear on the femoral head, making it misshapen and with a loss of its rounded appearance; flattened or shallow acetabulum, with irregular outline; CCO; new bone formation on the acetabulum and on femoral head and neck; acetabular rim wear; subchondral bone sclerosis along the cranial acetabular edge; CFHO. In the FL view, the presence of CCO and CFHO was recorded. An example of CCO and CFHO, in VD and FL views, are presented in Figs 1 and 2, respectively.

Stance analysis was conducted with a weight distribution platform (Companion Stance Analyzer; LiteCure LLC, Newark, Delaware, United States). The equipment was placed in the center of an observation room, at least 1-meter feet from the walls. Complying with the manufacturer's guidelines, the platform was calibrated at the beginning of each testing day and zeroed before each data collection. After an acclimatization period, animals were then encouraged to stand on to the weight distribution platform. To secure a correct position, the patient's

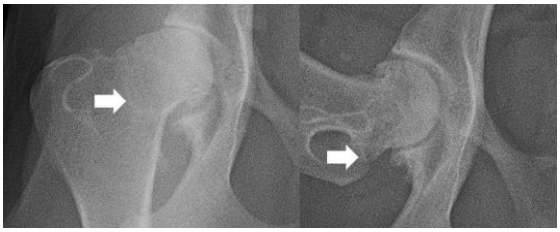

Fig 1. An example of a hip with caudolateral curvilinear osteophyte (arrow) on a ventrodorsal (left) and frog leg right (views).

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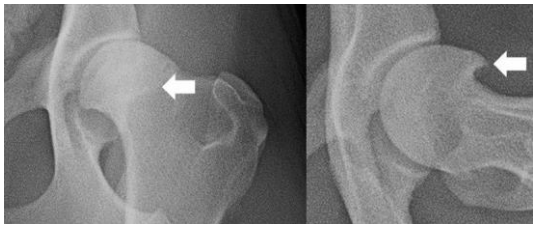

Fig 2. An example of a hip with circumferential femoral head osteophyte (arrow) on a ventrodorsal (left) and frog leg (right) views.

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trainer helped ensure it placed one foot on each quadrant of the platform while maintaining a natural stance with its center of gravity and stability (measured by the platform) near the plat- form's middle. When required, gentle restraint was used to maintain the patient's head in a natural, forward-facing position. Normal pelvic limb evaluation is considered 20% of the total weight [33]. A Gulick II measuring tape was used to determine thigh girth. Measurements were made at a distance of 70% thigh length, measured from the greater trochanter's tip, with the leg in an extended position. Animals were placed in lateral recumbency in a relaxed posi- tion [34]. ROM of the hip joints was obtained with a goniometer at extension and flexion with a flexed stifle [35].

Statistical analysis

Normality was assessed with a Shapiro-Wilk test, and each measured parameter was compared with the contralateral limb with a Paired Samples T-Test. Measured parameters by breed and sex were compared with an Independent Samples T-Test. Evaluation of the results of radio- graphic imaging, digital thermography, and physical examinations were conducted without knowledge of the results of the remaining evaluations. Correlation between parameters was assessed with Pearson correlation coefficient. All results were analyzed with IBM SPSS Statis-

tics version 20, and a significance level of $p<0.05$ was set.

Results

A sample of 50 police working dogs, of both genders (30 males and 20 females), with a mean age of 6.5±2.2 years and bodyweight of 26.7±5.3kg, were analyzed. Four breeds were repre- sented: German Shepherd Dogs (GSD, n = 17), Belgian Malinois Shepherd Dogs (BM, n = 15), Labrador Retriever (LR, n = 10), and Dutch Shepherd Dog (DSD, n = 8). They were used for four different purposes: Use of force (n = 20), drug detection (n = 14), search and rescue

(n = 7), explosives detection (n = 5), and tactical intervention (n = 4). Considering OFA grad-ing of hip joints, 35 animals were classified as mild (70%), 10 as moderate (20%), and 5 as severe (10%).

Measured age and weight values, individual limb weight distribution, thigh girth, and joint range of motion are presented in Table 1. No significant differences were observed when com- paring overall measurements of left and right limbs. Significant differences were observed in weight and thigh girth in both right and left pelvic limbs ($p<0.01$) when comparing males to females, with male dogs having higher values. Comparing breeds, LR were significantly older

than other animals ($p<0.01$), and GSD were significantly heavier than BM ($p<0.01$) and LR ($p<0.01$). GSD also had a significantly higher left thigh girth than BM ($p<0.01$), LR ($p<0.01$) and DSD ($p = 0.04$), and right thigh girth than BM ($p<0.04$) and LR ($p<0.01$). DSD had higher left joint flexion than BM and GSD ($p = 0.04$), while LR had a larger joint flexion angle

Table 1. Mean scores (±standard deviation) of overall weight and age, individual stance analysis, thigh girth and range of motion (extension and flexion) measurements, and by breed and sex, of left and right pelvic limbs.

	Weight	Age	Stance Analysis		Thigh Girth		Joint Extension		Joint Flexion	
	(kg, mean±SD)	(yrs, mean±SD)	(%, mean±SD)		(cm, mean±SD)		(˚, mean±SD)		(˚, mean±SD)	
			Left	Right	Left	Right	Left	Right	Left	Right
Overall	26.7±5.3	6.5±2.2	19.2±4.8	18.7±4.2	30.6±2.9	30.4±2.6	149.2±9.5	150.6±7.1	55.6±4.1	56.2±4.6
German Shepherd Dog	29.9±6.4	5.7±1.8	20.0±3.9	18.4±3.6	32.5±3.5	31.8±2.7	151.1±8.1	151.6±5.9	55.2±3.5	57.1±3.5
Belgian Malinois Shepherd Dog	27.5±4.1	5.3±1.4	17.8±5.5	19.8±5.9	29.9±2.7	29.9±2.2	146.7±7.1	150.6±4.9	54.3±4.6	56.1±6.1
Labrador Retriever	24.3±2.5	8.7±2.5	19.9±5.5	18.6±3.2	28.5±2.5	28.5±2.3	147.0±14.4	148.5±10.8	55.8±3.8	54.3±3.7
Dutch Shepherd Dog	27.5±4.1	5.3±1.4	19.0±4.4	17.4±2.9	30.2±2.1	30.7±2.1	152.5±8.8	151.5±7.9	58.5±3.7	56.5±4.2
Male	29.3±5.4	6.2±2.4	19.0±5.5	19.3±4.8	31.7±2.9	31.2±2.5	149.2±7.6	151.1±4.9	55.2±3.8	57.0±4.7
Female	23.5±2.8	6.9±2.5	19.4±3.5	17.8±3.1	28.8±2.0	28.9±2.2	149.2±12.1	150.0±9.6	56.2±4.5	54.9±4.2

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(p = 0.05). Sex showed a correlation with weight (r = 0.5, p<0.01). Breed showed a moderate correlation with thigh girth (r = 0.4, p<0.01 for the left pelvic limb and r = 0.3, p = 0.04 for the right pelvic limb), as did sex (r = 0.5, p<0.01 for both limbs) and high correlation with weight (r = 0.8, p<0.01 for both limbs). A correlation was observed between joint extension and age (r = -0.4, p<0.04 for the left pelvic limb and r = 0.3, p<0.02 for the right pelvic limb) and weight distribution (r = 0.5, p<0.01 for the left pelvic limb).

Regarding radiographic findings, absolute frequencies and percentages in the VD and FL views of the left and right pelvic limbs are presented in Table 2. Breed variations in Orthopedic Foundation for Animals hip scores are shown in Table 3. Comparing contralateral limbs, only the presence of CFHO, observed in the VD view, was significantly different (p = 0.03). DSD had significant differences in the frequency of CFHO in the VD view compared to GSD

(p = 0.03) and BM (p = 0.04) (left and right pelvic limbs, respectively). On the FL view, differ-ences were observed in the frequency of CFHO of BM and GSD (p = 0.02) and DSD

(p = 0.04), both on the left pelvic limb.

A difference in the frequency of the presence of a worn cranial effective acetabular rim angle was observed in the right pelvic limb of GSD and LR (p = 0.04). Breed showed a moderate correlation with the presence of CFHO in the VD view (r = 0.302, p<0.05 for the left pelvic limb). A moderate correlation was observed between age and the presence of CCO on the FL view (r = -0.408, p<0.01 for both pelvic limbs), an irregular, misshapen femoral head

(r = 0.302, p = 0.02 for the left pelvic limb), and new bone formation on the acetabulum and femoral head and neck (r = 0.312, p = 0.03 for the right pelvic limb). The joint extension was

Table 2. Absolute frequencies and percentages of radiographic findings in the ventrodorsal and frog-leg views of the left and right pelvic limbs.

Radiographic finding	Left				Right			
	Present		Absent		Present		Absent	
Irregular wear on the femoral head, making it misshapen and with a loss of its rounded appearance	48	96%	2	4%	47	94%	3	6%
Flattened or shallow acetabulum, with irregular outline	32	64%	18	36%	28	56%	21	42%
Caudolateral curvilinear osteophyte (CCO)	17	34%	33	66%	18	26%	32	64%
New bone formation on the acetabulum and on femoral head and neck	41	82%	9	18%	45	90%	5	10%
The angle formed at the cranial effective acetabular rim is worn away	36	72%	14	28%	41	82%	9	18%
Subchondral bone sclerosis along the cranial acetabular edge	49	98%	1	2%	49	98%	1	2%
Circumferential femoral head osteophyte (CFHO)	18	36%	32	64%	40	80%	10	20%
CCO on the Frog Leg view	18	36%	32	64%	15	30%	35	70%
CFHO on the Frog Leg view	45	90%	5	10%	43	86%	7	14%

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Table 3. Breed variations in Orthopedic Foundation for Animals hip scores.

	OFA hip grade					
	Mild		Moderate		Severe	
Overall	35	70%	10	20%	5	10%
German Shepherd Dog	9	53%	5	29%	3	18%
Belgian Malinois Shepherd Dog	13	87%	2	13%	0	0%
Labrador Retriever	7	88%	1	13%	0	0%
Dutch Shepherd Dog	6	60%	2	20%	2	20%

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high or moderate correlated with the presence of CCO in an FL view ($r = -0.506$, $p = 0.01$ for the left pelvic limb and $r = -0.439$, $p < 0.01$ for the right pelvic limb) and on the VD view($r = -0.315$, $p < 0.04$ for the right pelvic limb). Joint flexion was moderate correlated with the presence of CFHO in the VD view on both limbs ($r = -0.312$, $p = 0.02$ for the left pelvic limb and $r = -0.304$, $p < 0.04$ for the right pelvic limb).

Considering animals that did or did not present CCO or CFHO on the left pelvic limb, significant differences were observed in joint extension when CCO was present in both the VD and FL views ($p < 0.01$ for both) and joint flexion when CFHO was present in a VD view ($p = 0.02$). On the right pelvic limb, a significant difference was observed in joint extension when CCO was present, in both the VD and FL views, compared to when it was absent ($p < 0.01$).

Discussion

Hip OA is common in large breeds such as German Shepherd Dogs and Labrador, amongst others [36]. This study describes and compares several clinical and radiographic findings of different individuals in each individual and between breeds with hip OA. It also describes as, in these animals, disease derived changes occur in similar degree in both limbs since no significant differences were observed when comparing contralateral limbs. The reason for this finding is unclear and may be associated with the natural progression of the disease or with a relatively early diagnosis, the effect of a possible unilateral, or even intermittent overloading is still not noticeable.

Most hip OA signs are observed in the older population, usually in animals over 8 years, with the disease now at a chronic stage [3]. The mean age of animals included in this study was lower (6.5) years, except for LR. This result is not in line with published studies and may be associated with the fact that these animals are active working dogs, where complaints due to musculoskeletal disease are noticed early on, from its toll on gait and performance [37]. The difference in age of LR is less apparent and may be due to breed conformation, leading to a better disease tolerance, as hip OA seems to be better tolerated by animals than OA in different joints [10]. Additional reasons may be related to a less physically demanding mission of these dogs (most were product detection dogs) than the remaining animals included in the sample (mostly in search and rescue and Use of force activities).

Some of the other registered differences could be expected. GSD were significantly heavier than other breeds (BM and LR) and with higher thigh girth. Male dogs also heavier than females and with higher thigh girth. This may account for a positive correlation between thigh girth, weight, sex, and breed. The effect of weight and growth rate has been studied concerning hip dysplasia from an early stage, with heavier dogs showing a higher incidence of OA at maturity and an earlier age [38, 39]. This can partially account for the higher number of males included in this sample since they tend to be heavier and, therefore, possibly more prone to

develop OA. Measuring thigh girth is also be a useful measurement, not only in the initial assessment but also as an outcome measure [34]. We have presented thigh girth measurements in dogs with hip OA, but further studies should include a control group with disease-free dogs to compare both groups' values. Coxofemoral ROM may also be diminished, particularly during extension, although this is not a universal finding [14, 40]. Normal ROM of the hip joint in military working German Shepherd Dogs are described as 44˚±6 at flexion and 155˚±6 at extension [41]. In Labrador Retrievers, normal ROM been described as 50˚±2 at flexion and 162˚±3 at extension in one report, and 49˚ at flexion and 159˚ at extension in another [35, 42]. We have measured lower values in GSD and LR, which could be expected due to OA. Exercise has been described as having a positive effect on the severity of lameness in LR with hip dysplasia, which increased with longer exercise duration [43]. Since the animals included in the sample are all active working dogs, this inverse relationship can be present. The joint extension was one of the measurements made with higher dispersion, which may confirm the fact that ROM changes are not a consistent finding. It showed a correlation with age, which may be attributed to disease progression, as joint extension appears to be limited by the joint capsule fibrosis. This reduction in joint extension in older dogs has been described before [43]. On the other hand, hip joint flexion seems to be related to muscle mass and could increase in dogs with OA due to a loss in muscle masses [44]. As the patients in our sample did not exhibit a high degree of muscle atrophy, this increase was not observed.

Normal weight distribution for the stance analyzer is the same as for a pressure-sensitive walkway—30/30/20/20 (left thoracic limb/right thoracic limb/ left pelvic limb/right pelvic limb) [45, 46]. It has been proposed that bodyweight distribution at a stance may be an equivalent or superior measurement of pain associated with hip OA than both VI and PVF [33, 47], and dogs presenting with the disease often have slightly abducted pelvic limbs, easily noticeable when the animal is standing, increasing acetabular coverage [10]. Overall weight distribution values per limb recorded in this study were lower than the described 20% expected level. When analyzing weight distribution in individual breeds, the exception was registered in the left pelvic limb of GSD, but this may be a compensation mechanism. No significant variations were observed between breeds. This measurement's value, particularly in response to treatment, should be the subject of further research since it was the measured parameter that recorded the least breed associated variations.

It is well established that radiographic signs' development occurs later than the structural changes associated with OA, and symptoms must be severe before being observed on the X-ray [14, 48]. CFHO is a marked radiopaque line encircling the junction between the femoral neck and the epiphysis, along with the insertion of the joint capsule [49]. When comparing radiographic findings between contralateral limbs, only the frequency of CFHO on the VD view was significantly different. Since this is one of the radiographic predictors of future OA development [21], and these animals were only now being diagnosed, future studies will have to address whether further asymmetries between limbs develop if it was only an incidental finding. Since CFHO is usually better observed on an FL view, and the same asymmetry was not observed on the FL, this might be the case. CFHO also correlated with joint flexion, which can be explained by the changes that occur in the joint capsule during OA progression, making it less flexible. GSD also had significantly higher frequencies of this finding than BM and DSD. Interestingly, no correlation was found between weight and any of the radiographic findings considered, as could be expected, and they may rather be linked to breed variations.

CCO arises at the femoral neck's caudodorsal part due to traction on the hip joint capsule, and it manifests as a radiopaque line. It correlates with hip subluxation and, therefore, represents a risk factor for OA development at a later stage in life [15]. A correlation between CCO and age of an irregular femoral head and new bone formation was observed and can further

attest to the relationship between CCO and OA signs' development. It also correlated with joint extension and, as seen with CFHO, may be due to the changes that occur in the joint capsule. This possibility is also reflected when considering the animals that presented CFHO or CCO and comparing them with those that did not, with significant differences in ROM evaluation, flexion, and extension.

This study presents some limitations. Ideally, a control group with non-lame dogs shouldbe included. The interest of each of the findings in the prognosis or treatment monitoring ofOA could not be determined since data was collected in a single evaluation moment. Future studies are required to evaluate these points. Additionally, the sample included a majority of dogs with mild OA. For that reason, further studies should include a larger number of dogs with moderate and severe OA to determine if similar results are obtained.

Conclusions

The present study showed that clinical and radiographic signs occur symmetrically in naturally occurring hip OA in police working dogs. It also describes the correlation between the evaluations performed, which can be useful in evaluating and early diagnosing hip OA, as differences between the most commonly used working dogs breeds.

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Author Contributions

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3. EVALUATION OF THE VARIATION IN THE SYNOVIAL FLUID CRP AND IL-1 LEVELS IN PATIENTS WITH HIP JOINT OA AFTER THE IA ADMINISTRATION OF THE DIFFERENT SUBSTANCES

The influence of IL-1 and C-reactive protein synovial levels in the clinical signs and metrology instruments results, in a naturally occurring canine osteoarthritis model - Submitted to Research in Veterinary Science – Impact factor 1.892, Quartile 1.

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Quartile 1

The influence of IL-1 and C-reactive protein synovial levels in the clinical signs and metrology instruments results, in a naturally occurring canine osteoarthritis model

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Abstract

We aimed to evaluate IL-1 and C-reactive protein (CRP) levels in the synovial fluid (SF) in a naturally occurring canine osteoarthritis (OA) model, and its relation with animals' clinical, radiographic and thermographic disease signs.

One hundred (N=100) joints of active police working dogs with hip OA were evaluated. SF IL-1 and CRP levels, weight distribution, joint range of motion, thigh girth, digital thermography and radiographic signs were recorded. Data from four Clinical Metrology Instruments (CMI) was collected. Results were compared by age, sex and OFA scores with the Independent Samples T-Test, ANOVA and Pearson correlation coefficient, with $p<0.05$.

The sample included 100 pelvic limbs, equally distributed between left and right pelvic limbs 30 males and 20 females, with a mean age of 6.5±2.4 years and bodyweight of 26.7±5.2kg. IL-1 levels, particularly above 200pg/mL, may be related to the development of caudolateral curvilinear osteophyte, which then expresses a toll on the patient's levels of pain and activity. It was unclear if the CRP levels were a consequence of inflammatory activity within the joint or a reflection of better prognosis. Increasing body weight was related to worse CMI scores.

We described the relation of IL-1 and CRP synovial concentrations levels with several clinical signs, diagnostic imaging, laboratorial findings and clinical metrology instruments results of animals with OA. Further studies are required to determine and possibly quantify the interest of each parameter for the prognosis and treatment monitoring.

Keywords: Dog; Osteoarthritis; Interleukin-1; C-reactive protein; Stance Analysis; Digital Thermography; Clinical Metrology Instruments.

Introduction

Osteoarthritis (OA) affects all mammals and is an important and costly disease, representing a significant burden to societies, as it affects the quality of life, performance and healthcare, posing significant welfare challenges and concern (Loeser et al., 2012; Venable et al., 2008). OA is a relatively low-grade inflammatory disease, where the inflammatory process affects the progression of the disease, frequently without systemic manifestation (Calich et al., 2010; Goldring and Goldring, 2004). Interleukin 1 (IL-1) has been pointed out as the most critical pro-inflammatory cytokine responsible for the catabolism in OA, with a relation with lameness duration (Fujita et al., 2006). However, some studies have reported low or undetectable IL-1 levels in OA animals (Vincent, 2019). C-reactive protein (CRP) is an acute-phase protein, produced during inflammatory reactions or tissue injury, which may also be produced at the level of the inflamed tissues (Bennett et al., 2013). CRP is

the most useful acute-phase protein in the dog, as in humans, with the advantage of its shifts being noted from a very early stage of the disease process (Bennett et al., 2013).

The dog is a frequent model for the study of OA, since the pathologic process, clinical presentation and response to treatment are very similar to that of humans, making it the closest to a gold standard. The naturally occurring canine model has the advantage of presenting a faster disease progression, with equivalent life stages to those of humans, while sharing many of the environmental variations that also influence human OA (Gregory et al., 2012; Kol et al., 2015; Meeson et al., 2019). Exploring dog OA in translation treatment under the One Medicine initiative will help improve the health and well-being of both species (Cimino Brown, 2017; Meeson et al., 2019).

Pelvic radiographs are a part of screening programs for hip dysplasia, but also the clinical assessment of OA and determination of treatment outcome (Puckler et al., 2016). The most common radiographic views are ventrodorsal (VD) hip extended view and the ventrodorsal flexed view (called frog-legged view, FL). The particular interest of the FL view is that it augments the visibility and assessment of the circumferential femoral head osteophyte (CFHO) and caudolateral curvilinear osteophyte (CCO), early radiographic signs related with the development of the clinical signs of hip OA (Mayhew et al., 2002; Powers et al., 2004). Weight distribution, off-loading or limb favouring at the stance is a standard assessment during the orthopaedic examination, as animals tend to bear less weight on a painful limb (Gordon-Evans, 2012). Stance analysis has been reported as a sensitive evaluation for detecting lameness in dogs (Clough and Canapp, 2018). Digital thermal imaging relies on heat generated during physiologic functions and its relation with skin temperature control (Hildebrandt et al., 2012). It is a reliable technique to assess inflammatory arthritis pain and able to differentiate normal from osteoarthritis subjects (Fokam and Lehmann, 2019). Pain and functional ability are the most relevant parameters in the evaluation of OA animals and for assessment of treatment efficacy (Wiegant et al., 2015). For that purpose, several clinical metrology instruments (CMI) have been developed. The most commonly used are the Canine Brief Pain Inventory (CBPI) and the Liverpool Osteoarthritis in Dogs (LOAD) (Lascelles et al., 2018; Walton et al., 2018). The CBPI is divided into two sections, a pain severity score (PSS) that assesses the magnitude of the animal pain, and a pain interference score (PIS) that assesses the degree in which pain affects daily activities (Upchurch et al., 2016). It has been demonstrated as not being associated with response bias (Essner et al., 2020). Other CMIs are also used to evaluate different dimensions of OA, like the Canine Orthopaedic Index (COI, divided into four scores: stiffness, gait, function and quality of life – QOL), and the Hudson Visual Analogue Scale (HVAS), developed assess the degree of lameness in dogs (Brown, 2014; Hudson et al., 2004). Mobility impairment and activity levels are associated with musculoskeletal pain in humans. Mobility changes, and improvements, in particular, have been

recommended as measures of outcome (Lascelles et al., 2018). Pedometers are simple and inexpensive devices, capable of measuring ambulatory activity with acceptable accuracy(Tudor-Locke et al., 2002). Additional evaluated parameters include the examination of muscle masses since muscular atrophy is a consistent finding in OA animals, and also the joint range of motion (ROM), including flexion and extension, with a restricted ROM being usually present (Hyytiäinen et al., 2013).

The goal of this study is to evaluate IL-1 and CRP levels in the synovial fluid (SF) in a naturally occurring canine OA model, and its relation with animals' clinical, radiographic and thermographic signs. We hypothesize that increased levels of IL-1 and CRP are related to more severe signs.

Materials and Methods

The study protocol was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-Estar dos Animais da Universidade de Évora, approval nº GD/32055/2018/P1, September 25th, 2018). Written, informed consent was obtained from the Institution responsible for the animals.

The sample comprised one hundred (N=100) joints of active police working dogs with hip OA. For the diagnosis, a complete history, physical, orthopaedic, neurological and radiographic examinations were obtained and had to be consistent with bilateral hip OA. Additionally, animals should have a bodyweight ≥15kg, be over two years and must not have received any medication or nutritional supplements for six weeks or more. Animals were excluded if suspected to have any other orthopaedic or concomitant disease, or if they were not tolerant of data collection. All evaluations were performed at the same moment by the same researcher.

Stance Analysis

Stance analysis was conducted with a weight distribution platform (Companion Stance Analyzer; LiteCure LLC®, Newark, Delaware, United States). According to the manufacturer's guidelines, the equipment was placed in the centre of a room, at least 1 meter from the walls. It was calibrated at the beginning of each day and zeroed before each data collection. Trainers encouraged animals to stand on the platform while ensuring the patient placed one foot on each quadrant of the platform. If required, gentle restraint was used to keep the patient's head facing forward. The left-right symmetry index (SI) was calculated with the following formula: SI=[(WBR-WBL)/((WBR+WBL)x0.5)]x100 (Volstad et al., 2017; Walton et al., 2013), where WBR is the value of weight-bearing for the right pelvic limb, and WBL is the value of weight-bearing for the left pelvic limb. Negative values were made positive. Since normal weight-bearing for a pelvic limb is 20%

(Clough et al., 2018), deviation from this value was also considered, calculated by subtracting WB to 20.

Digital thermography

Digital thermography images were collected after 30 minutes, during which animals were allowed to calmly walk in a controlled temperature room (set at 21°C). With the animal positioned in a symmetrical upright standing position, a dorsoventral thermographic image was obtained, including the area from the last lumbar vertebra to the first coccygeal vertebra at a minimum at a distance of 60cm(Vainionpää et al., 2013). A lateral view was also obtained, at a distance of 60cm, with the greater trochanter in the centre. All images were produced with a FLIR ThermaCAM E25® model. The range of temperature was set at 15-40°CC and emissivity at 0.98. Data from the thermographic images were analyzed with the free software Tools (FLIR Systems, Inc), and the Rainbow HC colour pallet was used.

Clinical Assessment

For the determination of thigh girth and joint ROM, the patient was placed in lateral recumbency, with the affected limb uppermost. With an extended leg, thigh girth determination was made with a Gullick II measuring tape, at a distance of 70% thigh length, measured from the tip of the greater trochanter. Hip joint ROM was obtained with a goniometer at extension and flexion with a flexed stifle.

Pedometers

Pedometers were worn around the patient's neck, attached to an adjustable lightweight collar. They were placed one week before the evaluation moment, and mean daily counts were considered and calculated by dividing the register number of steps by the number of days considered.

Clinical metrology instruments

At the evaluation moment, trainers received the published instructions for HVAS, CBPI, COI and LOAD, and then completed an online copy of each for them. The CMIs were completed in sequence by the same handler in a quiet room with as much time as needed to answer all items.

Radiographic examination

Radiographic studies were conducted under light sedation, using a combination of medetomidine (0.01mg/kg) and butorphanol (0.1mg/kg), given intravenously. VD extended legs and FL views were obtained. The presence of seven radiographic OA signs was assessed: irregular wear on the femoral head, making it misshapen and with a loss of its rounded appearance; a flattened or shallow acetabulum, with irregular outline; CCO; new bone formation on the acetabulum and femoral head and neck; a worn away angle formed at the cranial effective acetabular rim; subchondral bone

sclerosis along the cranial acetabular edge and CFHO(Armbrust, 2009; Fortrie et al., 2015; Puckler et al., 2016; Smith et al., 2011). In the FL view, the presence of CCO and CFHO was also recorded.

Determination of synovial IL-1 and CRP concentrations

With the patient positioned in lateral recumbency with the affected joint uppermost, a small window of 4x4cm area surrounding the greater trochanter was clipped and aseptically prepared. An assistant positioned the limb in a neutral, parallel to the table position. For the collection of synovial fluid, a 21-gauge with 2.5" length needle was introduced just dorsal to the greater trochanter and perpendicular to the long axis of the limb, until the joint was reached. Determination of CRP and IL-1β concentrations were made using the DuoSet Ancillary Canine IL-1β Reagent kit (R&D Systems, United Kingdom), read with a FLUOstar OPTIMA (BMG Labtech), and Fuji Dri-Chem Slides VC-CRP PS (FUJIFILM Europe GmbH), read with a DRIChem NX500i (FUJIFILM Europe GmbH).

Statistical Analysis

Normality was assessed with a Shapiro-Wilk test, and each measured parameter was compared with an Independent Samples T-Test or ANOVA. Correlation between parameters was assessed with the Pearson correlation coefficient. Multiple regression was run to predict evaluated parameters from CRP serum and synovial IL-1 and CRP concentrations and to predict synovial IL-1 and CRP concentrations from age, sex, body weight and OFA score. All results were analyzed with IBM SPSS Statistics version 20, and a significance level of $p<0.05$ was set.

Data availability

The data used in this study is a property of the Guarda Nacional Republicana, a governmental police force from Portugal and, by law, confidential. The authors obtained specific approval in order to use the data. The data request may be sent to the Divisão de Medicina Veterinária (cari.dsad.dmv@gnr.pt). Other researchers, who meet the criteria for access to confidential data, can access data in the same manner as the authors.

Results

The sample included 100 pelvic limbs of police working dogs, equally distributed between left and right pelvic limbs, with a mean age of 6.5±2.4 years and a bodyweight of 26.7±5.2kg. Considering OFA hip grading, 70 joints were classified as mild (70%), 20 as moderate (20%) and ten as severe (10%). Both genders were represented (60 limbs from males and 40 from females), and male dogs showed significantly higher mean temperature on the lateral view than females ($p=0.05$) and also higher flexion values ($p=0.05$).

Overall IL-1 concentration in the synovial fluid was 161.8pg/mL (±66.5) and of CRP was 2.5mg/dL (±1.9). Serum CRP concentration was 0.8mg/dL (±0.5). Clinical findings with different cut

off points for IL-1 and CRP concentration in synovial fluid are presented in table 1. CMI results are presented in Table 2. Variables considered in multiple regression statistically significantly predicted IL-1 synovial concentration $F(5,85)=2.826$, $p=0.03$, $R2=0.117$, with OFA hip score ($p<0.01$) adding statistically significantly to the prediction. Considering a 100ng/ml cut off point for IL-1 concentration, no significant differences were observed. With a 200ng/ml cut off, animals with higher values showed higher frequency in the presence of CCO on the VD view ($p<0.01$), worse OFA hip grades ($p<0.01$) and lower serum CRP concentration ($p=0.02$). Considering a 0.3ng/dL cut off point for CRP concentration, animals below this range had worse PSS ($p=0.04$), PIS ($p<0.01$) and function scores ($p<0.01$). With a 1ng/dL cut off, animals with higher values had higher pedometer counts ($p=0.02$), lower CFHO frequency ($p<0.01$), better OFA hip grade ($p<0.01$), PIS ($p<0.01$) and function scores ($p<0.01$). Considering a 2ng/dL cut off value, animals with higher values had higher pedometer counts ($p=0.02$), higher mean and maximal thermography values on the lateral view ($p=0.05$ and $p<0.01$, respectively), increased frequency of CCO and CFHO on the VD view ($p<0.01$, for both), better OFA hip grade ($p<0.01$), and higher serum CRP concentration ($p=0.02$). IL 1 synovial levels added statistically ($p<0.01$) significantly to the prediction of thigh girth $F(3,59)=11.019$, $p<0.01$, $R2=0.359$ and joint extension thigh girth $F(3,58)=3.367$, $p=0.03$, $R2=0.148$.

Clinical findings for different OFA hip grades, according to the presence of CCO and CFHO, with different cut off points for weight, are presented in table 3. CMI results are presented in Table 4. Grouping joints by OFA hip grading, animals with a severe grade were significantly older than those with mild ($p<0.01$) and moderate ($p<0.01$) grades, and worse SI and deviation than mild ($p=0.03$ and $p=0.01$, respectively). On the digital thermography DV view, mild hip grades showed higher values than moderate grades ($p=0.03$). Mild grades had higher IL-1 concentration than moderate grades ($p<0.01$), and better HVAS scores than moderate ($p<0.01$) and severe ($p<0.01$) hip grades. They also had better PSS ($p<0.01$), PIS ($p<0.01$), LOAD ($p<0.01$), function ($p<0.01$), gait ($p<0.01$), QOL ($p<0.01$) and COI ($p<0.01$) scores than severe hip grades. Also, lower frequencies of CCO on the VD ($p=0.05$ and $p<0.01$ for moderate and severe, respectively) and FL ($p=0.03$ and $p<0.01$ for moderate and severe, respectively) views. Moderate had better LOAD ($p<0.01$), stiffness ($p=0.02$), function ($p<0.01$), gait ($p=0.02$), QOL ($p<0.01$) and COI ($p<0.01$) scores than severe hip grades. IL 1 synovial levels added statistically ($p=0.01$) significantly to the prediction of OFA hip grade $F(1,90)=6.332$, $p=0.01$.

Joints which exhibited CCO on the VD view had worse OFA hip grade ($p<0.01$), lower levels of synovial IL-1 concentration ($p=0.02$), higher serum CRP ($p<0.01$), and worse PSS ($p=0.05$), LOAD ($p<0.02$), function ($p=0.05$), gait ($p=0.01$) and COI ($p=0.04$) scores. IL 1 synovial levels added statistically ($p<0.01$) significantly to the prediction of the presence of CCO on a VD view

F(3,59)=11.019, p=0.01. Joints with CFHO on the VD view showed lower pedometer counts (p=0.02), worse OFA hip grade (p<0.01), and worse HVAS (p<0.01), PSS (p<0.01), PIS (p<0.01), LOAD (p=0.02), stiffness (p=0.02), function (p<0.01), gait (p=0.02), QOL (p=0.02) and COI (p<0.01) scores. Considering the FL view, joints where CCO was identified had worse SI (p<0.01), deviation (p=0.03) and OFA hip grade (p<0.01). IL 1 synovial levels added statistically (p<0.01) significantly to the prediction of the presence of CCO on an FL view F(3,59)=3.047, p=0.01. No significant variations were observed when CFHO was considered in this view.

Different cut off points were considered in regard to weight. With a 25kg cut-off, heavier animals had lower pedometer counts (p=0.03), higher SI (p<0.01), lower mean and maximal temperature on the lateral view (p<0.01 for both), and worse HVAS (p<0.01), PSS (p<0.01), PIS (p<0.01), LOAD (p<0.01), stiffness (p<0.01), function (p<0.01), gait (p<0.01), QOL (p<0.01) and COI scores (p<0.01). Setting a 30kg cut-off, value heavier animals had lower mean and maximal temperature on the lateral view (p<0.01 for both), worse flexion (p<0.01) and lower IL-1 concentration level (p=0.04). For a 35kg cut-off, heavier animals had lower SI (p<0.01), lower mean and maximal temperature on the lateral view (p<0.01 for both), worse flexion (p<0.01), higher frequency of CCO on a FL view (p<0.01), lower IL-1 concentration level (p<0.01) and CRP serum concentration (p<0.01), and worse HVAS (p=0.05), LOAD (p=0.05), stiffness (p=0.03), gait (p<0.01), QOL (p=0.03) and COI scores (p=0.03).

Correlations between measured parameters are presented in Table 5. In Table 6, the correlation between measured parameters and the different Clinical Metrology Instruments are also presented.

Table 1 – Mean values (±standard deviation) of overall weight, age, symmetry index (SI), deviation from normal weight-bearing, mean and maximal thermography values on ventrodorsal and lateral views, thigh girth, range of motion (extension and flexion) measurements, and synovial interleukin-1 (IL-1) and C-Reactive Protein (CRP), by different cut-off values for synovial IL-1 and CRP concentration.

		Age	Pedometer	Symmetry Index	Deviation	Thermography (ventrodorsal)		Thermography (lateral)		Thigh Girth	Joint Flexion	Joint Extension	IL-1	SF CRP	Serum CRP
		years	mean daily steps			mean. °C	maximal °C	mean. °C	maximal °C	cm	degrees	degrees			
IL-1 with 100pg/ml cut off	above	6.6±2.4	441.5±462.5	14.5±20.9	1.9±2.2	25.4±1.3	26.8±1.2	28.3±2.1	30.6±2.2	33.8±19.3	50.7±3.4	150.7±11.3	-	0.2±0.9	0.6±0.5
	bellow	6.6±1.9	325.7±261.7	16.5±20.9	3.0±5.3	26.0±0.8	27.5±0.8	27.9±1.1	30.2±0.9	32.1±2.3	51.6±2.6	153.3±2.1	-	0.5±0.6	0.4±0.3
IL-1 with 200pg/ml cut off	above	6.5±2.3	407.5±522.2	14.4±21.2	2.3±2.8	25.9±1.1	27.2±1.1	28.4±1.6	30.6±1.8	48.3±45.1	50.1±5.7	145.0±27.4	-	0.0±0.0	0.4±0.3
	bellow	6.7±2.4	439.5±442.5	14.7±20.8	2.0±2.6	25.4±1.3	26.8±1.2	28.2±2.1	30.6±2.2	48.3±45.1	50.9±2.8	151.8±3.3	-	0.2±0.9	0.6±0.5
CRP with 0.3 mg/dL cut off	above	5.0±2.4	814.0±1114.4	3.1±2.9	1.0±0.7	26.2±1.4	27.6±1.2	28.6±0.5	31.0±0.7	32.5±3.1	50.8±1.1	151.8±3.0	113.5±25.6	-	0.9±0.8
	bellow	6.5±2.2	458.4±477.0	12.5±19.7	1.7±2.6	25.4±1.3	26.8±1.3	28.2±2.0	30.4±2.1	36.3±22.7	50.8±3.3	150.7±13.3	169.3±69.1	-	0.6±0.5
CRP with 1mg/dL cut off	above	5.5±2.5	1602.0±450.2	3.9±2.6	1.3±0.5	26.2±1.4	27.6±1.2	28.7±0.5	31.0±0.7	31.6±2.7	50.5±1.0	150.8±2.2	117.3±27.9	-	1.1±0.8
	bellow	6.4±2.3	443.48±475.3	12.3±19.8	1.7±2.6	25.4±1.3	26.8±1.3	28.2±2.0	30.4±2.1	35.3±22.5	50.8±3.3	150.8±13.2	168.2±69.1	-	0.6±0.5
CRP with 2mg/dL cut off	above	4.0±1.4	1602.0±450.3	2.7±3.8	1.0±0.0	26.3±1.9	27.5±1.6	29.0±0.3	31.6±0.1	30.8±1.1	52.0±1.4	150.5±3.5	132.3±28.3	-	1.5±1.1
	bellow	6.4±2.3	443.5±475.3	12.1±19.4	1.67±3.6	25.5±1.3	28.8±1.3	28.2±1.9	30.4±3.0	35.2±2.2	50.8±3.2	150.8±12.9	166.0±69.0	-	0.6±0.5

Table 2 – Mean values (±standard deviation) of clinical metrology instruments scores by different cut-off values for synovial IL-1 and CRP concentration. CBPI – Canine Brief Pain Inventory; PIS – Pain Interference Score; PSS – Pain Severity Score; HVAS – Hudson Visual Analogue Scale; LOAD – Liverpool Osteoarthritis in Dogs; COI – Canine Orthopedic Index; QOL – Quality of Life.

		HVAS	CBPI		LOAD	COI				
			PSS	PIS		Stiffness	Function	Gait	QOL	Total
		(0-10)	(0-10)	(0-10)	(0-52)	(0-16)	(0-16)	(0-20)	(0-12)	(0-64)
IL-1 with 100ng/ml cut off	above	6.6±1.5	3.3±2.3	3.3±2.6	13.2±11.2	3.5±4.1	3.7±4.6	4.9±5.4	3.9±2.9	16.1±16.8
	bellow	6.8±1.5	2.9±2.3	3.2±2.4	12.0±11.9	3.5±4.2	3.8±4.3	4.7±5.5	3.8±3.2	15.8±16.9
IL-1 with 200ng/ml cut off	above	7.0±1.0	2.8±1.8	2.6±1.7	10.4±7.5	2.6±3.1	2.3±2.8	3.0±3.8	3.1±2.1	11.1±11.1
	bellow	6.6±1.5	3.4±2.4	3.5±2.7	13.6±11.7	3.7±4.3	4.0±4.8	5.2±5.6	4.0±3.1	16.9±17.4
IL-1 with 300ng/ml cut off	above	7.5±0.9	1.7±1.1	1.7±1.5	4.1±3.4	0.3±0.5	0.0±0.0	0.0±0.0	2.0±2.0	2.3±1.9
	bellow	6.6±1.5	3.4±2.4	3.5±2.6	13.9±11.3	3.8±4.2	4.1±4.6	5.3±5.4	4.0±2.9	17.2±16.8
CRP with 0.3 mg/dL cut off	above	7.7±1.1	1.6±1.3	1.3±0.6	5.2±6.1	0.8±1.8	0.4±0.9	1.8±4.0	2.2±1.1	5.2±7.7
	bellow	6.8±1.3	3.2±2.1	3.2±2.3	11.9±10.3	3.1±3.7	2.9±3.9	4.3±4.9	3.4±2.7	13.8±14.9
CRP with 1 mg/dL cut off	above	7.5±1.2	1.7±1.4	1.4±0.7	5.5±7.0	1.0±2.0	0.4±1.0	2.3±4.5	2.5±1.0	6.3±8.5
	bellow	6.8±1.3	3.1±2.1	3.1±2.3	11.8±10.2	3.1±3.7	2.9±3.9	4.2±4.9	3.4±2.7	13.6±14.9
CRP with 2 mg/dL cut off	above	7.0±1.7	2.4±1.9	1.7±0.9	9.0±9.9	2.0±2.8	1.0±1.4	4.5±6.4	3.0±1.4	10.5±12.0
	bellow	6.9±1.3	3.1±2.3	3.1±2.3	11.5±10.2	2.9±3.7	2.8±3.7	4.1±4.9	3.3±2.7	13.2±14.9

Table 3 – Mean values (±standard deviation) of overall weight, age, symmetry index (SI), deviation from normal weight-bearing, mean and maximal thermography values on ventrodorsal and lateral views, thigh girth, range of motion (extension and flexion) measurements, and synovial interleukin-1 (IL-1) and C-Reactive Protein (CRP), by different OFA hip grades, presence of caudolateral curvilinear osteophyte (CCO) and circumferential femoral head osteophyte (CFHO) and different cut-off values for weight.

		Age	Pedometer	Symmetry Index	Deviation	Thermography (ventrodorsal)		Thermography (lateral)		Thigh Girth	Joint Flexion	Joint Extension	IL-1	SF CRP
		years	mean daily steps			mean. °C	maximal °C	mean. °C	maximal °C	cm	degrees	degrees		
Mild		6.3±2.1	567.6±525.5	11.2±17.7	1.5±1.9	25.7±1.2	27.1±1.1	28.4±1.7	30.7±1.8	35.8±23.7	51.0±3.3	150.9±13.8	178.3±78.6	0.3±1.0
Moderate		6.4±2.5	294.0±270.5	16.6±21.6	2.4±3.5	24.9±1.1	26.5±1.2	27.9±2.3	30.6±2.3	31.7±3.5	51.1±3.5	151.7±3.6	130.6±30.5	0.1±0.2
Severe		8.4±2.7	281.0±350.4	25.1±27.2	3.5±2.9	25.4±1.5	26.6±1.4	28.1±2.9	30.2±3.2	28.9±2.1	49.5±2.9	149.3±3.4	144.7±22.9	0.0±0.0
CCO in a VD view	absent	6.1±2.3	437.5±423±.5	13.5±20.4	1.9±2.2	25.6±1.2	26.9±1.2	28.5±1.9	30.8±1.8	35.6±2.4	50.7±3.5	150.3±13.8	174.6±75.1	0.1±0.4
	present	7.1±2.6	428.4±503±.9	16.5±21.5	2.3±3.2	25.3±1.4	26.8±1.2	27.8±2.3	30.2±2.5	30.8±3.4	50.9±3.1	151.7±3.7	141.8±45.8	0.3±1.3
CFHO in a VD view	absent	5.9±2.1	653.9±556.2	10.3±16.7	1.6±1.9	25.6±1.2	27.0±1.1	28.5±1.9	30.7±1.8	37.8±27.6	50.8±3.7	150.1±16.2	163.7±70.9	0.4±1.1
	present	7.2±2.5	327.5±353.7	18.0±23.0	2.4±2.9	25.4±1.3	26.7±1.3	28.0±2.2	30.5±2.4	30.6±3.2	50.8±3.1	151.5±3.5	160.3±64.0	0.0±0.1
CCO in a FL view	absent	4.2±1.7	425.7±458.6	6.8±9.9	1.3±4.5	25.7±1.2	26.9±1.2	28.6±2.0	30.8±2.1	37.8±27.7	51.1±3.8	150.5±16.3	172.5±78.2	0.1±0.5
	present	7.1±2.7	440.8±454.2	20.1±24.4	2.5±3.0	25.4±1.3	26.8±1.2	28.0±2.0	30.4±2.2	30.6±2.9	50.6±2.9	151.1±3.4	154.8±57.4	0.3±1.1
BW with 25kg cut off	above	6.8±2.3	359.7±414.8	16.7±22.3	2.3±2.8	25.5±1.2	26.8±21.2	27.8±1.9	30.1±2.0	35.1±2.1	50.9±3.6	150.5±12.1	163.0±65	0.2±0.9
	bellow	6.4±2.6	701.6±496.7	5.9±8.2	0.9±1.3	25.6±1.6	27.0±1.3	29.9±1.7	32.3±1.6	28.7±1.7	50.4±2.2	152.2±2.3	160.3±75.4	0.32±0.9
BW with 30kg cut off	above	5.9±1.7	189.6±149.1	11.3±16.4	1.6±1.9	25.4±1.2	26.9±1.3	27.3±1.9	29.6±1.9	33.0±2.4	51.8±2.2	152.5±3.1	144.9±56.7	0.3±1.2
	bellow	7.2±2.6	501.8±483.9	16.9±23.1	2.3±2.9	25.6±1.3	26.9±1.1	28.7±1.9	31.1±2.0	34.2±2.4	50.1±3.8	149.7±13.8	174.2±71.8	0.1±0.5
BW with 35kg cut off	above	7.0±0.0	101.0±0.0	2.3±2.5	1.5±0.9	25.3±0.4	26.5±0.5	26.1±1.2	28.6±1.3	35.8±1.7	52.8±2.6	154.5±2.1	135.8±21.9	0.0±0.0
	bellow	6.7±2.5	449.1±455.7	15.9±21.4	2.1±2.7	25.5±1.3	26.9±1.3	28.5±1.9	30.8±2.1	33.5±1.9	50.5±3.3	150.4±11.4	165.8±70.4	0.2±0.9

Table 4 – Mean values (±standard deviation) of clinical metrology instruments scores, by different OFA hip grades, presence of caudolateral curvilinear osteophyte (CCO) and circumferential femoral head osteophyte (CFHO) and different cut-off values for weight. CBPI – Canine Brief Pain Inventory; PIS – Pain Interference Score; PSS – Pain Severity Score; HVAS – Hudson Visual Analogue Scale; LOAD – Liverpool Osteoarthritis in Dogs; COI – Canine Orthopedic Index; QOL – Quality of Life.

		HVAS	CBPI		LOAD	COI				
			PSS	PIS		Stiffness	Function	Gait	QOL	Total
		(0-10)	(0-10)	(0-10)	(0-52)	(0-16)	(0-16)	(0-20)	(0-12)	(0-64)
Mild		7.0±1.1	2.9±2.1	2.7±2.1	10.1±8.3	2.6±3.2	2.4±3.3	3.5±4.2	3.1±2.3	11.6±12.4
Moderate		6.3±1.6	3.5±2.3	3.8±2.7	13.1±11.7	3.8±4.3	4.3±5.3	5.3±5.9	3.9±3.2	17.3±18.4
Severe		5.5±1.6	4.7±2.8	5.1±3.2	24.6±12.9	7.0±5.4	8.3±4.9	9.6±6.5	6.7±3.6	31.6±19.8
CCO in a VD view	absent	6.8±1.5	2.9±2.1	2.9±2.3	11.0±9.3	2.9±3.7	3.0±4.9	3.7±4.6	3.5±2.6	13.2±14.3
	present	6.4±1.3	3.9±2.5	3.9±2.8	16.4±12.9	4.6±4.6	4.9±5.4	6.6±6.1	4.5±3.3	20.6±18.9
CFHO in a VD view	absent	7.1±1.1	2.5±1.8	2.4±1.7	9.9±7.5	2.4±2.9	2.1±2.9	3.3±4.0	3.0±2.0	10.8±11.3±
	present	6.3±1.6	3.9±2.5	4.1±2.9	15.6±12.3	4.5±4.7	5.1±5.2	6.1±6.1	4.6±3.4	20.2±19.0
CCO in a FL view	absent	6.9±1.2	2.9±2.1	2.8±2.1	12.4±8.7	3.1±3.1	2.9±3.4	4.4±4.5	3.4±2.3	13.8±12.9
	present	6.4±1.6	3.5±2.5	3.7±2.8	13.7±12.8	3.9±4.8	4.4±5.2	5.2±6.1	4.2±3.4	17.8±16.7
BW with 25kg cut off	above	6.4±1.5	3.7±2.4	3.8±2.6	14.7±11.7	4.2±4.3	4.4±4.8	5.7±5.6	4.4±3.0	18.8±17.4
	bellow	7.6±0.8	1.6±0.9	1.4±0.5	6.8±4.8	1.0±1.4	1.1±1.9	1.4±2.8	1.8±1.3	5.3±6.2
BW with 30kg cut off	above	6.7±1.4	3.2±2.1	3.2±2.3	12.6±10.7	3.1±3.4	3.2±3.8	4.9±4.9	4.1±2.5	15.2±14.2
	bellow	6.6±1.5	3.3±2.4	3.4±2.7	13.4±11.5	3.9±4.5	4.1±5.0	4.9±5.8	3.8±3.2	16.6±18.2
BW with 35kg cut off	above	5.8±1.0	4.4±1.9	4.0±2.0	17.8±6.7	5.2±2.0	5.0±3.4	8.0±2.4	5.2±1.7	23.4±9.3
	bellow	6.7±1.5	3.1±2.3	3.2±2.6	12.5±11.5	3.3±4.3	3.6±4.7	4.5±5.6	3.7±3.1	15.1±17.2

Table 5 – Correlation between measured parameters

Measure		Age	Weight	Pedometer	SI	Deviation	Termo DV	Termo DV max	Termo Lat	Termo Lat max	Thigh girth	Flexion	Extension	CCO VD	SF IL-1	SF CRP
Age	r_s	1	-0.08	-0.08	0.29	0.32	0.27	0.24	0.22	0.26	-0.07	-0.17	-0.03	0.19	0.22	-0.15
	Sig.		0.47	0.47	0.01*	0.01*	0.02*	0.03*	0.04*	0.02*	0.49	0.11	0.76	0.06	0.84	0.24
Weight	r_s	-0.08	1	-0.32	0.14	-0.34	-0.02	0.00	-0.43	-0.42	0.07	0.19	0.061	0.03	-0.15	-0.05
	Sig.	0.47		0.03*	0.21	0.74	0.83	0.97	0.01*	0.01*	0.49	0.06	0.57	0.89	0.17	0.71
Pedometer	r_s	-0.08	-0.32	1	-0.32	-0.32	-0.32	0.13	-0.43	0.42	-0.18	0.19	0.06	0.03	-0.12	0.37
	Sig.	0.47	0.03*		0.03*	0.03*	0.03*	0.41	0.00*	0.00*	0.24	0.07	0.57	0.79	0.43	0.04
SI	r_s	0.29	0.14	-0.32	1	0.71	-0.26	-0.22	-0.31	-0.23	-0.13	-0.18	-0.01	0.071	0.04	0.09
	Sig.	0.01*	0.21	0.03*		0.00*	0.02*	0.04*	0.01*	0.04*	0.25	0.10	0.96	0.52	0.73	0.46
Deviation	r_s	0.32	-0.34	-0.32	0.71	1	-0.09	-0.08	-0.21	-0.12	-0.07	-0.09	-0.03	0.08	-0.06	-0.05
	Sig.	0.01*	0.74	0.03*	0.00*		0.40	0.52	0.07	0.31	0.53	0.43	0.81	0.46	0.57	0.69
Termo DV	r_s	0.27	-0.02	-0.32	-0.26	-0.09	1	0.91	0.39	0.30	-0.02	0.12	0.03	-0.11	0.09	0.05
	Sig.	0.02*	0.83	0.03*	0.02*	0.40		0.00*	0.00	0.00*	0.85	0.31	0.77	0.32	0.41	0.72
Termo DV max	r_s	0.24	0.00	0.13	-0.22	-0.08	0.91	1	0.38	0.33	0.03	0.07	-0.01	-0.06	0.07	0.06
	Sig.	0.03*	0.97	0.41	0.04*	0.52	0.00*		0.00*	0.00*	0.81	0.53	0.99	0.59	0.51	0.66
Termo Lat	r_s	0.22	-0.43	-0.43	-0.31	-0.21	0.39	0.38	1	0.94	-0.12	-0.11	-0.10	-0.17	0.03	0.07
	Sig.	0.04*	0.01*	0.00*	0.01*	0.07	0.00	0.00*		0.00*	0.29	0.32	0.93	0.12	0.79	0.60
Termo Lat max	r_s	0.26	-0.42	0.42	-0.23	-0.12	0.30	0.33	0.94	1	-0.15	-0.14	0.01	-0.14	0.04	0.09
	Sig.	0.02*	0.01*	0.00*	0.04*	0.31	0.00*	0.00*	0.00*		0.18	0.22	0.89	0.22	0.69	0.46
Thigh girth	r_s	-0.07	0.07	-0.18	-0.13	-0.07	-0.02	0.03	-0.12	-0.15	1	-0.48	-0.90	-0.13	0.46	0.05
	Sig.	0.49	0.49	0.24	0.25	0.53	0.85	0.81	0.29	0.18		0.01*	0.00*	0.23	0.00*	0.63
Flexion	r_s	-0.17	0.19	0.19	-0.18	-0.09	0.12	0.07	-0.11	-0.14	-0.48	1	0.65	0.02	-0.15	0.02
	Sig.	0.11	0.06	0.07	0.10	0.43	0.31	0.53	0.32	0.22	0.01*		0.00	0.83	0.15	0.89
Extension	r_s	-0.03	0.061	0.06	-0.01	-0.03	0.03	-0.01	-0.10	0.01	-0.90	0.65	1	0.06	-0.31	-0.01
	Sig.	0.76	0.57	0.57	0.96	0.81	0.77	0.99	0.93	0.89	0.00*	0.00		0.57	0.00*	0.93
CCO VD	r_s	0.19	0.03	0.03	0.07	0.08	-0.11	-0.06	-0.17	-0.14	-0.13	0.02	0.06	1	-0.24	0.13
	Sig.	0.06	0.89	0.79	0.52	0.46	0.32	0.59	0.12	0.22	0.23	0.83	0.57		0.02*	0.29
SF IL-1	r_s	0.22	-0.15	-0.12	0.04	-0.06	0.09	0.07	0.03	0.04	0.46	-0.15	-0.31	-0.24	1	-0.12
	Sig.	0.84	0.17	0.43	0.73	0.57	0.41	0.51	0.79	0.69	0.00*	0.15	0.00*	0.02*		0.34

Table 6 – Correlation between measured parameters and Clinical Metrology Instruments.

Measure		Age	Weight	Pedometer	SI	Deviation	Termo DV	Termo DV max	Termo Lat	Termo Lat max	Thigh girth	Flexion	Extension	CCO VD	SF IL-1	SF CRP
HVAS	r_s	-0.36	-0.19	-0.19	-0.25	-0.32	0.09	0.14	0.20	0.16	0.12	0.09	-0.01	-0.13	0.12	0.13
	Sig.	0.01*	0.07	0.19	0.02*	0.00*	0.43*	0.21	0.07	0.16	0.28	0.39	0.95	0.22	0.26	0.29
PSS	r_s	0.39	0.16	0.13	0.24	0.27	0.00	-0.12	-0.12	-0.09	-0.14	-0.06	0.04	0.21	-0.09	-0.15
	Sig.	0.00*	0.12	0.40	0.03	0.02*	0.99	0.27	0.27	0.41	0.18	0.61	0.74	0.05*	0.37	0.23
PIS	r_s	0.41	0.19	0.06	0.30	0.31	-0.04	-0.1	-0.1	-0.06	-0.14	-0.09	0.01	0.19	-0.09	-0.17
	Sig.	0.00*	0.08	0.71	0.00*	0.01*	0.74	0.37	0.37	0.62	0.19	0.39	0.98	0.07	0.36	0.18
LOAD	r_s	0.51	0.14	0.01	0.35	0.39	0.09	0.05	-0.05	-0.04	-0.13	-0.07	-0.01	0.24	-0.14	-0.14
	Sig.	0.00*	0.19	0.95	0.00*	0.00*	0.41	0.65	0.68	0.70	0.25	0.54	0.94	0.02*	0.21	0.28
COI	r_s	0.58	0.16	0.05	0.32	0.39	0.14	0.09	-0.01	-0.02	-0.13	-0.11	-0.01	0.22	-0.14	-0.11
	Sig.	0.00*	0.15	0.75	0.00*	0.00*	0.22	0.41	0.94	0.89	0.23	0.30	0.93	0.04	0.21	0.37
Stiffness	r_s	0.58	0.12	0.11	0.30	0.39	0.13	0.08	0.02	0.05	-0.09	-0.12	-0.03	0.19	-0.10	-0.13
	Sig.	0.00*	0.27	0.45	0.01*	0.00*	0.24	0.48	0.85	0.65	0.36	0.26	0.79	0.06	0.36	0.31
Function	r_s	0.62	0.93	-0.05	0.35	0.39	0.13	0.09	0.05	0.08	-0.14	-0.15	-0.02	0.21	-0.15	0.14
	Sig.	0.00*	0.39	0.73	0.00*	0.00*	0.24	0.42	0.64	0.46	0.21	0.18	0.89	0.05*	0.18	0.28
Gait	r_s	0.52	0.19	0.05	0.27	0.36	0.13	0.08	-0.05	-0.04	-0.14	-0.09	0.01	0.26	-0.15	0.09
	Sig.	0.00*	0.08	0.75	0.01*	0.00*	0.27	0.49	0.59	0.74	0.21	0.41	0.96	0.01	0.18	0.45
QOL	r_s	0.55	0.22	0.11	0.33	0.38	0.18	0.13	-0.06	-0.05	-0.14	-0.07	0.00	0.16	-0.11	-0.08
	Sig.	0.00*	0.04*	0.46	0.00*	0.00*	0.11	0.09	0.59	0.68	0.19	0.54	1.00	0.13	0.32	0.53

Discussion

Osteoarthritis is the most commonly diagnosed joint disease both in human and veterinary medicine(Anderson et al., 2018). To our knowledge, this is the first study to describe the influence of IL-1 and CRP synovial concentrations levels in several clinical signs, diagnostic imaging, laboratorial findings and clinical metrology instruments results of animals with OA, characterizing the multiple dimensions of the disease.

It is well established that OA is a complex low-grade inflammatory process, which affects the progression of the disease (Loeser et al., 2012). IL-1 drives this process and increases the expression of inflammatory genes and mediators, with median detected concentrations of IL-1β concentrations of 109pg/mL in knees with mild OA and 122pg/mL in moderate OA, in an animal model(McNulty et al., 2013). We have recorded mean values of 178.3pg/mL for joints with mild OA, 130.6pg/mL for joints with moderate OA and 144.7pg/mL for joints with severe OA. Concentration levels were significantly higher in mild cases comparing with moderate ones, which may account for the also significantly higher thermographic evaluation. Regression analysis determined that the OFA hip score could be determined from IL-1 levels. The reason for higher levels in less severe OA grades is still unclear, but maybe an indicator of higher inflammatory activity in the early stages of the disease. This, however, did not translate in more significant disease-related impairment, since animals with mild OA exhibited better scores in several CMIs. This is line with what has been previously described, with no relationship being identified between pro-inflammatory and anti-inflammatory biomarker concentrations and gait asymmetry in dogs with OA (Allen et al., 2019).

In some cases, even if in cases without overt lameness, subtle shifts in body weight distribution at a stance may be present (Clough et al., 2018; Seibert et al., 2012). This was observed in the present sample, with severe OA cases showing worse SI and deviation than mild and moderate cases. Synovial IL-1 concentration showed no correlation with measured clinical parameters other than a weak, significant correlation with joint extension, but significantly added to the prediction of thigh girth and joint extension. It also correlated with the presence of CCO on a VD view and added to the prediction of its occurrence in both considered radiographic projections. CCO, in turn, correlated with PSS, LOAD and function scores. Cases were CCO was present had higher IL-1 and serum CRP levels, and also worse several CMI scores. IL-1 levels may be related to the development of this radiographic sign, which then expresses a toll on the patient's levels of pain and activity. This relation is also observed when comparing animals with an IL-1 concentration cut off point of 200pg/mL since animals with higher concentrations showed a higher frequency of CCO and worse hip grades.

The potential interest in determining CRP levels is the advantage of it being an objective and quantitative marker of inflammation, not biased by treatment with NSAIDs nor glucocorticoids

(Bennett et al., 2013; Borer et al., 2003; Kjelgaard-Hansen et al., 2013; Kum et al., 2013). It has been measure in dogs with stifle OA, with concentration levels being higher in cases of OA secondary to natural occurring cranial cruciate ligament rupture when compared to normal stifles. However, in cases of OA from other anatomical locations, synovial CRP levels were lower (Boal and Miguel Carreira, 2015). In this study, we have observed CRP synovial levels, similar to what has previously recorded in dogs with knee OA (2.5mg/dL in this study vs 2.22 mg/dL) (Boal and Miguel Carreira, 2015). Registered serum levels were higher than what has been recorded in a cohort of dogs with OA (0.8mg/dL in this study vs 0,05mg/dL) (Fujiki et al., 2007). It was interesting to observe that higher values of synovial CRP corresponded to better OFA hip grades, higher activity levels as measure with the pedometer and PIS and function scores. However, they also had higher thermography values, which is usually related to increased inflammatory activity. We also reported higher serum CRP concentration levels than what has been previously reported (Fujiki et al., 2007). In the same study, CRP levels increased after treatment (Fujiki et al., 2007). It is unclear if higher CRP levels are a consequence of high activity levels, micro-lesions within the joint and inflammatory activity within the joint, thus leading to the progression of the disease in animals still at an early stage or, on the other hand, if higher CRP levels reflect a better prognosis since higher levels after treatment have been previously recorded. Further studies must address this question.

Pain is the most relevant clinical sign of OA and a hallmark of the disease (Piel et al., 2014). Together with functional ability, they are of the most critical parameters in the evaluation of OA treatment efficacy, with canine studies offering valuable data that may translate to humans (Robertson-Plouch et al., 2019; Wiegant et al., 2015). An additional observation was related to the relationship between increasing body weight and worse scores with different CMIs, evaluating different components of OA. At the 35kg cut-off value, the highest considered, animals additionally showed worse clinical and imaging findings, higher thermography values, worse flexion and higher frequency of CCO.

Further studies, including a control group, with non-lame dogs, would be of interest to validate this comparison. Also, we only collected data in a single moment, and therefore cannot comment on the interest of each of the findings for the prognosis or treatment monitoring of OA. These aspects should be addressed in future studies.

Conclusions

Intra-articular CS may be a treatment option for some dogs with naturally occurring OA, being particularly useful in terms of reducing pain and return to function, with an added cost-effectiveness

when compared to other therapeutic options. Further studies are required, aimed at determining which are the better candidates for the treatment, and to evaluate alternative drugs and drug dosages.

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4. VALIDATION OF THE USE OF DIGITAL THERMOGRAPHY AND WEIGHT-BEARING EVALUATION IN OA ASSESSMENT

Evaluation of digital thermography imaging to assess and monitor treatment of police working dogs with naturally occurring hip osteoarthritis – Published in BMC Veterinary Research – Impact factor 1.860, Quartile 1.

Thermographic imaging of police working dogs with bilateral naturally occurring hip osteoarthritis. Published in Acta Veterinaria Scandinavica – Impact factor 1.590, Quartile 1.

Evaluation of four clinical metrology instruments for the assessment of osteoarthritis management in a naturally occurring canine model - Submitted to Scientific Reports – Impact factor 3.998, Quartile 1.

Characterization weight-bearing compensation in dogs with bilateral hip osteoarthritis - Submitted to Frontiers in Veterinary Science – Impact factor 2.140, Quartile 1.

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Evaluation of digital thermography imaging to assess and monitor treatment of police working dogs with naturally occurring hip osteoarthritis

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Abstract

Background: In dogs, thermal imaging has been documented only recently, but a growing interest in this modality has led to studies using thermography to assess pathologies in the canine hip, stifle, elbow, intervertebral disc, and bone neoplasia. This study aimed to evaluate the use of digital thermography in assessing and evaluatingtreatment responsein dogs with hip osteoarthritis (OA) and comparing its results with an objective measure and two clinical metrology instruments. In an experimental, randomized, double-blinded study, one hundred hip joints of fifty police working dogs with bilateral hip OA were evaluated. A dorsoventral and lateral thermographic image were obtained on days 0, 8, 15, 30, 90, and 180. Mean and maximal temperatures were determined. Additionally, the animal's weight-bearing distribution and radiographic examination of the hip joint (extended legs ventrodorsalview) were performed. Copies of the Canine Brief Pain Inventory (CBPI) and Canine Orthopaedic Index (COI) were obtained. Results were analyzed by ANOVA, followed by an LSD post-hoc test, and correlations were assessed withSpearman correlation coefficient, with $p < 0.05$.

Results: Values recorded on the lateral view were higher than those on the dorsoventral view. No differences or correlations were found between Orthopedic Foundation for Animals hip grades and temperature. Digital thermographic images showed a weak significant correlation with weight-bearing evaluations ($r = 0.13$, $p < 0.01$) and different clinical metrology instruments scores ($r = -0.25$, $p < 0.01$ for pain severity score, and $r = -0.21$, $p = 0.04$ for gait). It also correlated with radiographic findings, specifically the circumferential femoral head osteophyte and caudolateral curvilinear osteophyte.

Conclusion: To our knowledge, this is the first study presenting the digital thermography assessment of Police working dogs submitted to treatment for hip OA. Digital thermography, mainly based on a lateral view evaluation, showed a weak significant correlation with stance analysis and clinical metrology instruments scores.

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Background

Digital thermography is a contact-free, non-invasive screening tool that can assess soft tissue injuries, includ- ing muscle strains, sprains, tendinopathies, and OA in humans, horses, cats, and dogs [1–3]. It relies on identifying changes in heat in tissue due to disruptions of tissue morphology and physiological functions, which in turn relate to skin temperature control [4–6]. The rationale behind its use is that an injury is often associ- ated with variations in blood flow on the affected site, changing the skin temperature [7]. Changes in blood flow rate, local structures of subcutaneous tissues, andthe sympathetic nervous system's activity are reflected on skin temperature through a complex system [1]. Inflammation in subcutaneous and deeper tissues re- flects temperature changes in superficial tissues. These changes are a product of the inflammation mechanism, which influences blood vessels' diameter, blood flow rate, and capillary permeability [8, 9]. Digital thermog- raphy can provide a reproducible screening tool by describing the specific changes in each disease process [10, 11]. It has been described as useful in humans, horses, and cats [1, 3, 12, 13]. There has been a growing interest in thermal imaging in dogs, with recent reports on thermography use to assess pathologies in the canine hip, stifle, elbow, intervertebral disc, and bone neoplasia published [2, 13–18]. These studies have described nor- mal thermal imaging of different body areas or diseases. Still, there are no studies available to our knowledge comparing the results of thermal imaging with other evaluation modalities and in the evaluation of response to treatment.

OA is the most prevalent joint disease in dogs, with an estimated prevalence of 20% [19–22]. Since the disease lacks obvious extra-articular manifestations, it is well suited to use a local therapy by intra-articular (IA) injection [23]. Commonly used IA treatment modalities include corticosteroids (as triamcinolone hexacetonide), hyaluronan, and autologous platelets [24–26]. Pain is the most relevant clinical sign of OA and a hallmark of the disease [27, 28]. Several clinical metrology instruments have been developed to assess pain and evaluate treatment response [29]. One of the best clinical metrology instrument created for dogs is the Canine Brief Pain Inventory (CBPI), divided into a pain severity score (PSS), to evaluate the overall pain magnitude and a pain interference score (PIS), to assesses the degree to which pain affects daily activities [30–34]. The Canine Orthopaedic Index (COI) is an additional clinical metrology instrument to evaluate other dimensions of OA's impact. It is divided into stiffness, gait, function, and quality of life scores [35–38]. A typical assessment performed during the orthopedic examination is evaluating weight distribution, off-loading, or limb favoring at the stance [39].

OA patients exhibit subtle shifts in body weight distribu- tion at a stance due to pain or instability [40, 41]. Stance analysis, which evaluates individual limb weight-bearing, is an objective measure, reported as sensitive for detect- ing lameness in dogs [41], and equivalent or superior measurement of hip OA-related pain associated with hip OA than vertical impulse and peak vertical force [41].

This study aimed to evaluate the use of digital thermography in assessing and evaluating treatment response in dogs with hip OA. As OA is a disease involving multiple dimensions, from changes in limb function, ability to conduct daily activities, and demeanor [32], we also aimed to compare the results of digital thermography to an objective measure (weight-bearing evaluation) and two clinical metrology instruments (the CBPI and the COI). We hypothesize that digital thermography results will correlate with the weight-bearing assessment results, the considered clinical metrology instruments, and radiographic examination.

Results

The sample from this study included 100 limbs of 50 Police working dogs with bilateral hip OA: 17 German Shepherd Dogs, 15 Belgian Malinois Shepherd Dogs, 10 Labrador Retriever, and 8 Dutch Shepherd Dog, 30 from males and 20 females, with a mean age of 6.5 ± 2.2 years, bodyweight of 26.7 ± 5.3 kg and body condition score of 4/9 [42]. Joints were classified as mild (n = 70), moderate (20), and severe (10), according to the Orthopedic Foun- dation for Animals hip grading scheme [43, 44]. Three images (1 DV and 2 LT, to have a DV and LT view from each joint) were obtained from each animal in six differ- ent evaluation moments (days 0, 8, 15, 30, 90, and 180), amounting to 900 images. All patients were followed up to the last evaluation day. At the initial evaluation (day 0), the mean and maximal temperature on the DV were 24.7 °C ± 1.7 and 25.8 °C ± 1.7, respectively. On the LT view, mean and maximal temperatures were 26.1 °C ± 2.3 and 28.1 °C ± 2.4, respectively. The thermographic evalu- ation results in each view by the Orthopedic Foundation for Animals hip grades are presented in Table 1. No significant differences or correlations were found between OFA hip grade and temperature.

Table 1 Mean and maximal thermographic evaluation values (± standard deviation) of ventrodorsal and lateral views, by Orthopedic Foundation for Animals hip grades at the initial evaluation

OFA hip grade	Dorsoventral view (°C, mean ± SD)		Lateral view (°C, mean ± SD)	
	Mean	Max	Mean	Max
Mild (*n* = 70)	24.9 ± 1.6	25.9 ± 1.6	26.2 ± 2.2	28.2 ± 2.1
Moderate (*n* = 20)	24.9 ± 1.8	25.9 ± 1.9	26.1 ± 2.6	28.2 ± 2.4
Severe (*n* = 10)	24.0 ± 1.8	25.0 ± 1.7	25.5 ± 2.6	27.2 ± 2.3

Mean and maximal values of ventrodorsal and lateral views on each evaluation day are presented in Table 2. Compared to the initial evaluation, significant variations in the thermographic evaluation were recorded mainly on the lateral view. During follow-up evaluations, significant differences in the result of treatment were registered with SI ($p < 0.01$) and deviation ($p < 0.01$). Correlations between thermography evaluation and weight-bearing evaluation, on the initial assessment and during the follow-up period, are presented in Table 3. Correlations between thermography evaluation and clinical metrology instrument scores on the first assessment and during the follow-up period are shown in Table 4. Considering radiographic findings, at the initial evaluation, maximal DV, mean and maximal LT thermographic evaluations showed a weak correlation with the presence of caudolateral curvilinear osteophyte on the ventrodorsal view ($r = -0.2$, $p = 0.05$; $r = -0.3$, $p < 0.01$ and $r = -0.2$, $p = 0.04$, respectively).

Discussion

Digital thermal imaging can be used to assess musculoskeletal conditions, including OA, based on the variations in blood flow that injury and inflammation generate, which can affect the skin temperature [7-9]. To our knowledge, this is the first study to describe the use of digital thermography in the initial evaluation and to monitor treatment outcome in dogs with OA.

Pain is the most relevant clinical sign of OA [27, 28], and it is a multi-dimensional experience with functional, sensory, evaluative, and affective components [45]. To capture information regarding this wide-ranging nature of the disease, we choose to compare digital thermog- raphy to an objective measure, stance analysis [41], directed at evaluating the function, and two different clinical metrology instruments, to assess pain and the ability to conduct specific daily activities. Previous re- ports indicate that SI is reliable indicators of clinical lameness in dogs [46]. Considering a cut-off point of 18% of weight-bearing for pelvic limbs seems to increase sensitivity and specificity [41, 47, 48]. For that reason, we compared thermography scores with both SI and deviation from the normal value of 20%. During the follow-up period, digital thermography results based on the lateral view, specifically the maximal value, showed low but significant correlations with stance analysis results, which provides evidence favoring using a lateral view rather than a dorsoventral when monitoring OA treatment. Still, the dorsoventral maximal value also showed a week significant correlation with SI. This is not entirely unexpected, as inflammatory mediators drive OA within the joint, and thermography has shown to be a reliable technique to assess inflammatory pain and differentiate normal from human osteoarthritis pa- tients [49-51]. The use of the maximal temperature value may better reflect this inflammatory process and nature. It may also present an additional advantage for less experienced operators than mean values. This last approach requires a more precise determination of anatomical areas of interest and is influenced by incorrect inclusion of measurements from non-affected tissues. It is also well established that the perception of pain and overall joint function is influenced by sensory innervation of the tissues that compose the joint, from the subchondral bone, periosteum, synovium to the capsule, and surrounding tissues, such as muscles [52, 53]. As LT views include a more significant amount of muscle masses than DV views, which are also involved in the disease process and under inflammation, this may ac- count for higher mean and maximal temperature values registered on a lateral view [51, 54]. It may also account for the weak significant correlation between LT evalua- tions and pain severity scores during the follow-up

Table 2 Mean and maximal thermographic evaluation temperature values (±standard deviation) of ventrodorsal and lateral views, on each evaluation moment. * indicates significant differences when compared with day 0 evaluation ($p < 0.01$). Negative values represent negative correlations between values

Instant	Dorsoventral view (°C, mean ± SD)		Lateral view (°C, mean ± SD)	
	Mean	Max	Mean	Max
0	24.9 ± 1.7	25.8 ± 1.7	26.1 ± 2.3*	28.1 ± 2.4*
8	24.0 ± 2.3	25.5 ± 2.2	30.9 ± 2.3*	34.6 ± 1.6*
15	26.6 ± 2.2	25.9 ± 2.3	29.5 ± 3.1*	34.3 ± 1.6*
30	24.8 ± 2.3	26.3 ± 2.3	29.6 ± 2.6*	33.6 ± 1.9*
90	25.8 ± 1.3	27.1 ± 1.4*	28.5 ± 2.1*	30.7 ± 2.3*
180	25.5 ± 1.3	26.9 ± 1.2*	28.2 ± 2.1*	30.6 ± 2.1*

Table 3 Correlation coefficients between thermography evaluation and weight-bearing evaluation (symmetry index and deviation from the normal 20% weight-bearing), on the initial evaluation and during the follow-up period. COI – Canine Orthopedic Index; PIS – Pain Interference Score; PSS – Pain Severity Score; QOL – Quality of Life; SI – Symmetry Index. [a] indicates a significant correlation

Initial evaluation				Follow up period	
Measure		SI	Deviation	SI	Deviation
Dorsoventral mean	r_s	0.22	0.06	−0.05	−0.03
	Sig.	0.83	0.59	0.27	0.49
Dorsoventral max	r_s	0.48	0.04	−0.12	−0.05
	Sig.	0.65	0.73	0.01[a]	0.27
Lateral mean	r_s	0.04	0.05	0.08	0.09
	Sig.	0.97	0.65	0.08	0.04[a]
Lateral max	r_s	0.03	0.07	0.13	0.10
	Sig.	0.75	0.51	< 0.01[a]	0.04[a]

Table 4 Correlation coefficients between thermography evaluation and clinical metrology instrument scores, on the initial evaluation and during the follow-up period. COI – Canine Orthopedic Index; PIS – Pain Interference Score; PSS – Pain Severity Score; QOL – Quality of Life; SI – Symmetry Index. [a] indicates a significant correlation

Evaluation moment	Measure		Score						
			PSS	PIS	COI	Stiffness	Function	Gait	QOL
T0	Dorsoventral mean	r_s	−0.01	−0.03	−016	−0.21	−0.11	−0.22	−0.03
		Sig.	0.89	0.78	0.13	0.04[a]	0.28	0.04[a]	0.78
	Dorsoventral maximal	r_s	−0,02	−0.03	−0.14	– 0.17	−0.11	– 0.19	−0.01
		Sig.	0.89	0.81	0.19	0.10	0.31	0.06	0.97
	Lateral mean	r_s	−0.04	−0.06	−0.12	– 0.10	−0.41	– 0.21	−0.02
		Sig.	0.73	0.59	0.28	0.32	0.69	0.04[a]	0.84
	Lateral maximal	r_s	−0.01	−0.01	−0.09	– 0.84	−0.07	– 0.17	0.03
		Sig.	0.99	0.98	0.36	0.42	0.48	0.09	0.81
Follow up period	Dorsoventral mean	r_s	−0.04	−0.04	−0.06	– 0.05	−0.04	– 0.06	−0.07
		Sig.	0.42	0.36	0.25	0.32	0.38	0.23	0.16
	Dorsoventral maximal	r_s	0.06	−0.01	−0.01	0.02	0.01	−0.06	−0.01
		Sig.	0.23	0.91	0.77	0.70	0.91	0.22	0.97
	Lateral mean	r_s	−0.10	−0.01	0.04	0.03	0.06	0.01	0.08
		Sig.	0.03[a]	0.82	0.37	0.59	0.22	0.79	0.08
	Lateral maximal	r_s	−0.25	−0.06	−0.01	−0.01	– 0.01	−0.1	0.3
		Sig.	< 0.01[a]	0.22	0.94	0.86	0.79	0.79	0.5

evaluations. An additional reason for these weak but significant correlations (also reflected in the scatter plot) may be related to the fact that different evaluation methods are capturing different dimensions of OA [32].

The thermographic evaluation also correlated with clinical metrology instruments scores, specifically gait and function, at the initial assessment. This can be explained by the fact that inflammation, whose effect is recorded by digital thermography, reflects affected tissues, their contribution to pain perception, and loss of normal function. These signs, related to an inability to perform normal daily activities, are most likely to present a patient for consultation [29]. As most joints represented in this sample were classified as mild OA, they are likely expressed mainly on scores that aim to measure functionality rather than overall demeanor, such as QOL. A weak significant correlation was ob- served with the PSS, but not with PIS, and only during the follow-up period. This is not entirely unexpected, as patients in this study high-drive working dogs, which tend to be stoic and usually show only subtle signs of pain, making its evaluation more challenging [55, 56].

In a human OA study, increased temperatures have been related to even slight degenerative changes and low temperatures with more severe disease cases [9]. In other reports, a correlation between increasing temperature and more severe radiographic changes has been described [57, 58]. Although no differences were found in the thermographic evaluation of different OFA hip grades, hip joints classified as severe did have lower values in all considered thermographic evaluations, which may signal a trend. With severe OA, a loss of the tissues that surround the joint also occurs [1, 51]. These factors may be responsible for the decrease in temperature ob- served in severe hip grades than moderate hip grades. The evaluation of more hips classified as severe would help clarify this fact, as the large majority of joints considered in this study were classified as mild. The circumferential femoral head osteophyte and the caudolateral curvilinear osteophyte are the two features that represent early radio- graphic signs that predict the development of the clinical symptoms of hip OA [43, 59–61]. This is supported by our results, as both showed a correlation with digital thermography evaluation and may be linked to the inflam- matory process that drives OA and is responsible for producing clinical signs.

This study evaluated digital thermography's ability to assess and assess treatment response in dogs with hip OA by com-paring digital thermography results with other commonly used and validated evaluation modalities. A limitation of the study is related to the fact that evaluated dogs were working dogs, which tend to be stoic, making it more challenging to assess these patients using the CBPI [55, 56]. Also, the majority of animals had mild or moderate OA. It would be of interest to include disease-free patients and a larger proportion of animals representing the remaining hip grades to describe their digital thermography evaluation and this evaluation technique's ability to differentiate between them.

Conclusions

To our knowledge, this is the first study presenting the digital thermography assessment of Police working dogs submitted to treatment for hip OA. Significant variations were observed in the thermographic evaluation of patients between initial and follow-up evaluations. Digital thermography, mainly based on an LT view evaluation, correlated with weight-bearing distribution and clinical metrology instruments scores. It also correlated with the presence of caudolateral curvilinear osteophyte on the ventrodorsal view at the initial assessment, a finding associated with the development of clinical symptoms of hip OA. Digital thermography may be an option for the screening of dogs with hip OA.

Methods

The sample comprised one hundred (N = 100) hip joints from fifty active police working dogs with naturally occurring bilateral hip OA, constituting a convenience sample of patients presenting for treatment in the Clinica Veterinária de Cães of the Guarda Nacional Republicana (Portuguese Gendarmerie), after they were first diagnosed. All patients were active working dogs and remained in ac- tive work during and after this study's conclusion. For this report, data from a longitudinal double-blinded, negative controlled study was used. Animals were signaled based on a diagnosis consistent with bilateral hip OA. For the diagnosis, the dog's history was considered, in addition to a difficulty to perform specific exercises (as rising, jump- ing, or maintaining obedience positions, leading to a worse performance), a physical examination consistent with hip OA (joint pain and stiffness, with a reduced range of motion), and an OFA hip scores of mild, moderate or severe. Additionally, they should be over 2 years and have a bodyweight ≥20 kg [62, 63]. They could not be on any medication or nutritional supplements for 6 weeks or more to allow a washout period [64]. Animals with any other suspected or documented orthopedic, neurological, or concomitant disease were excluded. Other conditions were ruled out through physical and radiographic examin- ation, complete blood count, and serum biochemistry. The same researcher examined all patients.

Using a statistical analysis software, patients were randomly assigned to a group and, on day 0 (treatment day), either received an intra-articular administration of 0.9% NaCl (control group) or treatment (a platelet concentrate - VPET®, Hylan G-F 20, stanozolol or triamcinolone hexacetonide), the same to both hips, according to the assigned group. All groups had the same number of joints (n = 20), and all administrations were performed by the same researcher, blinded to the given group. No other medications/treatments were administered during the follow-up period.

Thermographic images, CBPI, COI, and weight-bearing evaluation results were recorded on days 0, 8, 15, 30, 90, and 180, corresponding to the day when the IA treatment response was evaluated. These evaluations were conducted before the IA administration. On each assessment, three images were taken sequentially: a dorsoventral view, a right lateral view, and a left lateral view, with a FLIR ThermaCAM E25® camera. A single researcher, blinded to the patient's assigned group, performed the thermographic evaluation. Before image collection, dogs were introduced in a room with a controlled temperature, set at 21 °C. They were allowed to walk calmly to adjust to room temperature for 30 min. During the actual image collec- tion, animals were positioned standing in an upright pos- ition, as symmetrically as possible. When required, the dog's trainer assisted in maintaining the animal's position. They were allowed to touch the dog's abdomen but not its torso. Each dorsoventral image included the last lumbar vertebra area to the first coccygeal vertebra (Fig. 1). This procedure has high repeatability between observers and cameras [13]. For the lateral views, the greater trochanter was located and placed in the image's center (Fig. 2).

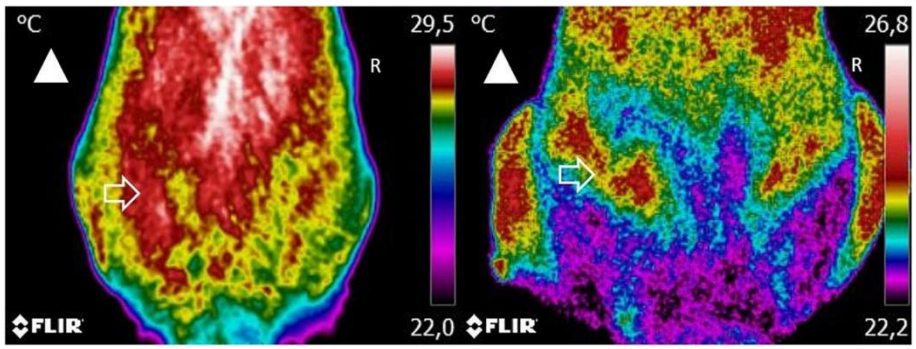

Fig. 1 A dorsoventral view of two dogs with moderate (left) and severe (right) osteoarthritis. Images include the area from the last lumbar vertebra to the first coccygeal vertebra at a minimum, at a distance of 60 cm. Arrowhead indicates the direction of the dog's head. Arrow indicates the anatomical location of the hip joint. Increased temperature areas are observed on the hip joint, but with surrounding lower temperatures on the patient with severe OA

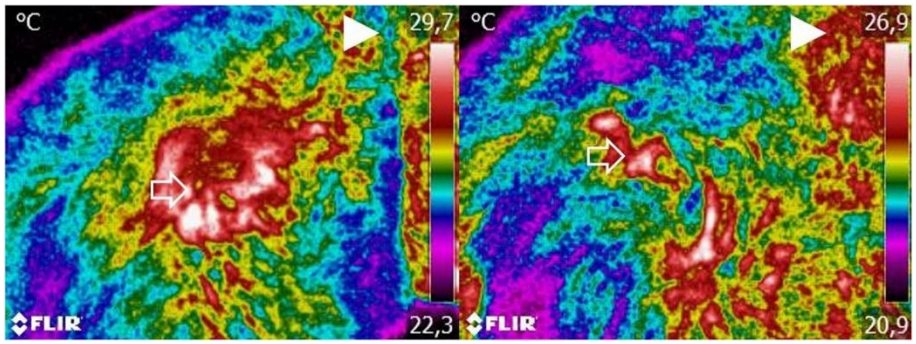

Fig. 2 A lateral view of the same two dogs represented in Fig. 1, with moderate (left) and severe (right) osteoarthritis. Images were taken with the greater trochanter in the center of the image at a distance of 60 cm. Arrowhead the direction of the dog's head. Arrow indicates the anatomical location of the hip joint. Increased temperature areas are observed on the hip joint, but with surrounding lower temperatures on the patient with severe OA

Figure 3 shows a dog at the time of initial evaluation and 30 days post-treatment. No fur clipping was performed before image collection since it can be harmful for thermographic evaluation, affecting reading stability for at least 60 min after clipping [14]. Image settings were adjusted to include a range of 15–40 °C and emissivity of 0.98. Thermographic images were analyzed with Tools (FLIR Systems, Inc), a Rainbow HC color pallet was selected, and equal-sized temperature boxes were placed on the hip joint's anatomical area. Mean and maximal temperatures were determined.

After image collection, handlers received the published instructions for CBPI and COI and completed an online copy of each for them. These were completed in sequence by the same handler, which was blinded to the group his/her dog was assigned, in each of the follow-up assessments, without knowledge of their previous answer. Weight-bearing distribution was obtained with the Companion Stance Analyzer (LiteCure LLC, Newark, Delaware, United States). The equipment was placed on a flat surface in the center of a room, at least 1 m from the walls, calibrated at the beginning of each day, and zeroed before each evaluation moment. Animals were encouraged by their handlers to stand on to the weight distribution platform, with one foot on each quadrant. Gentle restraint was used to maintain the patient's head in a natural, forward-facing position. For each patient, 20 measurements were conducted, and a mean value was obtained. Normal weight distribution for each limb is considered 20% of the total weight [41], and we also considered the deviation from this value, obtained by subtracting the weight-bearing of the limb to 20. Additionally, a left-right symmetry index (SI) was calculated, using the following formula: $SI = [(WB_R\text{-}WB_L)/ ((WB_R + WB_L) \times 0.5)] \times 100$ (WB_R = weight-bearing of the right pelvic limb and WB_L = weight-bearing for the left pelvic limb) [32, 65]. Negative symmetry index values were transformed to positive values. Radiographic images were conducted under light sedation, using a combination of medetomidine (0.01 mg/kg) and buthorphanol (0.1 mg/kg), given intravenously. A ventrodorsal extended view was obtained, as described elsewhere [43]. Since sedation can influence blood circulation and body temperature, this

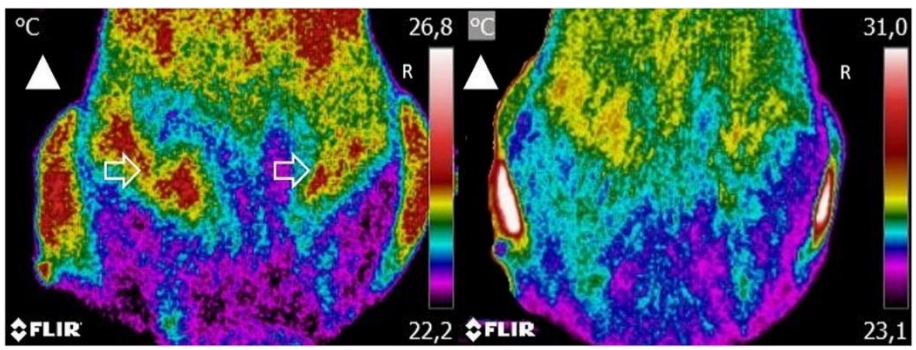

Fig. 3 A dorsoventral view of the same dog at the time of initial evaluation (left) and 30-days post-treatment (right). Arrowhead the direction of the dog's head. Arrow indicates the anatomical location of the hip joint. Arrow indicates the anatomical location of the hip joint. Lower temperatures are observed at 30-days post-treatment

procedure was conducted after weight-bearing and digital thermography evaluations. On day 0, the body condition score was determined, according to the Laflamme scale [42].

Normality was assessed with a Shapiro-Wilk test. Mean and maximal values obtained on the dorsoventral view at the initial evaluation and during the follow-up period were compared with those obtained on the lateral view with ANOVA, followed by an LSD post-hoc test, or the Wilcoxon test, as appropriate for the data distribu- tion. Correlations were assessed with the Spearman correlation coefficient. Results were analyzed with IBM SPSS Statistics version 20, $p < 0.05$.

Abbreviations

CBPI: Canine Brief Pain Inventory; COI: Canine Orthopedic Index; DV: Dorsoventral view; OA: Osteoarthritis; PIS: Pain Interference Score; PSS: Pain Severity Score

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Authors' contributions

JCA designed the protocol, conducted treatments, and prepared the manuscript. PJ and AS selected patients and conducted treatments. CL and LMC revised the protocol and prepared the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study is a part of a project approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval n° GD/32055/2018/P1, September 25th, 2018). Written, informed consent was obtained from the Institution responsible for the animals (Guarda Nacional Republicana, Portuguese Gendarmerie) through dispatch of the Doctrine and Training Commander n°327/16, dated September 16th, 2016.

Consent for publication

Not applicable.

Competing interests

Companion, LiteCure LLC, provided the Stance Analyser used in this study and the digital thermography camera was supplied by Specman, Lda.

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RESEARCH Open Access

Thermographic imaging of police working dogs with bilateral naturally occurring hip osteoarthritis

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Abstract

Background: Digital thermal imaging is a physiologic, non-invasive, contactless, and non-radiating diagnostic tool that can assess a wide range of musculoskeletal conditions, including hip osteoarthritis (HOA). Fifty police working dogs were evaluated to compare the dorsoventral (DV) and lateral (LT) thermographic images in dogs with naturally occurring bilateral HOA. A DV, and left and right lateral LT images were obtained for each animal in six different moments. They were positioned standing in a symmetrical upright position for the DV view. Each image included the area from the last lumbar to the first coccygeal vertebrae. Each LT view was set with the greater trochanter in the centre of the image. Images were taken with a thermographic camera from a distance of 60 cm. Mean and maximal temperatures were recorded, analyzed with ANOVA, dependent samples t-test, and Spearman correlation, with $P < 0.05$.

6.5 ± 2.2 years and bodyweight of 26.7 ± 5.3 kg. The overall value recorded on the DV view was 25.3º ± 9.1 and 28.4º ± 2.8 on the lateral view. These were significantly different ($P < 0.01$) and with a low correlation ($r = 0.10$, $P = 0.03$). German Shepard dogs showed significantly lower values on all views than other breeds ($P < 0.01$), and heavier dogs had higher values on the lateral view.

Conclusions: This is the first study that describes digital thermography's diagnostic use to evaluate working dogs with naturally occurring HOA, comparing two different views. Future studies should address each one's value in the diagnosis and response to treatment of this disease.

Background

Hip osteoarthritis (HOA) is a common problem in the canine population. The severity of clinical signs is variable, and many cases are subclinical [1]. Hip dysplasia is a significant predisposing factor, and other factors include age, prior joint injury, obesity, genetic predisposition, and activity levels. Overall, osteoarthritis accounts for at least 80% of cases of lameness and joint diseases in companion animals [2 – 5].

Digital thermal imaging is a physiologic, non-invasive, contactless, and non-radiating diagnostic tool that relies on heat resulting from physiological functions related to skin temperature control [6 – 8]. Skin temperature reflects a complex system that depends on blood-flow rate, local structures of subcutaneous tissues, and the sympathetic nervous system's activity. Inflammation in subcutaneous and deeper tissues can be reflected by superficial tissue temperature changes due to changes in blood vessel diameter, blood flow rate, and increased capillary

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permeability [9, 10]. Digital thermal imaging can be used to assess a wide range of musculoskeletal condi- tions, including OA, by identifying tissue' s tempera- ture changes. It is also useful to monitor rehabilitation progress, and, unlike other medical modalities, it is not related to morphology [7, 11, 12]. A 180×180 pixel reso- lution has been deemed enough to provide reliable results for medical uses, but a higher resolution (320 × 240) means smaller changes can be detected [13]. It has been described as useful in several species, from humans to horses and cats, but its clinical utility has rarely been studied in companion animals [7, 13 – 15]. By correlating changes in temperature patterns with various diseases, degenerative, or injury processes, digital thermography can provide a reproducible diagnostic tool, particularly in early disease phases [16 – 18]. Canine thermal imag- ing has been documented recently [11], with a growing interest reflected in an increase in the number of studies assessing thermography use. These reports present this diagnostics modality to evaluate a wide range of patholo- gies in different joints and the inter-vertebral disc. Type and coat colour are variables that must be taken consid- ered, as its influence on the evaluation results has been documented [12, 13, 19 – 23].

Methods

This study aimed to compare a dorsoventral (DV) and a lateral (LT) thermography image views of police work- ing dogs presenting with HOA. We hypothesize that both views present similar results. The study proto- col was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem- estar dos Animais da Universidade de Évora, approval nº GD/32,055/2018/P1, September 25th, 2018). Writ-

ten, informed consent was obtained from the institution responsible for the dogs (Guarda Nacional Republicana, Portuguese Gendarmerie). The sample comprised 50 active police working dogs with naturally occurring bilat- eral HOA, signaled from the population of police work- ing dogs of the Guarda Nacional Republicana. These animals were submitted to treatment for HOA and to be included in the study, they should have a body- weight ≥ 15 kg, be over 2 years, and on no medication or nutritional supplements for six weeks or more. The study was conducted over 180 days, with images collected on days 0, 8, 15, 30, 90, and 180. On each day, three images were taken sequentially from each animal: a dorsoventral view, a right lateral view, and a left lateral view. All images were obtained with a FLIR ThermaCAM E25® camera. Before the images were obtained, dogs were allowed to walk calmly around a room with a steady tem- perature, set at 21 ºC, to adjust to room temperature for 30 min. For the dorsoventral view, animals were positioned standing in an upright position, as sym- metrically as possible. If needed, the trainer could help place the dog by holding the dog under the abdomen but without touching the dogs torso. Each thermo- graphic image included the area from the last lumbar vertebra to the first coccygeal vertebra at a minimum at a distance of 60 cm (Fig. 1). This procedure has been previously described, with high repeatability between observers and cameras [14]. Each lateral view was set with the greater trochanter in the centre, also at a dis- tance of 60 cm. The range of temperature was set at 15 – 40°C and emissivity at 0.98.

Data from the thermographic images was analyzed with the free software Tools (FLIR Systems, Inc), and the Rainbow HC collor pallet was used. Both hips' mean temperatures on the two views were evaluated by placing temperature boxes of equal size on the ana- tomical area of the hip joint (Fig. 2). The maximal tem- perature within the box was also registered since the goal was to record signs of inflammation, a hallmark of OA. Normality was assessed with a Shapiro – Wilk test, and mean values obtained on the dorsoventral view were compared with those obtained on the lateral view with ANOVA, followed by an LSD post-hoc test. The same procedure was conducted for maximal tempera- tures. Temperature values were compared by age, sex, breed, and body weight using a paired samples t-test. For weight, cut-off values of 20, 25, 30, and 35 kg were evaluated. Correlations were assessed with the Pearson correlation coefficient. All results were analyzed with IBM SPSS Statistics version 20, and a significance level of $P<0.05$ was set.

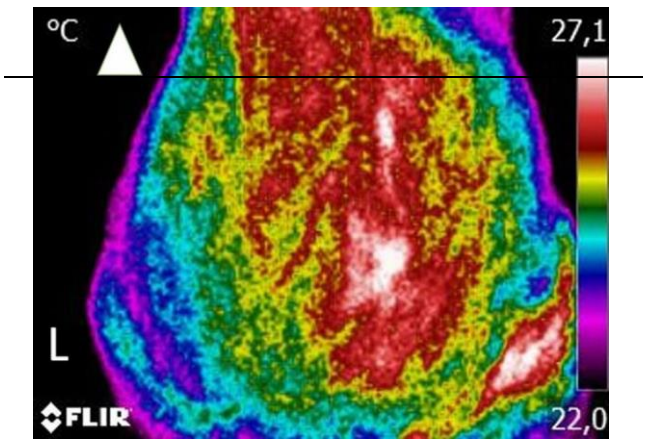

Fig. 1 Example of a digital thermography dorsoventral view. Each image included the area from the last lumbar vertebra to the first coccygeal vertebra at a minimum at a distance of 60 cm. Arrowhead indicates cranial direction

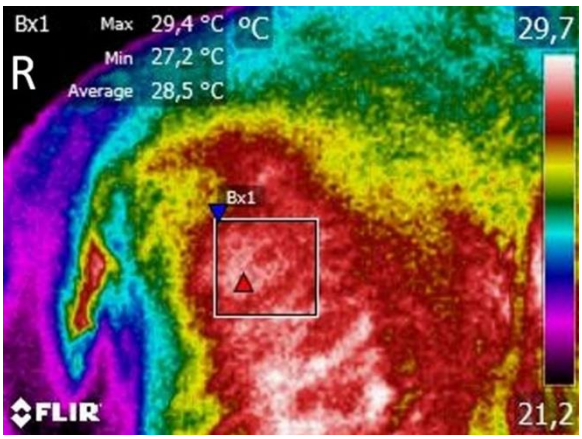

Fig. 2 Example of data analysis on a digital thermography lateral view. A temperature box is placed over the anatomical area of the hip joint, and temperature values recorded.

Results

The sample included 50 police working, and four breeds were represented: German Shepherd dogs (GSD, n = 17), Belgian Malinois Shepherd dogs (BM, n = 15), Labrador Retriever (LR, n = 10), and Dutch Shepherd dog (DSD, n = 8). Thirty were males and 20 females, with a mean age of 6.5 ± 2.2 years and bodyweight of 26.7 ± 5.3 kg. Each joint was considered a sample unit and evaluated individually, and classified as mild (n = 70), moderate (20), and severe (10) according to the Orthopedic Foundation for Animals scoring [24]. Three images were obtained from each animal in six different evaluation moments, amounting to 900 images. Mean and maximal values of overall body weight and age, by breed and sex, are presented in Table 1. Mean and maximal values of overall, left, and right pelvic limbs thermography values on the DV and LT views, by breed and sex, are presented in Table 2.

Table 1 Mean and maximal values (standard deviation) of overall body weight and age by breed and sex

	Bodyweight (kg, mean ± SD)	Age (yrs, mean ± SD)
Overall	26.7 ± 5.3	6.5 ± 2.2
German Shepherd dog	29.9 ± 6.4	5.7 ± 1.8
Belgian Malinois Shepherd dog	27.5 ± 4.1	5.3 ± 1.4
Labrador Retriever	24.3 ± 2.5	8.7 ± 2.5
Dutch Shepherd dog	27.5 ± 4.1	5.3 ± 1.4
Male	29.3 ± 5.4	6.2 ± 2.4
Female	23.5 ± 2.8	6.9 ± 2.5

Overall mean values observed in the DV and LT views were significantly different ($P < 0.01$), and so were maximal values ($P < 0.01$). Mean and maximal values showed low correlations ($r = 0.10$, $P = 0.03$ and $r = 0.15$, $P < 0.01$, respectively). Comparing mean and maximal temperatures by individual pelvic limb, significant differences were observed on the left hip on the DV and LT views ($P < 0.01$). The same was true for the right hip ($P < 0.01$). Still, a correlation was found between left hip mean values on DV and LT views ($r = 0.43$, $P < 0.01$), left hip's maximal values on DV and lateral views ($r = 0.16$, $P < 0.01$), and right hip maximal values on DV and LT views ($r = 0.14$, $P = 0.02$).

When comparing male and female dogs, significant differences were observed for mean values of the left and right hips on the LT view ($P < 0.01$ and $P = 0.03$, respectively). When animals were grouped by body weight, with the cut-off set at 20 kg, significant differences were observed on the left hip maximal value on the DV view ($P = 0.04$), on the mean and maximal values of the left hip on the LT view ($P < 0.01$ for both), and on the mean and maximal values of the right hip on the LT view ($P < 0.01$ and $P = 0.03$, respectively). Similar results were observed with the cut-off set at 25 kg, 30 kg, and 35 kg, with significant differences observed on the mean and maximal values of the left hip on the LT view ($P < 0.01$ for both) and on the mean and maximal values of the right hip on the LT view ($P < 0.01$ for both). Bodyweight showed a correlation with mean and maximal values recorded on the left LT view ($r = -0.34$, $P < 0.01$ and $r = -0.30$, $P < 0.01$, respectively), and on the right LT view ($r = -0.30$, $P < 0.01$ and $r = -0.23$, $P < 0.01$, respectively).

Comparing animals by breed, mean and maximal values for the left hip on the DV view was significantly different for GSD and BM than all other breeds ($P < 0.01$). No differences were observed between RL and DSD. A similar trend was observed on the LT for mean values, with significant differences observed between all breeds ($P < 0.01$), except DSD and RL and BM. For maximal values, GSD registered different values compared to all other breeds ($P < 0.01$). For the right hip on the DV view, no significant differences were observed between breeds. For the maximal value, significant differences were observed between GSD and all other breeds ($P < 0.01$), and BM and RL ($P = 0.02$). On the lateral view, GSDs mean values were again significantly different from other breeds ($P = 0.01$). Differences were also observed between BM and RL ($P < 0.01$) and RL and DSD ($P = 0.03$). For maximal values, GSD registered different values compared to all other breeds ($P < 0.01$).

Table 2 Mean and maximal (± standard deviation) values of overall, left, and right pelvic limbs thermography values on the DV and LT views by breed and sex

	Dorsoventral view (º, mean ± SD)					Lateral view (º, mean ± SD)					
	Overall	Overall max	Left	Left max	Right	Right max	Overall	Overall max	Left	Left max	Right	Right max
Overall	25.3 ± 9.1	26.3 ± 1.9	24.9 ± 1.9	26.3 ± 1.9	25.6 ± 1.2	26.2 ± 2.0	28.4 ± 2.8	31.9 ± 3.1	28.7 ± 2.9	31.9 ± 3.2	29.0 ± 2.8	32.1 ± 2.9
German Shepherd dog	24.1 ± 1.9	25.3 ± 1.8	24.1 ± 1.8	25.3 ± 1.8	24.0 ± 1.9	25.3 ± 1.8	27.1 ± 2.6	30.8 ± 3.4	26.7 ± 2.5	30.6 ± 3.6	27.4 ± 2.6	31.1 ± 3.1
Belgian Malinois Shepherd dog	26.0 ± 2.0	26.4 ± 1.9	24.8 ± 1.8	26.4 ± 1.9	27.2 ± 2.3	26.4 ± 1.9	29.1 ± 2.6	32.3 ± 3.0	28.9 ± 2.6	32.2 ± 3.0	29.2 ± 2.6	32.4 ± 2.9
Labrador Retriever	25.8 ± 1.7	27.1 ± 1.7	25.8 ± 1.6	27.1 ± 1.6	25.8 ± 1.7	27.1 ± 1.7	30.7 ± 2.4	32.7 ± 2.7	30.6 ± 2.4	32.6 ± 2.7	30.8 ± 2.4	32.9 ± 2.7
Dutch Shepherd dog	25.7 ± 2.1	26.9 ± 2.1	25.7 ± 2.0	26.9 ± 2.1	25.7 ± 2.1	26.9 ± 2.1	29.7 ± 2.4	32.7 ± 2.7	29.7 ± 2.3	32.7 ± 2.7	29.6 ± 2.5	32.6 ± 2.7
Male	24.9 ± 2.1	26.1 ± 2.1	24.8 ± 2.1	26.1 ± 2.1	24.7 ± 2.1	26.1 ± 2.1	28.6 ± 2.9	31.8 ± 3.2	28.4 ± 2.9	31.7 ± 3.3	28.7 ± 2.9	31.9 ± 3.1
Female	25.6 ± 1.9	26.5 ± 1.9	25.1 ± 1.8	26.5 ± 1.8	26.9 ± 1.9	26.5 ± 1.9	29.4 ± 2.7	32.3 ± 3.0	29.3 ± 2.7	32.1 ± 3.1	29.4 ± 2.7	32.4 ± 2.9

Discussion

Digital thermography provides a visual map of the skin temperature distribution. A difference of more than 1 °C between similar areas or tissues is considered significant, and the identification of inflammation, characterized by, among others, an increase in temperature, is a critical step in determining the appropriate treatment [7, 15]. In dogs, thermography has been able to identify animals with dysplastic elbows and cranial cruciate ligament deficiency, compared with sound animals [20, 22]. All animals included in this study had bilateral HOA, and therefore we only evaluated contralateral differences. When comparing values for the hip joint's anatomical region on the DV and LT views, significant differences were observed. The main reason for this difference may be the larger amount of muscle masses included in the LT view area, thus contributing to higher temperature values. Still, the values observed on different views showed some correlation. This effect was also observed when comparing dogs with different cut-off points for bodyweight and males and females (male dogs being heavier), with increasing body weight always accounting for higher temperature levels. These differences were observed on mean and maximal scores. A thermographic value of 28.47 °C ± 0.45 on an LT view has been described in healthy LR. However, this value was for a region of interest that encompassed the most proximal third of the pelvic limb and not the hip joint area specifically [19]. Similar values (28.4 °C ± 2.8) were found when the overall sample was considered, but higher values in the LR dogs (30.7 °C ± 2.4). This difference may be associated with OAs changes, but further studies should address this hypothesis.

Working and sporting dog owners and trainers are frequently reluctant to clip their dogs, and it has not been proven as necessary for the thermographic evaluation of structures in dogs. Fur clipping can be harmful during a thermographic evaluation since a minimum of 60 min after clipping is required for stable readings to be acquired [19]. Still, the coat's type and color are variables that must be taken into account, and their influence evaluated, as it tends to be predictable [12, 20, 23]. All of the breeds included in this study had short hair, and some had a double coat. The majority of GSD included were sable, BM fawn, LR yellow, and DSD bridle. Comparing thermography evaluation by breed, the most consistent difference was the lower values observed in GSD, even though these were heavier dogs. The reason for this is unclear but may be related to coat characteristics since GSD usually have a very thick undercoat. Some coats can also make it harder to locate the area of interest, mainly when the hair is very long and/or thick [14, 25]. Strong knowledge of the area of interest's anatomical landmarks is paramount to obtain valuable and correct information from the thermographic images [13]. This identification was not particularly problematic in this study but would undoubtedly be more challenging in longhaired or overweight/obese dogs. These variations in coat further stress the importance of guaranteeing an adjustment period in a temperature-controlled room. In horses, it has been suggested that there is no need for such an adjustment or equilibration time before thermographic imaging. This could be because a horse is much bigger and produces more heat than a dog [26], and also, with less variation in coat types. For those reasons, this suggestion probably cannot be applied to dogs. We also chose to register and analyse maximal scores as higher temperature levels could be related to inflammation. For that reason, it may better reflect the events that occur during OA development.

Conclusions

This is the first study presenting two different digital thermography views of dogs diagnosed with HOA. Significantly higher values were recorded on an LT view. Coat characteristics influenced thermographic evaluations. Further studies are required to determine each views interest and the influence of different coats in HOA treatment monitoring.

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Authors' contributions
JCA designed the protocol, conducted treatments, and prepared the manuscript. PJ and AS selected patients and conducted treatments. CL and LMC revised the protocol and prepared the manuscript. All authors have read and approved the final version of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The study protocol was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval n° GD/32,055/2018/P1, September 25th, 2018). Written, informed consent was obtained from Guarda Nacional Republicana, Portuguese Gendarmerie, through dispatch of the Doctrine and Training Commander n°327/16, dated September 16th, 2016.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Quartile 1

Evaluation of four clinical metrology instruments for the assessment of osteoarthritis management in a naturally occurring canine model

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Abstract

We aimed to test construct and criterion validity of four Clinical Metrology Instruments (CMIs) for the evaluation of naturally occurring canine OA model. Fifty Police working dogs with bilateral hip OA were assessed in a prospective, randomized, double-blinded study. Patients were evaluated using a stance analyzer in six different moments, divided over a 180-day period. Pedometer step count, weight-bearing symmetry index and deviation from normal weight-bearing were calculated and used as criterion validity. In each evaluation moment, a copy of the Hudson Visual Analogue Scale, Canine Brief Pain Inventory, Liverpool Osteoarthritis in Dogs and Canine Orthopedic Index were completed by the dogs' handlers. Correlations between CMIs were evaluated as construct validity. Further evaluation was performed with the Kaiser-Meyer-Olin measure of sampling adequacy, Eigenvalue and scree-plot analysis. Internal consistency was tested with Cronbach's α.

A significant weak correlation was found between all CMIs and stance analysis symmetry index measure and deviation, indicating criterion validity. A significant weak correlation was also found between pedometer count and LOAD plus COI. Cronbach's α was 0.80 for HVAS, 0.98 for CBPI, 0.97 for LOAD and 0.98 for COI. A significant strong correlation was observed between CMIs, indicating construct validity.

We presented criterion and construct validity of these CMIs, which are able to capture various dimensions of OA. They can be used for the evaluation of naturally occurring canine osteoarthritis models.

Keywords: Osteoarthritis, Pain, Dog, Animal Model, Clinical Metrology Instruments.

Introduction

Osteoarthritis (OA) is an important and costly disease in humans and dogs [27,45], being the most commonly diagnosed joint disease both in human and veterinary medicine. It represents a significant burden to societies, as it affects the quality of life, performance and implies a high cost in terms of healthcare [12,52]. The dog is the closest to a gold standard model, due to anatomical resemblance, disease progression, same shared environment and lifestyle, with the advantage of providing a faster disease progression [16,23,29–31,33,37]. Exploring spontaneous dog OA under the One Medicine initiative can help improve the health and well-being of both humans and dogs [31].

Pain is the most relevant clinical sign of OA and a hallmark of the disease [38,52]. Since it is central in both human and veterinary clinical practice, the current therapeutic goal for both is the management of pain and associated loss of function [7,9]. Several clinical metrology instruments (CMI) have been developed in order to evaluate pain and assess outcome. As a whole, they show

discriminatory, responsiveness and criterion validity as measures of pain and impairment in performing daily activities, and represent a patient-centred approach [17,31,42]. Two of the most widely used are the Canine Brief Pain Inventory (CBPI) and the Liverpool Osteoarthritis in Dogs (LOAD) [18,25,48,49]. The CBPI encompasses two sections, a pain severity score (PSS) and a pain interference score (PIS). The first assesses the magnitude of the pain of the animal and the second assesses the degree in which pain affects daily activities [44]. The Canine Orthopaedic Index (COI) has been developed to assess 4 dimensions of OA in dogs: stiffness, gait, function and quality of life [3]. The Hudson Visual Analogue Scale (HVAS) has been compared with force plate analysis, shown to be repeatable and valid to assess the degree of mild to moderate lameness [21].

Weight distribution and off-loading or limb favouring at the stance is a commonly used subjective assessment during orthopaedic examination [26]. Patients with OA may not be overtly lame but exhibit subtle shifts in body weight distribution at a stance [11,41]. Stance analysis has been reported as sensitive for detecting lameness in dogs [10]. It has been proposed that bodyweight distribution at a stance may be an equivalent or superior measurement of hip OA pain than both vertical impulse and peak vertical force [11,24]. Mobility impairment and decreased activity are associated with musculoskeletal pain in humans, and improved results in regards to mobility have been recommended as measures of outcome [25]. Pedometers are inexpensive, simple devices that measure ambulatory activity with acceptable accuracy [43].

The aim of this study was to compare HVAS, CBPI, LOAD and COI with each other, to evaluate construct validity. We also aimed to compare these CMIs to objective measures (weight-bearing and pedometers step counts) to test criterion validity and compare changes in a follow-up study. In addition, we wanted to test factor analysis of the CMIs. We hypothesized that correlation would be found between the four considered CMIs, and also between them and the considered objective measures.

Materials and Methods

The study protocol was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval nº GD/32055/2018/P1, September 25th, 2018), and comply with relevant institutional and national guidelines for the care and use of animals. Written, informed consent was obtained from the Institution responsible for the animals. A sample of 50 Police working dogs (N=50) of both sexes was used, a convenience sample selected for a longitudinal double-blinded, negative controlled study, evaluating intra-articular treatment modalities in a naturally occurring canine OA model. Data from this study was used for the present analysis, with information collected at the initial diagnostics

evaluation being used as a cross-sectional cohort, while follow-up evaluations, assessing response to treatment, constituted the longitudinal cohort. Inclusion and exclusion criteria were defined by research activity other than that currently reported and are summarized in table 1.

Table 1 – Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Mobility impairment, as described by the trainer and detect by the assisting veterinarian;	Suspected or diagnosed neurological/musculoskeletal disorder other than hip OA;
Bodyweight ≥15kg;	Documented or suspected presence of concomitant disease;
Age ≥1 year;	Receiving any other drugs;
Radiographic evidence of bilateral OA of the hip joint;	Results of routine blood testing outside normal limits;
Not to be on any medication or nutritional supplements for six weeks or more;	

The study was conducted over a period of 180 days, and data was gathered on days 0, 8, 15, 30, 90 and 180. On day 0 (treatment day), patients either received an intra-articular administration of 0.9% NaCl (control group) or a treatment (a platelet concentrate, Hylan G-F 20, triamcinolone hexacetonide or stanozolol), according to the assigned group. All groups had the same number of animals. No other medications/treatments were administered during the follow-up period. In all evaluations moments, a copy of all CMIs was completed by the handlers, stance analysis was performed, and pedometer count was recorded.

Before completion of an online copy of the HVAS, CBPI, COI and LOAD, handlers received the published instructions for each of them. The CMIs were completed in sequence by the same handler in each of the follow-up assessments, without knowledge of their previous answer, in a quiet room with as much time as needed to answer all items. As CBPI has two sections (PSS and PIS), and COI has four dimensions (stiffness, function, gait and QOL), were considered all sections and dimensions in the analysis.

Stance analysis was conducted with a weight distribution platform (Companion Stance Analyzer; LiteCure LLC®, Newark, Delaware, United States). According to manufacturer's guidelines, the platform was placed in the center of a room, at least 1 meter from the walls, and calibrated at the beginning of each day and zeroed before each data collection. After a period of familiarization with the space, animals were encouraged by their handlers to stand on to the weight distribution platform, with one foot on each quadrant of the platform, while maintaining a natural stance with their centre of gravity and stability (measured by the platform) near the middle of the platform. Gentle restraint was used to maintain the patient's head in a natural, forward-facing

position. Evaluations were conducted in the morning, and no exercise or activity was performed before it, as exercise can exacerbate pelvic limb lameness and a potential factor of variation during gait evaluation [1]. For all animals, at least 20 measurements were performed, and the mean value determined. Handlers were unaware of the evaluation results before completing the CMIs. Normal weight distribution for the pelvic limbs was considered 40% (20% right pelvic limb + 20% left pelvic limp) of the total weight [11].

Pedometers were worn around the dog's neck, attached to an adjustable lightweight collar, so that they detected and counted forelimb steps only, associated with greater accuracy at a walk, trot or run [8]. They were placed one week before the first evaluation moment, in order to determine a baseline value, and then maintained up to the 30th-day post-treatment. For the 90th and 180th post-treatment days evaluation, the animals worn the pedometer for a week before that evaluation moment. Mean daily counts were considered and calculated by dividing the register number of steps by the number of days considered.

To test criterion validity, CMI scores were compared with the left-right symmetry index (SI), calculated with the following formula: SI=[(WBR-WBL)/((WBR+WBL)x0.5)]x100 [46,47,49], where WBR is the value of weight-bearing for the right pelvic limb, and WBL is the value of weight-bearing for the left pelvic limb. Negative values were made positive. As normal weight-bearing for the pelvic limbs is 40%, we also considered the deviation from this value, obtained subtracting WBR+WBL to 40. To additionally test validity, we compared CMI scores against changes in activity, considered as changes in mean daily step count with the pedometers.

We compared results of the CMIs using repeated-measures ANOVA, with a Huynh-Feldt correction and tested construct validity by comparing individual CMI scores against all others. The correlation was assessed with Spearman's rank correlation coefficient, with $p<0.05$. Additionally, we performed factor analysis for all CMIs, using the Kaiser-Meyer-Olin (KMO) measure of sampling adequacy, with adequacy considered >0.6. Eigenvalue and scree-plot analysis were used to assess extracted values, and item loading on the extracted components was based on a varimax-rotated model of factor analysis. A communality cut-off value of 0.4 was considered. Internal consistency for all CMIs was tested with Cronbach's α.

Results

The sample included 50 Police working dogs, of both sexes (30 males and 20 females), with a mean age of 6.5±2.4 years and bodyweight of 26.7±5.2kg. Four breeds were represented: German Shepherd Dogs (n=17), Belgian Malinois Shepherd Dogs (n=15), Labrador Retriever (n=10), and

Dutch Shepherd Dog (n=8). In the six considered evaluation moments, a response for all CMIs was obtained for every animal, accounting to 300 responses for each CMI.

Correlations for the cross-sectional cohort are presented in table 2. A significant strong correlation was observed between all CMIs. A significant weak correlation was observed between SI and COI, and its dimensions Stiffness, Function and Gait. A significant moderate correlation was also observed pedometer step count and LOAD, COI, Stiffness, Function, Gait and QOL. Scatterplot of COI versus SI is presented in figure 1. Correlations for the longitudinal cohort between changes of different evaluations performed are presented in table 3.

Significant strong correlations were observed between all CMIs. Additional, significant weak correlations were observed between SI and all other evaluations except pedometer step count. The same was found for deviation. Scatterplot of LOAD versus HVAS, PIS, PSS and COI are presented in figures 2, 3, 4 and 5, respectively. Scree plots for HVAS, CBPI, LOAD and COI are presented in figures 6, 7, 8 and 9, respectively.

Comparing CMI results, Mauchly's test rejected the present of sphericity ($\chi 2(35)=5189$, $p<0.01$). The difference between mean scores was significantly different, with $F(1.18, 320548)=257845$, $p<0.01$. Cronbach's α was 0.80 for HVAS, 0.98 for CBPI, 0.97 for PIS, 0.98 for PSS, 0.97 for LOAD, 0.98 for COI, 0.97 for stiffness, 0.97 for function, 0.96 for Gait and 0.85 for QOL. Kaiser-Meyer-Olkin factor analysis was 0.88 for HVAS, 0.94 for CBPI, 0.96 for LOAD and 0.96 for COI. As all values were above 0.8, factor analysis was conducted for the four CMIs. Scree plots for HVAS, CBPI, LOAD and COI are presented in figures 6, 7, 8 and 9, respectively. For HVAS, two factors with eigenvalues>1 were extracted. These factors accounted for 60.2% and 13.3%, respectively, of the total variance. Based on the varimax-rotated solution, loading for these items was performed. All items loaded heavily on the first component, with communalities ranging between 0.68 and 0.91. For the second component, not all items showed significant communalities, with values ranging from -0.39 to 0.65. For CBPI, a single factor was extracted, which accounted for 90.9% of the total variation. All other items loaded heavily on this component, with communality being >0.93. For LOAD, two factors were extracted and were responsible for 76.1% and 10.5% of the total variance, respectively. Other items loaded mainly on the first component, with commonalties ranging from 0.71 to 0.94. For COI, also only one factor was extracted, accounting for 79.5% of the total variation. All other items loaded on this component, with communalities ranging from 0.46 to 0.89.

Table 2 – Correlations for the cross-sectional cohort. CBPI – Canine Brief Pain Inventory; COI – Canine Orthopedic Index; HVAS – Hudson Visual Analogue Scale; LOAD – Liverpool Osteoarthritis in Dogs; PIS – Pain Interference Score; PSS – Pain Severity Score; QOL – Quality of Life; SI – Symmetry Index. * indicates significant correlation.

Measure		SI	Deviation	Pedometer	HVAS	PIS	PSS	LOAD	COI	Stiffness	Function	Gait	QOL
SI	r_s	1.00	.072	-.040	-.203	.036	.196	.300	.324	.026	.367	.324	.177
	Sig.		.617	.823	.161	.300	.178	.036*	.023*	.318	.010*	.023*	.224
Deviation	r_s	.072	1.00	-.200	-.151	.198	.210	.169	.240	.180	.233	.229	.265
	Sig.	.617		.256	.200	.174	.147	.245	.096	.216	.107	.114	.066
Pedometer	r_s	-.040	-.200	1.00	.326	.-323	-.323	-.425	-.523	-.531	-.499	-.427	-.530
	Sig.	.823	.256		.060	-.063	.078	.012*	.002*	.000*	.003*	.012*	0.01*
HVAS	r_s	-.203	-.151	.326	1.00	-.806	-.775	-.698	-.643	-.640	-.578	-.577	-.652
	Sig.	.161	.200	.060		.000*	.000*	.000*	.000*	.000*	.000*	.000*	.000*
PIS	r_s	.036	.198	.-323	-.806	1.00	.966	.831	.842	.808	.774	.793	.801
	Sig.	.300	.174	-.063	.000*		.000*	.000*	.000*	.000*	.000*	.000*	.000*
PSS	r_s	.196	.210	-.323	-.775	.966	1.00	.784	.811	.772	.722	.770	.800
	Sig.	.178	.147	.078	.000*	.000*		.000*	.000*	.000*	.000*	.000*	.000*
LOAD	r_s	.300	.169	-.425	-.698	.831	.784	1.00	.936	.918	.910	.854	.848
	Sig.	.036	.245	.012*	.000*	.000*	.000*		.000*	.000*	.000*	.000*	.000*
COI	r_s	.324	.240	-.523	-.643	.842	.811	.936	1.00	.964	.937	.954	.905
	Sig.	.023*	.096	.002*	.000*	.000*	.000*	.000*		.000*	.000*	.000*	.000*
Stiffness	r_s	.026	.180	-.531	-.640	.808	.772	.918	.964	1.00	.885	.907	.836
	Sig.	.318	.216	.001*	.000*	.000*	.000*	.000*	.000*		.000*	.000*	.000*
Function	r_s	.367	.233	-.499	-.578	.774	.722	.910	.937	.885	1.00	.830	.810
	Sig.	.010*	.107	.003*	.000*	.000*	.000*	.000*	.000*	.000*		.000*	.000*
Gait	r_s	.324	.229	-.427	-.577	.793	.770	.854	.954	.907	.830	1.00	.809
	Sig.	.023*	.114	.012*	.000*	.000*	.000*	.000*	.000*	.000*	.000*		.000*
QOL	r_s	.177	.265	-.530	-.652	.801	.800	.848	.905	.836	.810	.809	1.00
	Sig.	.224	.066	0.01*	.000*	.000*	.000*	.000*	.000*	.000*	.000*	.000*	

Table 3 – Correlations for the longitudinal cohort. CBPI – Canine Brief Pain Inventory; COI – Canine Orthopedic Index; HVAS – Hudson Visual Analogue Scale; LOAD – Liverpool Osteoarthritis in Dogs; PIS – Pain Interference Score; PSS – Pain Severity Score; QOL – Quality of Life; SI – Symmetry Index. * indicates significant correlation.

Measure		SI	Deviation	Pedometer	HVAS	PIS	PSS	LOAD	COI	Stiffness	Function	Gait	QOL
SI	r_s	1.00	.261	-.040	-.152	.196	.130	.230	.211	.177	.227	.195	.202
	Sig.		.000*	.823	.013*	.001*	.033*	.000*	.001*	.004*	.000*	.001*	.001*
Deviation	r_s	.261	1.00	-.200	-.127	.141	.112	.133	.174	.129	.156	.191	.179
	Sig.	.000*		.256	.038*	.021*	.068	.030*	.004*	.035*	.010*	.002*	.003*
Pedometer	r_s	-.040	-.200	1.00	.063	-.147	-.118	-.168	-.183	-.167	-.260	-.135	-.122
	Sig.	.823	.256		.412	.055	.125	.028*	.017*	.029*	.001*	.078	.111
HVAS	r_s	-.152	-.127	.063	1.00	-.830	-.814	-.736	-.716	-.695	-.057	-.677	-.732
	Sig.	.013*	.038*	.412		.000*	.000*	.000*	.000*	.000*	.355	.000*	.000*
PIS	r_s	.196	.141	-.147	-.830	1.00	.956	.844	.853	.828	.805	.808	.813
	Sig.	.001*	.021*	.055	.000*		.000*	.000*	.000*	.000*	.000*	.000	.000*
PSS	r_s	.130	.112	-.118	-.814	.956	1.00	.807	.817	.792	.755	.783	.785
	Sig.	.033*	.068	.125	.000*	.000*		.000*	.000*	.000*	.000*	.000*	.000*
LOAD	r_s	.230	.133	-.168	-.736	.844	.807	1.00	.954	.928	.919	.912	.863
	Sig.	.000*	.030*	.028*	.000*	.000*	*		.000*	.000*	.000*	.000*	.000*
COI	r_s	.211	.174	-.183	-.716	.853	.817	.954	1.00	.966	.952	.965	.916
	Sig.	.001*	.004*	.017*	.000*	.000*	.000*	.000*		.000*	.000*	.000*	.000*
Stiffness	r_s	.177	.129	-.167	-.695	.828	.792	.928	.966	1.00	.906	.919	.847
	Sig.	.004*	.035*	.029*	.000*	.000*	.000*	.000*	.000*		.000*	.000*	.000*
Function	r_s	.227	.156	-.260	-.643	.805	.755	.919	.952	.906	1.00	.872	.836
	Sig.	.000*	.010*	.001*	.000*	.000*	.000*	.000*	.000*	.000*		.000*	.000*
Gait	r_s	.195	.191	-.135	-.677	.808	.783	.912	.965	.919	.872	1.00	.965
	Sig.	.001*	.002*	.078	.000*	.000*	.000*	.000*	.000*	.000*	.000*		.000*
QOL	r_s	.202	.179	-.122	-.732	.813	.785	.863	.916	.847	.836	.965	1.00
	Sig.	.001*	.003*	.111	.000*	.000*	.000*	.000*	.000*	.000*	.000*	.000*	

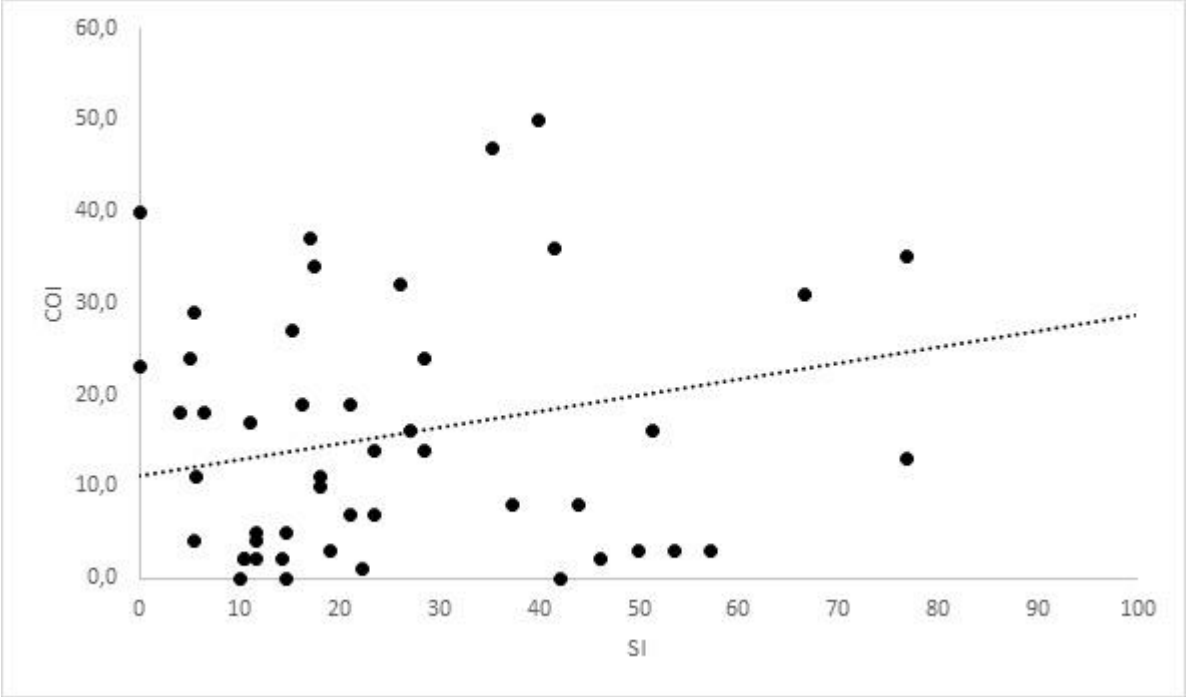

Figure 1 – Scatterplot of COI (Canine Orthopedic Index) versus SI (symmetry index) in the cross-sectional cohort. A significant weak correlation was observed ($r=0.21$, $p<0.01$).

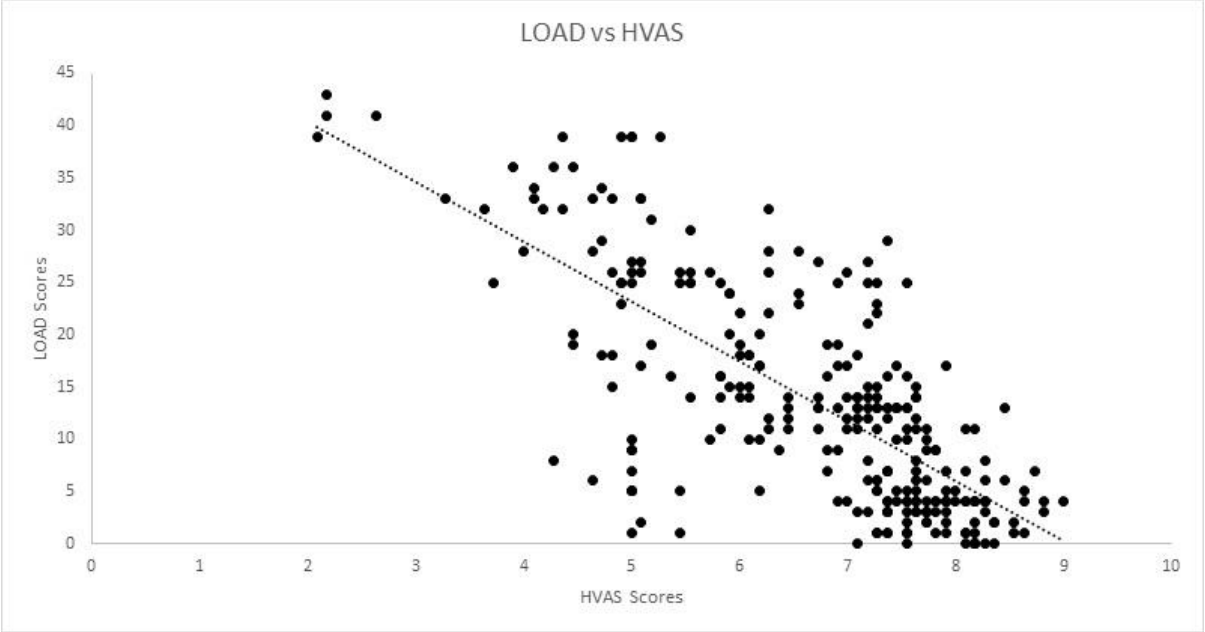

Figure 2 – Scatterplot of LOAD (Liverpool Osteoarthritis in Dogs) and HVAS (Hudson Visual Analogue Scale) in the longitudinal cohort. A significant strong correlation was observed ($r=-0.736$, $p<0.01$).

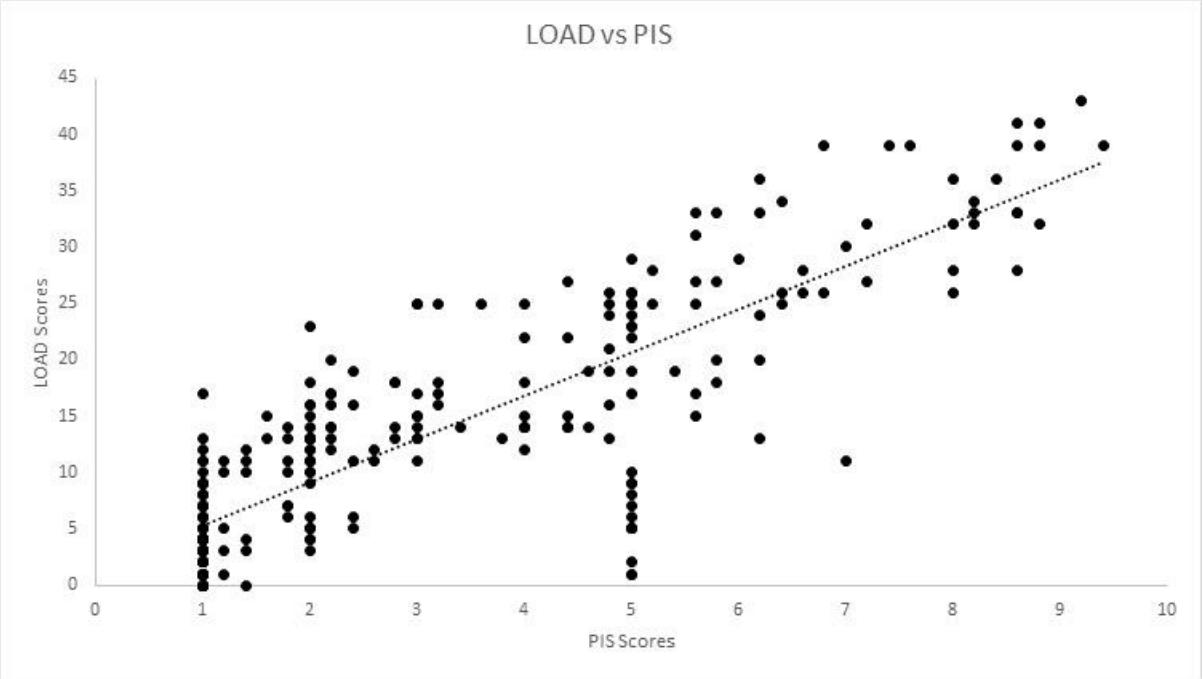

Figure 3 – Scatterplot of LOAD (Liverpool Osteoarthritis in Dogs) and PIS (Pain Interference Score) in the longitudinal cohort. A significant strong correlation was observed ($r=-0.844$, $p<0.01$).

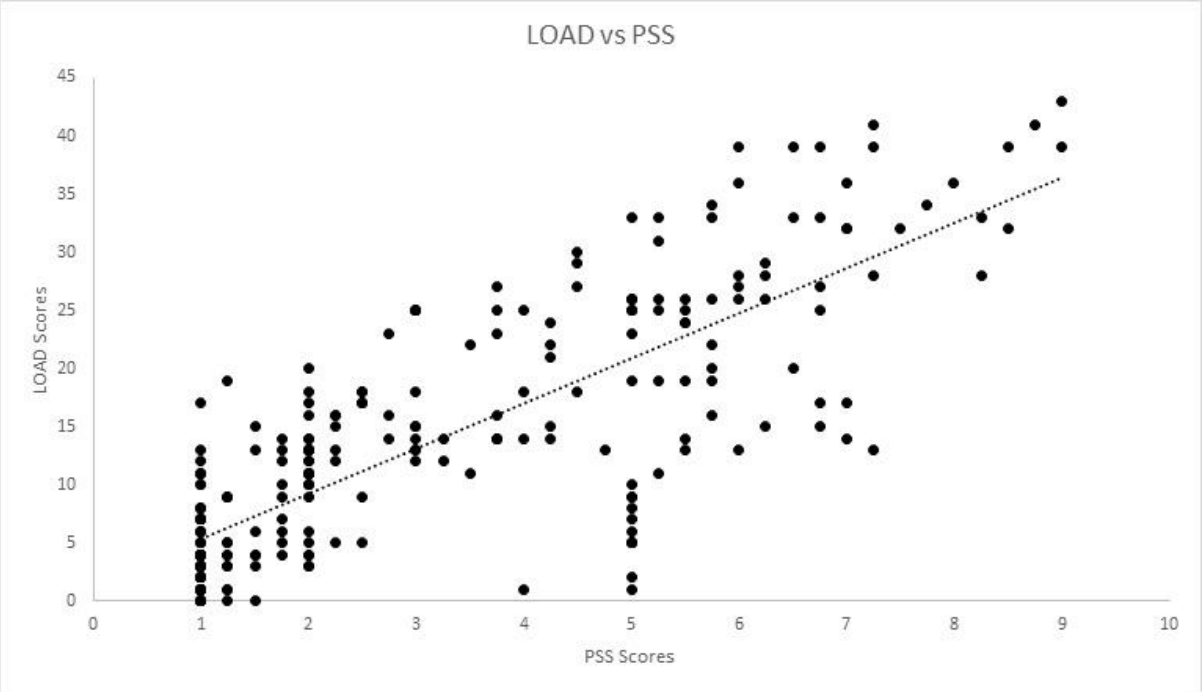

Figure 4 – Scatterplot of LOAD (Liverpool Osteoarthritis in Dogs) and PSS (Pain Severity Score) in the longitudinal cohort. A significant strong correlation was observed ($r=-0.807$, $p<0.01$).

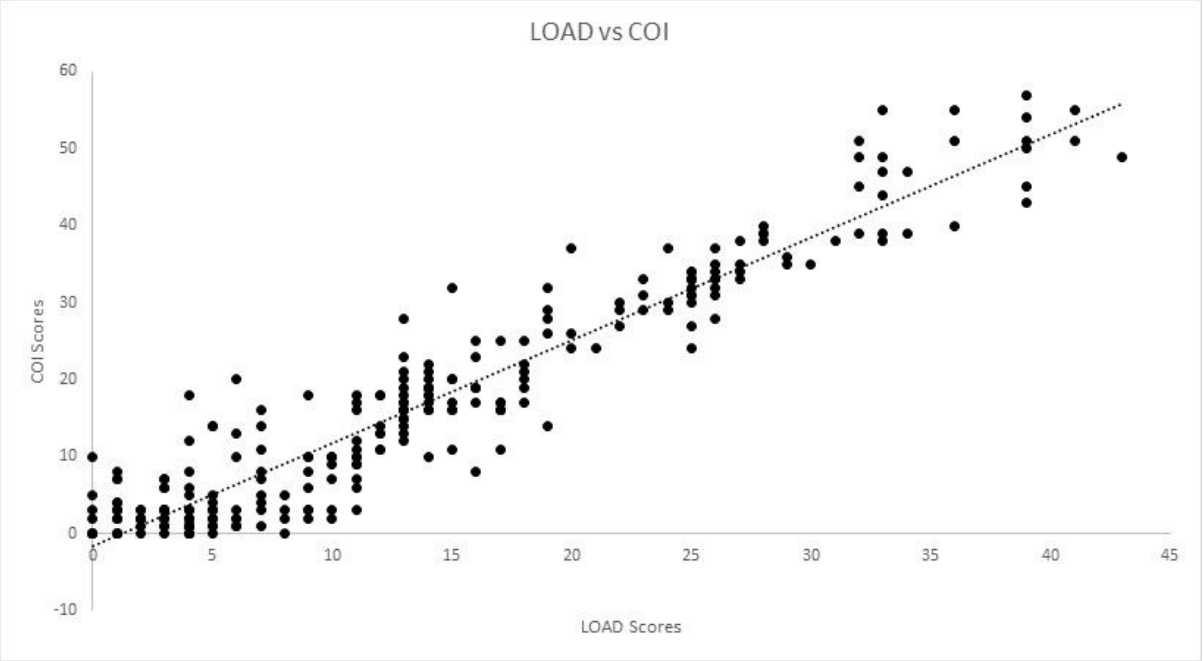

Figure 5 – Scatterplot of LOAD (Liverpool Osteoarthritis in Dogs) and COI (Canine Orthopedic Index) in the longitudinal cohort. A significant strong correlation was observed ($r=-0.954$, $p<0.01$).

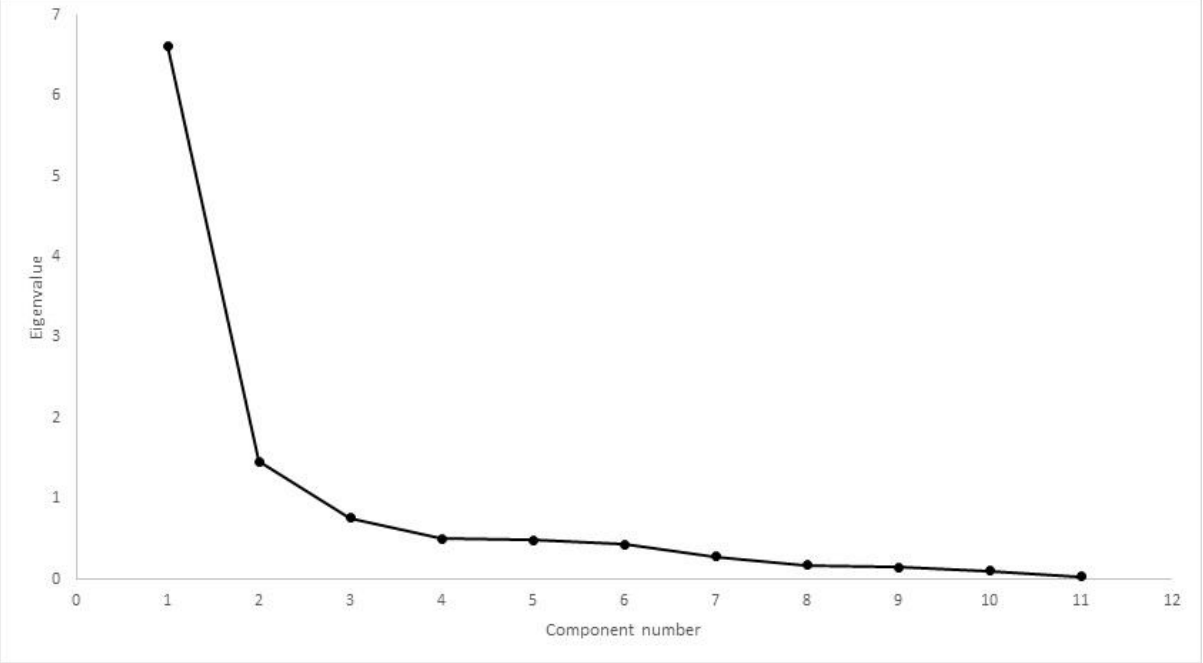

Figure 6 – Scree plot of factor analysis of HVAS (Hudson Visual Analogue Scale). Two factors had Eigenvalues >1, with a discernible "shoulder" observed.

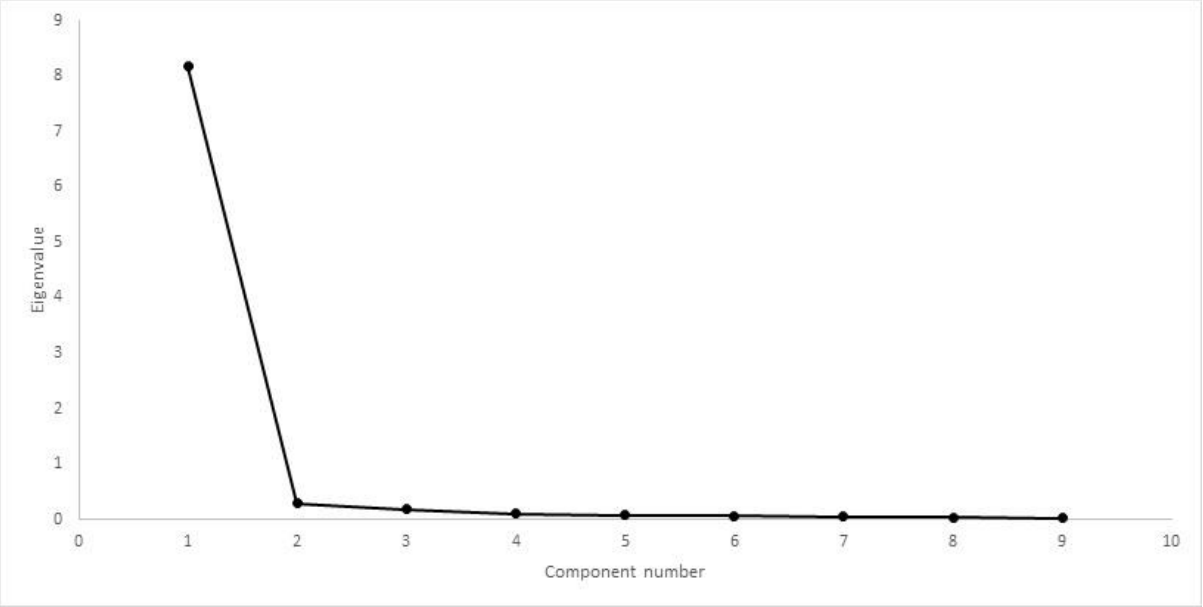

Figure 7 – Scree plot of factor analysis of CBPI. One factor had Eigenvalues >1, with a discernible "shoulder" observed.

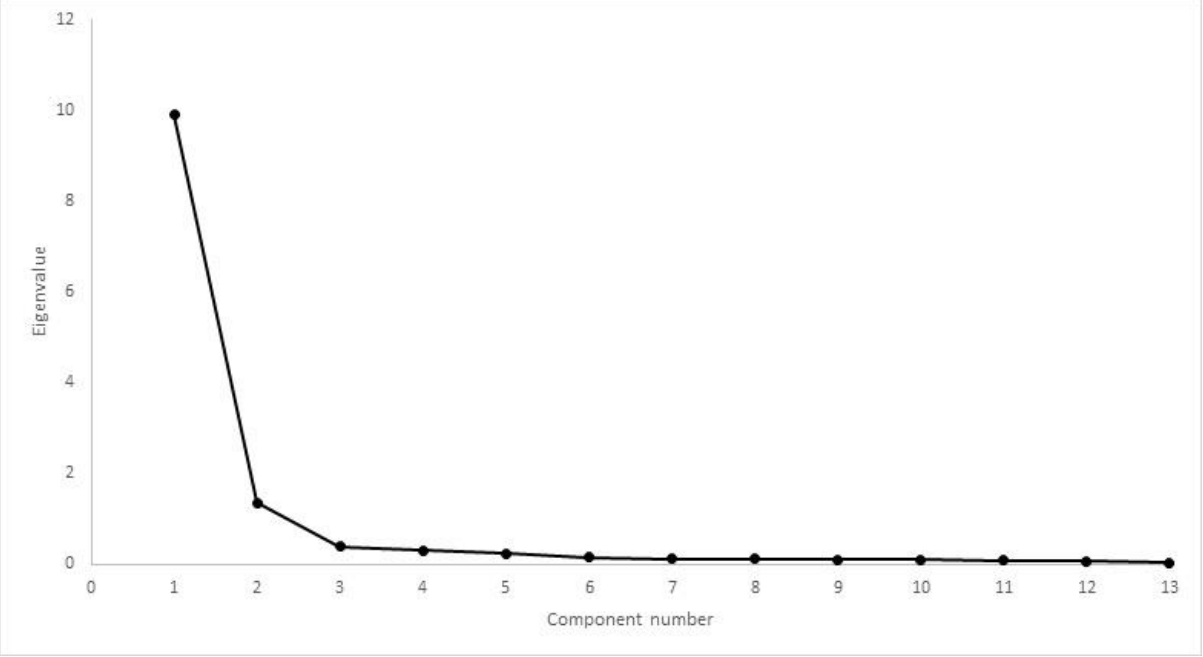

Figure 8 – Scree plot of factor analysis of LOAD. Two factors had Eigenvalues >1, with a discernible "shoulder" observed.

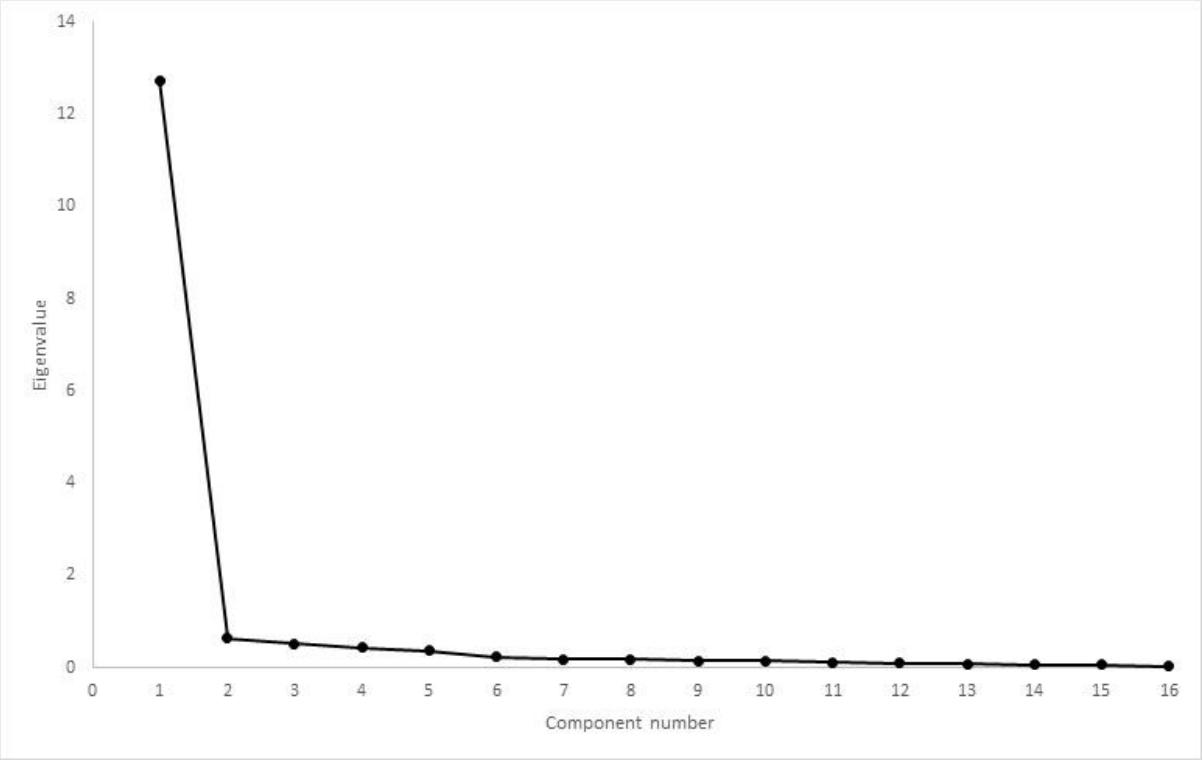

Figure 9 – Scree plot of factor analysis of COI. One factor had Eigenvalues >1, with a discernible "shoulder" observed.

Discussion

A good understanding and evaluation of canine pain, and its toll on daily activities, is paramount to the development of treatments of chronic conditions, such as OA [15,22]. To our knowledge, this study first presents evidence of criterion validity for HVAS and COI. It has been described for LOAD and CBPI [49] but, for the first time, we compared the scores of these four CMIs with stance analysis and mean daily step count, as an outside measure of disease. We also presented construct validity for all of the instruments, as well as their internal consistency.

The evaluation of instrument validity provides evidence that it measures what it is supposed to measure. One of the assessments to make is construct validity, which can be performed by testing the agreement of the instrument with a recognized "gold standard" [40,49]. Weight distribution platform, as a pressure-sensitive walkway, can provide accurate and consistent measures of weight distribution with no significant difference between devices [2]. It has been proposed that bodyweight distribution at a stance may be an equivalent or superior measurement of pain associated with hip OA than both vertical impulse and peak vertical force [11,24]. Previous reports indicate that SI is reliable indicators of clinical lameness in dogs [36]. The CBPI has been reported as being able to detect a measurable effect for individual animals assessment, with results correlating with PVF [4–6,14,38,49,51]. LOAD scores also show a correlation with peak vertical forces generated by a force

platform [18,49], as has the HVAS [39]. Significant weak correlations of LOAD and CBPI with SI peak vertical force have also been described, but these dogs had OA of a single joint [49]. In the present report, all animals had bilateral disease, and we used as an external measure of disease weight-bearing using a stance analyzer. We found a significant but weak correlation between SI and COI, stiffness, function and gait scores in the cross-sectional cohort longitudinal cohort. In the longitudinal cohort, this significant weak correlation was observed with all CMI. The fact that the correlations found are weak is not completely unexpected, as CMIs aim to evaluate OA as a whole, from its signs to the impact it has on the animal's life, while stance analysis is directed at evaluating function. This may explain why, in the cross-sectional cohort, correlations occur precisely with the scores that aim to evaluate more functional parameters of the disease. An additional possibility is the fact that all animals exhibited bilateral disease, which may lead to less marked asymmetries between contralateral limbs. We still chose to use this evaluation, as even bilateral disease does not necessarily show equal signs in both limbs. Also, and since all animals had bilateral hip OA, we performed a second analysis with the stance analyzer, to measure the deviation from the normal weight distribution on the pelvic limbs. This evaluation also showed a significant weak correlation with all CMIs scores, except for PSS. The weight-bearing evaluation also has the advantage of allowing comparisons between different breeds and can still evaluate dogs that are unable to trot [49]. A second external measure of disease, which was mean daily step counts, was used. Mobility impairment and decreased activity are associated with musculoskeletal pain in humans, with improved results regarding mobility have been recommended as measures of outcome [25]. Pedometers have the advantage of activity monitoring over a prolonged period in the patient's home environment [8,48]. In normal dogs, pedometer steps can reasonably estimate distance travelled, and recording data over several days, in opposition to single-day recordings, has demonstrated to help avoid anomalies in other species [13,50]. We observed a significant moderate correlation with LOAD and COI in the cross-sectional cohort and a weak correlation with the same CMIs in the longitudinal cohort. The reason for a weaker correlation on the longitudinal cohort may be associated with the fact that the animals of this sample are active working dogs. This means that the overall activity of these animals is not only voluntary activity but also varies with operational activity. For example, if one of the search and rescue dogs is engaged in a real situation, and not just training, during the pedometer evaluation week, it will increase the activity count. This "individual" effect may be balanced in this analysis with the fact that the work/training/rest routines are quite balanced within the population so that the animals do not go through long periods where they kennelled or under intense workloads. We anticipated that an increase in patient complaints (reflected in CMI scores) would correspond to higher activity levels (reflected in higher pedometer counts), and vice versa. However, a negative correlation was found,

and pedometer counts followed the significant variations observed in these two CMIs scores, meaning that activity levels (measured by the pedometer count) increase, CMIs scores reduce (corresponding to better scores). A possible explanation for this may be an increase in spontaneous and playing activity, since an animal that has less pain will probably move more and be more willing to play with the handler, which is also more likely to engage in these activities, seeing the animal more active.

Construct validity can be assessed through factor analysis or by comparing the results of different instruments. Internal consistency is most frequently tested using Cronbach's α [40,42,49]. A strong correlation was observed between CMIs in both cohorts. Previous reports have observed moderate to good correlation between LOAD and CBPI [34,49]. Each CMI was designed based on different animal populations, encompassing different breeds, sizes and OA in different joints. In addition, OA is a multidimensional disease, and different CMIs may evaluate or capture different components of the disease. Factor analysis for some of the CMIs extracted a different number of components when compared with previous reports: of LOAD, 2 components were extracted, compared with the 3 described components [49]; of CBPI, 1 component was extracted, similar to one report [49], but differing with the 2 components in another [5]; and of COI, 1 component was extracted, in contrast with 4 component in a previous study [3]. Different factor analysis with a different population is not unusual. In terms of composition, our sample is homogeneous compared with those of previous reports, with fewer breeds, similar in size and conformation, all with bilateral OA of the same joint, and with similar levels of activity. Another possible explanation may be related to the proxy completing the CMIs. It has been described that quantifying pain and attributing it a score is subjective and, therefore, may lack validity when performed by individuals unfamiliar with signs of pain [19,20]. In our study, the proxy for all dogs were experience handlers, used to observe working and sporting dogs, particularly their own, and detect changes in movement pattern, performance losses and, possibly, changes in response to treatment. Alternative construct validity was performed through factor analysis. Factors extracted with eigenvalues greater than one or through scree-plot analysis were the same: two for HVAS and COI and one for CBPI and COI. Item loading of the components for HVAS and LOAD identified items that could be described with "ability to exercise/how often does the dog stop during exercise", "mobility/attitude" and "stiffness/disability". Even though CBPI and COI share some similar items with HVAS and LOAD, as the ability to rise or jump, stiffness and demeanour, this has not resulted in the extraction of more components. For CBPI, this has been described before [49]. Still, we presented enough data that shows that these CMIs address the clinical manifestations of OA and are able to detect changes as a result of treatment.

Conclusions

In this study, we determined criterion and construct validity of four different CMIs. These instruments were able to capture the various aspects of what constitutes the multi-dimensional experience that is OA. Therefore, they are valid tools to be used for the evaluation of naturally occurring canine osteoarthritis models.

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Quartile 1

Characterisation of weight-bearing compensation in dogs with bilateral hip osteoarthritis

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Abstract

Osteoarthritis (OA) is the most commonly diagnosed joint disease in veterinary medicine, and weight distribution, off-loading or limb favouring at the stance is a commonly used subjective assessment during the orthopaedic examination. To describe the weight-bearing compensation that occurs in police working diagnosed with bilateral hip OA, fifty police working dogs with bilateral hip OA were evaluated with a weight distribution platform at the time of initial evaluation and after different intra-articular treatments. Six evaluation sessions were performed, spread by 180 days. Results were compared by breed, age, sex, weight and OFA scores with the Independent Samples T-Test, one-way ANOVA and Pearson correlation coefficient, with $p<0.05$.

Animals had a mean age of 6.5±2.4 years and bodyweight of 26.7±5.2kg. No significant differences were observed when comparing weight-bearing for different breeds, sex, hip grades or different cut-offs for weight during the initial evaluation. A weight shift from pelvic to thoracic limbs was observed, with a weak significant correlation found between a pelvic limb and the opposing contralateral thoracic limb. During the follow-up period, Labrador Retrievers showed higher symmetry index and deviation from normal values than German Shepherd Dogs and Dutch Shepherd Dogs, as did male comparing with females dogs. At this period, the symmetry index showed a significant, week correlation with body weight. Weight-bearing of all limbs correlated with the remaining limbs, reflecting a more balanced weight distribution.

This study describes weight-bearing redistribution in dogs with bilateral hip OA, providing important information for the evaluation of this prevalent condition, but also in response to treatment.

Keywords: Dog; Osteoarthritis; Hip; Weight bearing; Stance Analysis.

Introduction

Gait analysis, and the measurement of ground reaction forces (GRF), are a well-established method to describe gait and severity of an existing lameness, with a 90% sensitivity and specificity[1–3]. It is an area of increasing interest, to assess response to surgical procedures, treatment outcome, orthopaedic conditions and breed differences[4–8]. Osteoarthritis (OA) represents at least 80% of the cases of lameness and joint diseases in companion animals, making it the most frequently diagnosed joint disease in veterinary medicine. There are several described risk factors, as having higher bodyweight, being of a specific breed, neutered and older than eight years[9–12]. Sporting and working animals are under increased risk, as they are exposed to chronic fatigue injuries, leading to tissue damage and ultimate failure[13]. Although measuring changes in limb function do not directly correlate to changes due to joint pain, one could expect function in the limb to have comparable changes as a consequence of it[14]. Ground reaction forces have been described as outcome measures

reflecting pain-related functional impairments in the context of OA, being abnormally lower[15–17]. The use of weight distribution platforms has been described, and presented as accurate and repeatable measurement, with a pressure-sensitive walkway as a reference[18]. Weight distribution platforms consist of a scale, with four different quadrants, in order to independently assess the four limbs[19].

When a limb is affected, significant adaptations occur on all limbs both at stance and swing phases. The described compensation mechanism is that most pronounced changes occur in the affected limb, followed by the contralateral limb, opposing the contralateral limb and, to a lesser extent, the opposing ipsilateral limb [20]. Animals with hip OA present complex changes in gait, which involve more joints than just the affected hip alone GRF of the lame limb in dogs with hip OA can be similar to those of the limbs of non-affected dogs, whereas the contralateral limb shows higher values[21]. Weight distribution, and off-loading or limb favouring at the stance is a commonly used subjective assessment during the orthopaedic examination, yet lameness may be difficult to detect during gait evaluation[22]. Stance analysis has been reported as being sensitive for detecting lameness in dogs, particularly in large breed dogs[23].

The study aims to describe weight-bearing compensation that occurs in police working diagnosed with bilateral hip OA. We also aimed to describe weight-bearing redistribution in response to treatment. We hypothesise that different breeds and patients with different hip grades, classified according to the Orthopedic Foundation for Animals (OFA) grading scheme, would show a different redistribution of body weight.

Materials and Methods

Fifty (N=50) active police working dogs, with bilateral hip OA, comprised the sample. They were selected from the population of police working dogs of the Guarda Nacional Republicana (Portuguese Gendarmerie Canine Unit) to take part in a longitudinal double-blinded, negative controlled study, evaluating the use of intra-articular treatments (platelet concentrate, Hylan G-F 20, triamcinolone hexacetonide, stanozolol and saline as a control) in the management of osteoarthritis. Patients were divided into five groups, of ten patients each, according to the treatment being administered: 2ml of 0.9%NaCl per hip joint; 20mg/1ml (Bluxam, Riemser Pharma GmbH) of triamcinolone hexacetonide per hip joint; stanozolol (Estrombol, Laboratório Fundacion) at 0.3mg/kg per hip joint; 2ml of Hylan G-F 20 (Synvisc®, Sanofi, Portugal) per hip joint; and 3ml of platelet concentrate per hip joint. A single administration was carried out, on day 0. At the time of the initial evaluation, dogs were scheduled to undergo treatment for hip OA. Diagnosis based on dog's history (difficulty rising, jumping and maintaining obedience positions, stiffness and decreased overall performance), physical examination (pain during joint mobilisation, stiffness and reduced range of

motion) and radiographic findings (OFA hip scores of mild, moderate or severe) consistent with bilateral hip OA. In order to be included in this study, the dog must have a bodyweight ≥15kg, be over two years and should not have received any medication or nutritional supplements for at least six weeks. The need for additional rescue analgesia was evaluated by the assisting veterinarian and recorded. Animals with suspect or confirmed orthopaedic disease other than OA or concomitant disease were excluded. Dogs not tolerant of data collection were also excluded. The same researcher performed all evaluations on days 0, 8, 15, 30, 90 and 180.

Weight-bearing distribution was performed with a weight distribution platform (Companion Stance Analyser; LiteCure LLC®, Newark, Delaware, United States). Following manufacturer's instructions, it was placed in the centre of a room, at least 1 meter from the walls, and calibrated at the beginning of each day and zeroed before each data collection. For data collection, animals were then encouraged by its trainers to stand on to the weight distribution platform, while ensuring that the patient placed one foot on each quadrant of the platform and maintained a natural stance with their centre of gravity and stability near the middle of the platform (measured by the platform). When required, gentle restraint was used to maintain the patient's head in a natural, forward-facing position. Normal weight distribution for each thoracic limb was considered as 30% of total weight, and 20% for each pelvic limb[19]. A left-right symmetry index (SI) was calculated for thoracic and pelvic limbs, with the following formula: SI=[(WBR-WBL)/((WBR+WBL)x0.5)]x100[24, 25]. WBR is the value of weight-bearing for the right limb, and WBL is the value of weight-bearing for the left limb. Negative values were made positive. We also considered deviations from normal weight-bearing for both thoracic and both pelvic limbs, considered as 60% and 40%, respectively. This was obtained subtracting WBR+WBL to 60 for the thoracic limbs and 40 for the pelvic limbs.

For each animal, a ventrodorsal radiographic view was obtained, to determine OFA hip grade. All radiographic studies were conducted under light sedation, using a combination of medetomidine (0.01mg/kg) and buthorphanol (0.1mg/kg), given intravenously.

Normality was assessed with a Shapiro-Wilk test, and measured parameters were compared with an Independent Samples T-Test or one-way ANOVA. Correlation between parameters was assessed with the Pearson correlation coefficient. All results were analysed with IBM SPSS Statistics version 20, and a significance level of $p<0.05$ was set.

Results

The sample for this study included 50 active police working dogs, 30 males and 20 females, representing four breeds: German Shepherd Dogs (GSD, n=17), Belgian Malinois Shepherd Dogs (BM, n=15), Labrador Retriever (LR, n=10), and Dutch Shepherd Dog (DSD, n=8). Animals had a

mean age of 6.5±2.4 years and bodyweight of 26.7±5.2kg, with both genders being represented (30 males and 20 females). In concern to OFA hip grading, 35 animals were classified as mild (70%), 10 as moderate (20%) and 5 as severe (10%). Mean values of overall weight, age and weight-bearing distribution of each limb by breed, sex and OFA hip grades at the moment of initial evaluation are presented in table 1. No significant differences were observed when comparing values for different breeds and sex, nor with different cut-offs for weight at the time of initial evaluation. Correlations for weight-bearing distribution for each limb at the moment of the initial evaluation is presented in table 2. Regarding SI and deviation, no significant differences were found comparing animals with the different weight cut off points, by breed, sex or OFA hip grade. A strong correlation was found between SI and deviation scores ($r=1.0$, $p<0.01$).

Considering the evolution of weight-bearing distribution during the follow-up period, a significant decrease in weight-bearing was observed in the combined thoracic limbs comparing with initial evaluation ($p<0.01$) and increase in the combined pelvic limbs ($p=0.05$). Correlations for weight-bearing distribution for each limb during follow-up evaluations are presented in table 3. During this period, significant differences were observed in the weight-bearing distribution of individual thoracic limbs considering a weight cut-off point of 20kg, but not on the combined value of both. No significant differences were found with the remaining cut-offs. LR showed significantly higher weight-bearing on the right thoracic limb than GSD ($p<0.01$) and BM ($p<0.01$). They also had significantly lower weight-bearing on the right pelvic limb and the combined value of both pelvic limbs than GSD ($p=0.02$ for both), BM ($p<0.01$ for both) and DSD ($p=0.02$ and $p=0.04$, respectively). Female dogs showed significantly lower weight-bearing on the left pelvic limb ($p<0.01$) and combined pelvic limbs ($p=0.01$), and higher right thoracic limb ($p<0.01$). Animals with a severe hip grade, showed significantly lower weight-bearing on the combination of both pelvic limbs when compared with mild and moderate hip grades ($p=0.02$ and $p<0.01$, respectively), and higher on the thoracic limbs ($p=0.05$ for mild and $p=0.03$ for moderate).

Table 1 – Mean values (±standard deviation) of overall weight, age and weight-bearing distribution of each limb by breed, sex and Orthopedic Foundation for Animals hip grades on initial evaluation.

	Weight	Age	Stance Analysis left thoracic limb	Stance Analysis right thoracic limb	Stance Analysis left pelvic limb	Stance Analysis right pelvic limb
	(kg, mean±SD)	(yrs, mean±SD)	(%, mean±SD)	(%, mean±SD)	(°, mean±SD)	(°, mean±SD)
Overall	26.7±5.3	6.5±2.2	31.6±6.2	30.7±6.7	19.0±4.4	18.7±4.2
German Shepherd Dog	29.9±6.3	5.7±1.8	31.7±5.4	29.9±5.6	20.0±3.9	18.4±3.6
Belgian Malinois Shepherd Dog	24.3±4.1	6.5±2.5	30.3±8.4	32.1±7.9	17.8±5.5	19.8±5.9
Labrador Retriever	24.3±2.5	8.7±2.4	31.9±4.9	30.5±5.7	19.9±4.4	18.6±3.2
Dutch Shepherd Dog	27.5±3.9	5.3±1.3	33.8±4.6	30.3±8.1	19.0±4.4	17.4±2.9
Male	29.0±5.4	6.2±2.3	31.3±7.1	30.7±7.4	19.0±5.5	19.3±4.8
Female	23.5±2.8	6.9±2.8	32.1±4.7	30.6±5.5	19.4±3.5	17.9±3.1
Mild	26.7±5.2	6.5±2.4	31.7±6.9	30.2±6.6	19.5±4.4	18.9±4.3
Moderate	26.5±5.4	6.4±2.4	30.5±5.3	32.7±8.3	17.7±6.9	19.1±4.8
Severe	26.4±5.4	6.5±2.5	33.6±1.1	30.0±2.6	19.8±1.3	16.6±2.1

Table 2 – Correlations for weight-bearing distribution for each limb on initial evaluation. LPL – left pelvic limb; LTL – left thoracic limb; RPL – right pelvic limb; RTL – right thoracic limb. * indicates significant correlation.

Limb		LTL	RTL	LPL	RPL
LTL	rs	1	-0.83	-0.08	-0.20
	Sig.		<0.01*	0.14	<0.01*
RTL	rs	-0.83	1	-0.29	-0.14
	Sig.	<0.01*		<0.01*	<0.01*
LPL	rs	-0.08	-0.29	1	-0.14
	Sig.	0.14	<0.01*		0.02*
RPL	rs	-0.20	-0.14	-0.14	1
	Sig.	<0.01*	<0.01*	0.02*	

Table 3 – Correlations for weight-bearing distribution for each limb during follow-up evaluations. LPL – left pelvic limb; LTL – left thoracic limb; RPL – right pelvic limb; RTL – right thoracic limb. * indicates significant correlation.

Limb		LTL	RTL	LPL	RPL
LTL	rs	1	-0.87	-0.09	-0.16
	Sig.		<0.01*	0.186	0.01*
RTL	rs	-0.87	1	-0.23	-0.15
	Sig.	<0.01*		<0.01*	<0.01*
LPL	rs	-0.09	-0.23	1	-0.14
	Sig.	0.19	<0.01*		0.03*
RPL	rs	-0.16	-0.15	-0.14	1
	Sig.	0.01*	<0.01*	0.03*	

During the follow-up period, and regarding SI, heavier animals (analysed with a 30 and 35kg cut-off points) had higher asymmetries on thoracic limbs ($p=0.02$ and $p=0.03$, respectively) and also on pelvic limbs ($p<0.01$ for both). The same animals showed significantly higher deviations for thoracic limbs ($p=0.02$ and 0.03, respectively). Comparing animals by breed, LR showed significant lower SI and deviation on both thoracic than GSD ($p=0.03$ and 0.04, respectively) and BM ($p<0.01$ for both). Concerning pelvic limbs, LR had higher SI than DSD ($p=0.02$) and deviation than GSD ($p=0.03$). Male dogs had significantly higher SI and deviation than females ($p<0.01$). SI showed a weak, significant correlation with weight ($r=-0.16$, $p<0.01$).

Discussion

This report characterises weight distribution variations in dogs with bilateral hip OA. Subtle changes in posture or weight-bearing may occur in the early stages of the disease process, which can be easily missed with the visual assessment[26–28]. Additionally, OA animals may not be overtly lame at a walk or a trot but exhibit subtle shifts in body weight distribution at a stance due to pain or instability associated with orthopaedic or neural disease[19, 29]. Weight distribution platform, as a pressure-sensitive walkway, can provide accurate and consistent measures of weight distribution with no significant difference between devices[18]. At the moment of the initial evaluation, a weight shift was observed from the pelvic to the thoracic limbs. For dogs presenting with pelvic limb-lameness, a load redistribution more by side-to-side compensation rather than pelvic-to-thoracic has been described[30, 31]. Our results contrast with this finding, which may be related to the fact that all animals exhibited bilateral disease, and therefore may try to relieve the pelvic limbs by transferring weight to the thoracic limbs. Also, a weak significant correlation was found between pelvic limbs with the opposing contralateral thoracic limb.

Interestingly, no significant differences were observed were comparing the various breeds represented in the sample. This is in contrast with what has been described for gait analysis, where significant breed differences have been reported, both at a walk and trot, a probable reflection of different conformations[32]. Besides, no significant differences were observed when comparing animals by sex or with the different weight cut off values. This may increase interest in weight distribution analysis as a diagnosis and response to treatment evaluation tool since it did not show significant variability for different sets of animals. Additionally, it does not require data normalisation to compare different animals, a requirement with gait analysis[33, 34].

Bodyweight distribution at a stance may has been proposed to be an equivalent or superior measurement of pain associated with hip OA than both VI and PVF, with highest sensitivity and specificity being set at a cut-off of 2[19, 35]. We have recorded values below and above this cut-off

value. This may be since all animals had bilateral disease, and a weight-bearing mean value above 18% is usually paired with a value below. Also, since these animals are active Police working dogs, it is possible that complaints and signs are detected in an earlier stage of the disease (the majority of animals were classified with a mild hip grade), and therefore still not exhibit compensation mechanisms to a large extent. Similar to what is observed with clinical signs of OA and vertical GRF[8], no correlation has been found between hip grade and weight-bearing, SI or deviation.

During the follow-up period, and in response to treatment, a significant decrease in weight-bearing in the thoracic limbs was observed, counteracted by an increase in the pelvic limbs, approaching the described normal 30/30/20/20 (left thoracic limb/right thoracic limb/ left pelvic limb/right pelvic limb)[18, 36]. During this stage, weight-bearing of all limbs correlated with the remaining limbs, which may reflect a more even, balanced, weight distribution. There may exist a tendency to see fewer improvements in male dogs concerning bodyweight distribution with pelvic limb pain relief, as they naturally tend to carry more weight on the thoracic limbs[29]. We have observed a different tendency, with female dogs exhibiting significantly lower pelvic limb weight distribution at this stage. In contrast, male dogs had higher SI and deviation values. This may be a reflection of the higher body weight of male dogs since body weight showed a significant correlation with SI. When comparing animals by breed, with LR showed lower pelvic limb weight distribution and higher SI and deviation that remaining breeds. On the other hand, SI and deviation values in the thoracic limb were significantly lower in LR, which may reflect breed-specific conformation.

This study presents some limitations, namely the lack of a disease-free group, with non-lame dogs. Also, since the sample was comprised exclusively by police working dogs, there is a need to evaluate animals of different breeds, conformations and activity levels. These limitations are due to the nature of the sample, comprised of dogs specifically presenting for treatment. Also, there is a need to compare different, well-established treatments, such as non-steroidal anti-inflammatory drugs. These commonly used therapies were not included in this study, as it aimed to evaluate only intra-articular treatment modalities.

Conclusions

This study describes weight-bearing redistribution in dogs presenting with bilateral hip OA, and in response to treatment. It provides important information for the evaluation of this prevalent condition, but also in response to treatment.

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5. DETERMINATION OF THE EFFECT OF 4 SUBSTANCES DELIVERED BY INTRA-ARTICULAR ADMINISTRATION IN PATIENTS WITH HIP JOINT OSTEOARTHRITIS;

Management of osteoarthritis using one intra-articular platelet concentrate administration in a canine osteoarthritis model – Published in The American Journal of Sports Medicine – Impact factor 6.060, Quartile 1.

Effect of a single intra-articular administration of stanozolol in a naturally occurring canine osteoarthritis model: a randomized trial - Submitted to Bone & Joint Research – Impact factor 3.532, Quartile 1.

Effect of a single intra-articular high molecular weight hyaluronan administration for the management of osteoarthritis in a naturally occurring canine osteoarthritis model – Published in the Journal of Orthopaedic Surgery and Research - Impact factor 2.145, Quartile 2.

The intra-articular administration of triamcinolone hexacetonide in the treatment of osteoarthritis. Its effects in a naturally occurring canine osteoarthritis model – Published in PLoS One – Impact factor 2.740, Quartile 1.

Management of Osteoarthritis Using 1 Intra-articular Platelet Concentrate Administration in a Canine Osteoarthritis Model

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Background: Osteoarthritis (OA) represents a significant burden to societies, as it affects quality of life and performance and implies a large cost in terms of health care. Autologous platelets are a regenerative treatment modality for OA that are thought to be a potential stimulation of the natural healing cascade.

Purpose: To describe the effect of the platelet concentrate V-PET in the management of OA in a naturally occurring canine model, using several outcome assessment modalities.

Study Design: Controlled laboratory study.

Methods: A total of 40 joints of active working police dogs with hip OA were randomly assigned to a control group (CG) and a platelet concentrate group (PCG; treatment) and evaluated. At treatment day (T0) and 8, 15, 30, 90, and 180 days after treat- ment, weight distribution, joint range of motion at flexion and extension, thigh girth, digital thermography, radiographic signs, 4 clinical metrology instruments, and synovial fluid interleukin 1 and C-reactive protein levels were recorded. Results were com- pared using repeated-measures analysis of variance with a Huynh-Feldt correction, paired-samples *t* test, or Wilcoxon signed rank test, with $P < .05$.

Results: Dogs were 6.5 6 2.4 years old (mean 6 SD) and had a body weight of 26.7 6 5.2 kg. At T0, 32 (80%) joints were graded as having mild OA, 6 (15%) as moderate, and 2 (5%) as severe. No differences were found between groups at T0. Between the PCG and CG, the symmetry index showed significant improvements in the PCG from 8 days ($P = .01$) to 180 days ($P = .01$). Joint flexion also improved in the PCG up to 90 days ($P < .05$) and extension improved up to 180 days ($P < .01$). Several clinical metrology instru- ment scores also improved up to 90 to 180 days after treatment. In the CG, radiographic signs progressed, while the PCG showed some improved signs. In both groups, increasing body weight and age corresponded with worse clinical and laboratory findings.

Conclusion: A single injection of platelet concentrate had a positive effect, lasting up to 6 months, on several clinical, imaging, and laboratory signs in a naturally occurring canine OA model.

Clinical Relevance: We characterized the effects of this platelet concentrate in dogs, considered the gold standard of the study of OA, with a group of working animals with similar high demands as athletes.

Keywords: animal model; dog; osteoarthritis; pain; autologous platelet concentrate; stance analysis; digital thermography; digital radiography; clinical metrology instruments; C-reactive protein; interleukin 1

Osteoarthritis (OA) affects all mammals, and it is an important disease in humans and dogs and a source of chronic pain, with the prevalence expected to rise due to a simultaneous increase in life expectancy and obesity.[2,31]

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OA represents a significant burden to societies, as it affects quality of life (QOL) and performance and implies a large cost in terms of health care.[13] The dog is a frequent animal model for the study of OA because the pathologic process, clinical presentation, and response to treatment are very similar to those observed in humans.[28] In addition, the nat- urally occurring canine model provides the advantage of presenting faster disease progression, with equivalent life stages to those in humans, while sharing many of the

environmental variations that also influence human OA, thus making it easier to study. For these reasons, the dog is the closest to a gold standard model.[23,26,35,37] Therefore, the study of canine OA can provide important information in translational medicine under the One Medicine Initiative, which will help improve the health and well-being of humans and dogs.[8,37]

Despite being a focus of extensive research, OA is a condition with limited treatment options presently available, and its management centers mainly on the alleviation of pain, improvement of function, and possible slowing of disease progression.[37,38] For that reason, there has been a growing interest in the development of treatments that preserve or restore cartilage by promoting its synthesis or inhibiting its breakdown.[21] Autologous platelets are a potential regenerative treatment modality for OA, used with the aim to stimulate the natural healing cascade and regeneration of tissues. Platelet-released growth factors include insulin-like growth factor 1, transforming growth factor *b*, platelet-derived growth factor, vascular endothelial growth factor, and basic fibroblast growth factor, which, by a supraphysiologic release directly at the treatment site, signal cells to proliferate and influence their maturation, differentiation, and tissue repair. Being an autologous product, it does not hold the risk of immune rejection or disease transmission.[11,39,46]

Pain and functional ability are the most relevant parameters in the evaluation of OA as an assessment of treatment efficacy.[56] For these assessments of the multiple dimensions of OA, several clinical metrology instruments (CMIs) have been developed. The most commonly used are the Canine Brief Pain Inventory (CBPI) and the Liverpool Osteoarthritis in Dogs (LOAD).[54,55] The CBPI is divided into 2 sections: a pain severity score (PSS) and a pain interference score (PIS).[50] Other validated CMIs include the Canine Orthopedic Index (COI; divided into 4 scores: stiffness, gait, function, and QOL), and the Hudson Visual Analog Scale (HVAS), developed to assess the degree of lameness in dogs.[6,25] The ventrodorsal hip extended view is the most common pelvic radiographic projection used for the clinical assessment of OA and determination of treatment outcome.[43] The circumferential femoral head osteophyte (CFHO) and caudolateral curvilinear osteophyte (CCO) are early radiographic signs related to the development of the clinical signs of hip OA.[33,42] Stance analysis has been reported as a sensitive evaluation for detecting lameness in dogs.[9] It evaluates weight distribution, and off-loading or limb favoring at the stance is a common assessment during orthopaedic examination since dogs commonly bear less weight on a painful limb.[22] Digital thermal imaging has been shown to be a reliable technique to assess inflammatory arthritis pain.[17] This evaluation method relies on heat generated during physiologic functions and its relation with skin temperature control.[24,44] Activity levels and mobility impairments are associated with musculoskeletal pain in humans. For that reason, mobility changes have been recommended as measures of outcome.[29] A simple and inexpensive method to assess mobility levels is through the use of pedometers since they are capable of measuring ambulatory activity with acceptable accuracy.[49] Since muscular atrophy is a consistent finding in dogs with OA, examination of muscle masses and restrictions in joint range of motion (ROM; at flexion and extension) is also usually performed.[32,57] Interleukin 1 (IL-1) has been identified as the most important proinflammatory cytokine responsible for the catabolism in OA, with a relation to lameness duration.[20,36] C-reactive protein (CRP) is an acute-phase protein produced during inflammatory reactions or tissue injury, which may also be produced at the level of the inflamed tissues, with the advantage of its shifts being noted from a very early stage.[5,14]

The goal of this study was to describe the effect of the platelet concentrate V-PET (Pall Corporation) in the management of OA in a naturally occurring canine model using several outcome assessment modalities. We hypothesized that it would be able to reduce the clinical signs of OA, as compared with a control group (CG), where no therapeutic options were used.

Abbreviations used in this article are defined in Table 1.

METHODS

The study protocol was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval GD/32055/2018/P1, September 25, 2018). Informed consent was obtained from the institution responsible for the animals (Guarda Nacional Republicana, Portugal). The sample comprised 40 joints of active working police dogs with bilateral hip OA diagnosed on the basis of history and physical, orthopaedic, neurologic, and radiographic examinations. To be included in this

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TABLE 1
Abbreviations

Abbreviation	Definition
Evaluation Moment	
CBPI	Canine Brief Pain Inventory
CCO	caudolateral curvilinear osteophyte
CFHO	circumferential femoral head osteophyte
CG	control group
CMI	clinical metrology instrument
COI	Canine Orthopedic Index
CRP	C-reactive protein
DV	dorsoventral
HVAS	Hudson Visual Analog Scale
IL-1	interleukin 1
LOAD	Liverpool Osteoarthritis in Dogs
Lt	lateral
OA	osteoarthritis
PCG	platelet concentrate group (treatment)
PIS	pain interference score
PSS	pain severity score
QOL	quality of life
ROM	range of motion
SI	symmetry index
VD	ventrodorsal

TABLE 2
Evaluation Modalities Used on Each Evaluation Moment[a]

	0 d[b]	8 d	15 d	30 d	90 d	180 d
Treatment	3					
Goniometry	3	3	3	3	3	3
Thigh girth measurement	3	3	3	3	3	3
HVAS	3	3	3	3	3	3
CBPI	3	3	3	3	3	3
COI	3	3	3	3	3	3
LOAD	3	3	3	3	3	3
Digital thermography	3	3	3	3	3	3
Pedometer	3	3	3	3	3	3
Stance analysis	3	3	3	3	3	3
Digital radiography	3			3	3	3
Synovial fluid						
CRP	3	3		3	3	3
IL-1	3	3		3	3	3
Routine blood testing	3			3	3	3

[a]CBPI, Canine Brief Pain Inventory; COI, Canine Orthopedic Index; CRP, C-reactive protein; HVAS, Hudson Visual Analog Scale; IL-1, interleukin 1; LOAD, Liverpool Osteoarthritis in Dogs.
[b]Treatment day (days are counted from treatment day).

study, dogs had to have a body weight ≤20 kg and age ≥2 years, and they should have not received any previous treatment for hip OA and not received any medication or nutritional supplements for ≥6 weeks. Dogs with other suspected or documented orthopaedic, neurologic, or concomitant disease or dogs that were not tolerant of data collection were excluded.

In a double-blinded study, after selection, dogs were randomly assigned to a CG (n = 20) or a treatment group (platelet concentrate group [PCG]; n = 20). The CG received an intra-articular administration of 3 mL of 0.9% NaCl, while the PCG received a single administration of 3 mL of a platelet concentrate produced using the com- mercially available V-PET kit according to the manufac- turer's instructions. Whole blood (55 mL) was collected from the jugular vein using a 60-mL syringe filled with 5 mL of anticoagulant citrate dextrose solution and intro- duced into the provided closed system. The blood flowed by action of gravity through the filter where platelets were concentrated. The final liquid product was collected using the provided syringe and administered within 10 minutes without activation. These procedures were conducted at room temperature without direct light exposure and took around 25 minutes from blood collection to administration. Intra-articular administrations were conducted under light sedation using a combination of medetomidine (0.01 mg/kg) and butorphanol (0.1 mg/kg) given intravenously. The dogs were positioned in lateral recumbency with the affected joint uppermost, and a small win- dow of 4 × 4–cm area surrounding the greater trochanter was clipped and aseptically prepared. With the help of an assistant, the limb was set in a neutral posi- tion, parallel to the table. A 21-gauge, 2.5-inch needle was introduced just dorsal to the greater trochanter and per- pendicular to the long axis of the limb, until the joint was reached.[52] Confirmation of correct needle placement was obtained through the collection of synovial fluid, and the treatment or saline was administered. After treatment, animals rested for 3 consecutive days and resumed their normal activity over a period of 5 days. An outline of evaluations performed and evaluation moments is presented in Table 2. All evaluations were performed by the same researcher throughout the study (J.C.A.). No additional treatments were administered, and no rehabilitation protocols were conducted.

Weight distribution evaluation was conducted using a weight distribution platform (Companion Stance Ana- lyzer; LiteCure LLC) following the manufacturer's guide- lines. For the evaluation, animals were encouraged by their trainers to stand on the platform with 1 foot on each quadrant of the platform. For all animals, at least 20 measurements were performed, and the mean value was determined. A left-right symmetry index (SI) was cal- culated using the following formula: $SI = [(WB_R - WB_L) / (WB_R + WB_L) \times 0.5)] \times 100$,[53,55] where WB_R is the value of weightbearing for the right pelvic limb and WB_L for the left pelvic limb. Negative values were made positive. Devi- ation from the normal value of 20% weightbearing for a pel- vic limb was also considered[10] and was calculated by subtracting weightbearing from 20.

Before digital thermography images were collected, dogs were allowed to walk calmly in a room with controlled temperature (21°C) for 30 minutes. Then, with the animal positioned in a symmetrical upright standing position, a dorsoventral (DV) thermographic image, including the area from the last lumbar vertebra to the first coccygeal vertebra, was obtained.[51] A lateral (Lt) view was also obtained with the greater trochanter in the center. All

images were taken at a distance of 60 cm (ThermaCAM E25; FLIR Systems). To analyze the images, the free soft- ware Tools (FLIR Systems) was used with a rainbow color palette. Temperature boxes of equal size were placed on the anatomic area of the hip joint on both views, and mean and maximal temperatures were determined. Thigh girth and joint ROM were determined with the dogs in lateral recum- bency with the affected limb uppermost. With use of a Gullick II measuring tape, thigh girth was determined at a distance of 70% thigh length, measured from the tip of the greater tro- chanter with an extended leg.[34] Hip joint ROM was obtained using a goniometer at extension and flexion with a flexed sti- fle.[30] These measurements were made in triplicate, and the mean value was calculated. Activity levels were measured using the pedometer worn around the dogs' neck and attached to an adjustable lightweight collar.[7] They were placed 1 week before the first evaluation moment to deter- mine a baseline value and maintained up to the 30th day after treatment. For evaluation at the 90th and 180th post-treatment days, animals wore the pedometer for a week before the evaluation moment. Mean daily counts were con- sidered and were calculated by dividing the registered num- ber of steps by the number of days considered. Each trainer completed the HVAS, CBPI, COI, and LOAD on evaluation days after receiving instructions. The CMIs were completed in sequence by the same handler in a quiet room with as much time as needed to answer all items.

For radiographic examination, which was conducted under the same light sedation induced for treatment admin- istration of synovial fluid collection, a VD extended-legs view was obtained. The presence of 7 radiographic OA signs was assessed: irregular wear on the femoral head, making it misshapen and have a loss of its rounded appearance; a flat- tened or shallow acetabulum, with irregular outline; CCO; new bone formation on the acetabulum and on the femoral head and neck; a worn-away angle formed at the cranial effective acetabular rim; subchondral bone sclerosis along the cranial acetabular edge; and CFHO.[4,18,43,48] Joints were also graded according to the Orthopedic Foundation for Animals scheme. A mild score corresponded to a partially subluxated femoral head, causing an incongruent and wid- ened joint space with a shallow acetabulum only partially covering the femoral head. In young dogs (24-36 months old), OA lesions may not be present. Moderate grades were attributed when significant subluxation was present and the femoral head was barely seated in a shallow acetab- ulum. Secondary remodeling along the femoral neck and head, acetabular osteophytes, and subchondral sclerosis were present. In severe cases, the femoral head was partly or completely out of a shallow acetabulum, with extensive secondary arthritic bone changes along the femoral head and neck head, acetabular rim changes, and large amounts of abnormal bone pattern changes. A full description of the Orthopedic Foundation for Animals hip grading scheme is available online (https://www.ofa.org/diseases/hip-dysplasia/grades). The same researcher, blinded to the group and identity of the dog, scored all joints (J.C.A.). A sample of synovial fluid was obtained for the determination of IL-1*b*, and CRP concentrations were made using the DuoSet Ancil- lary Canine IL-1*b* Reagent kit (R&D Systems) and read using FLUOstar OPTIMA (BMG Labtech), Fuji Dri-Chem Slides VC-CRP PS (FUJIFILM Europe GmbH), and DRI-Chem NX500i (FUJIFILM Europe GmbH).

Normality was assessed using a Shapiro-Wilk test. Group results were compared in each evaluation moment, and each measured parameter was compared with the result observed at treatment day. To assess the effect of different parameters on the dogs' clinical evolution, results were compared by sex, age, body weight, and presence of CCO and CFHO using a paired-samples *t* test, repeated- measures analysis of variance, Huynh-Feldt correction, or Wilcoxon signed rank test. All results were analyzed using IBM SPSS Statistics Version 20 (IBM Corp), and a significance level of $P \setminus .05$ was set.

RESULTS

The sample included 40 pelvic limbs of working police dogs, equally distributed between left and right pelvic limbs, with a mean 6 SD age of 6.5 6 2.4 years and body weight of 26.7 6 5.2 kg. Based on the Orthopedic Foundation for Animals hip grading at T0, 32 joints were classified as mild (80%), 6 as moderate (15%), and 2 as severe (5%). Both sexes were represented (26 limbs from males and 14 from females). No differences were found between groups at the initial evaluation. The composition of the platelet concentrate and whole blood is presented in Table 3.

After treatment, increased lameness was observed in 8 dogs, which resolved spontaneously within 48 hours.

Clinical and Laboratory Findings

When results between groups were compared using repeated-measures analysis of variance with a Huynh-Feldt correction, significant differences were found concerning body weight (F[3.29, 92.3] = 5.9; P= .001), maximal temper- ature on the DV view (F[3.6, 87.2] = 5.5; P = .001), mean temperature on the Lt view (F[4.8, 129] = 44.3; P = .001), maximal temperature on the Lt view (F[4.57, 123.4] = 101.6; P= .001), joint flexion (F[3.6, 107.5] = 11.9 (P= .001), IL-1 synovial concentration (F[2.4, 76.9] = 6.4; P = .001), and SI (F[3.73, 111.8] = 3.3; P = .016). Evolution of SI is presented in Figure 1.

Significant differences were also observed with different CMIs: PSS (F[3.1,81.2] = 3.3; P= .015), PIS (F[3.4, 87.5] = 4.3; P= .006), LOAD (F[2.16, 56.2] = 1.5; P= .022), stiffness (F[2.7, 71] = 1.3; P = .028), function (F[5, 130] = 2.4; P = .040), gait (F[2.5, 65.7] = 1.8; P= .016), and COI (F[5, 130] = 2.0; P= .007). V alues recorded for the initial evalu- ation and its variations throughout the study are pre- sented in Tables 4 and 5.

Radiographic Findings

Table 6 presents the frequency of different radiographic findings at the initial and final evaluations.

In the CG, animals that presented CCO on the radio- graphic VD view did not show significant differences ver- sus those that did not. At the 30-day evaluation, animals

TABLE 3
Composition of Whole Blood and Platelet Product[a]

	Whole Blood		Platelet Concentrate	
Parameter	Mean	SD	Mean	SD
Platelets	293.35	65.71	1144.06	256.26
Red blood cells, $\times 10^6$/mm^3	6.65	0.92	4.19	0.58
Hematocrit, %	45.01	6.87	22.05	3.37
White blood cells	10.47	3.93	19.90	8.25
Lymphocytes	2.20	1.01	5.50	2.52
Monocytes	0.74	0.41	0.82	0.46
Neutrophils	6.97	0.78	11.08	1.24
Eosinophils	0.54	0.78	0.82	1.20
Basophils	0.02	0.02	0.00	0.00

[a]Values are presented as $\times 10^3$/mm^3 unless noted otherwise.

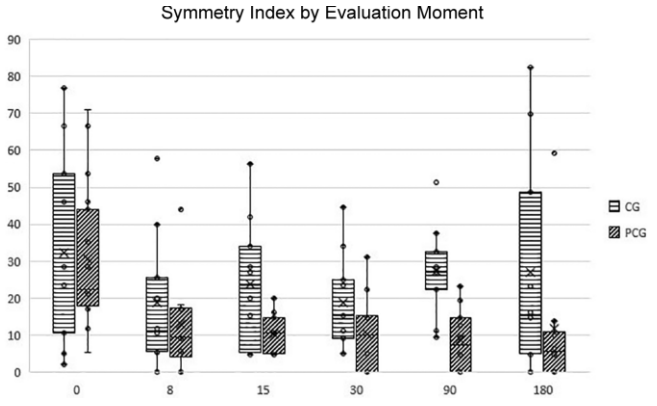

Figure 1. Evolution of the symmetry index in the control group (CG) and platelet concentrate group (PCG; treatment). Box plots represent the median and 25th and 75th percen- tiles, and whiskers represent the 10th and 90th percentiles. Circles denote outliers; x represents the mean.

with CCO had lower mean thermographic evaluation on the Lt view ($P = .04$), worse joint flexion ($P = .03$), and higher IL-1 and lower CRP concentration levels ($P = .04$ for both). Animals with CCO had higher maximal thermo- graphic evaluation on the Lt view at the 90-day evaluation ($P = .05$) and higher CRP at the final evaluation ($P = .02$). In the PCG, dogs with CCO at the initial evaluation had worse PSS ($P = .01$), PIS ($P = .02$), stiffness ($P < .01$), and gait ($P = .02$). At the first treatment assessment, at 8 days, they had lower mean thermographic values on the DV view ($P = .04$) and maximal values on the Lt view ($P = .05$), with worse PIS ($P < .01$), LOAD ($P = .01$), func- tion ($P = .01$), gait ($P < .01$), and COI ($P = .05$). At 15 days, the same dogs had worse PSS ($P < .01$), PIS ($P < .01$), and gait ($P < .01$). At the 30-day evaluation, those with CCO at the initial evaluation had lower pedometer counts ($P = .02$) and worse HVAS ($P = .04$), PSS ($P = .01$), PIS ($P < .01$), LOAD ($P = .05$), stiffness ($P < .01$), function ($P = .03$), gait ($P = .04$), and COI ($P = .03$). At 90 days, they had higher SI ($P = .03$), higher mean and maximal thermo- graphic values on the Lt view ($P = .03$ and $P < .01$, respec- tively), and worse HVAS ($P = .04$) and PSS ($P = .01$). At the final evaluation, higher maximal thermographic values on the DV view ($P = .02$) were observed.

Dogs in the CG with CFHO on a VD view at the initial evaluation had worse joint extension at the 8-day evalua- tion ($P < .01$) and worse HVAS ($P = .02$), PSS ($P = .01$), and PIS ($P = .03$). At 15 days, they had a lower mean ther- mographic evaluation on the Lt view ($P = .02$) and worse PSS ($P = .02$) and PIS ($P = .05$). The lower mean thermo- graphic evaluation on the Lt view was also observed at 30 days ($P = .01$). At 90 days, these dogs had worse HVAS scores ($P = .02$). At the final evaluation, they had a lower maximal thermographic evaluation on the Lt view ($P = .04$) and worse PSS ($P = .05$) and PIS ($P = .03$). In the PCG, dogs with CFHO had better PSS ($P = .02$) and PIS ($P = .02$). At 8 days, the same dogs had lower thigh girth ($P = .05$) but better PSS ($P = .05$), PIS ($P < .01$), LOAD ($P = .02$), function ($P = .01$), gait ($P = .01$), and COI ($P = .01$). Again at 15 days, they had better PSS ($P = .05$), PIS ($P = .01$), and function ($P = .04$). At 30 days, these dogs showed higher pedometer counts ($P < .01$) and better HVAS ($P = .04$), PSS ($P < .01$), function ($P < .01$), and gait ($P < .01$). This trend with CMI scores was again observed at 90 days, with better HVAS ($P = .03$), PSS ($P < .01$), PIS ($P < .01$), LOAD ($P < .01$), stiffness ($P = .05$), function ($P < .01$), gait ($P < .01$), QOL ($P = .01$), and COI ($P < .01$). At the final evaluation moment, only a lower mean thermographic value on the DV view ($P = .05$) and a better PSS score ($P = .01$) were observed.

Comparisons by Sex

In the CG, when animals were grouped by different find- ings, male dogs had significantly higher body weight for all evaluation moments ($P = .01$). At the initial evaluation, males had lower values for all thermographic evaluations ($P < .01$) and higher PIS ($P = .04$). At 8 days, the same was true regarding thermographic evaluation ($P < .01$), except for a maximal value on the Lt view and lower joint extension values ($P < .01$). At 15 days, males still showed lower joint extension ($P = .04$) and higher PIS ($P = .03$). At the 30-day evaluation, females showed higher thermo- graphic maximal values on the Lt view ($P < .01$). At 90 days, male dogs had higher thigh girth ($P = .03$) and worse PSS and PIS ($P = .01$). In the final evaluation moment, male dogs had lower extension values ($P = .02$) and worse HVAS ($P = .02$), PSS ($P < .01$), PIS ($P < .01$), stiffness ($P = .02$), function ($P = .02$), gait ($P < .01$), QOL ($P = .02$), and COI ($P = .01$). In the PCG at the initial evaluation, male dogs had higher thigh girth ($P < .01$) than did females. Males had lower mean thermographic values on the Lt view at the 8-day evaluation moment ($P = .02$) and higher thigh girth at 15 days ($P = .02$). At 30 days, males had lower mean and maximal thermographic values on the DV view ($P < .01$ for both) and Lt view ($P < .01$ and $P = .02$, respectively) in addition to higher thigh girth ($P < .01$). At 90 days, males had a higher mean temperature value on the Lt view again ($P = .03$) and a lower IL-1 con- centration ($P = .03$). At the final evaluation moment, males

TABLE 4
Parameters Evaluated at the Initial Evaluation and at 8 and 15 Days[a]

Treatment Day					8 d							15 d						
CG			PCG		CG			PCG				CG			PCG			
Modality		Mean	SD	Mean	SD	Mean	SD	*P* Value	Mean	SD	*P* Value	*P* Value	Mean	SD	*P* Value	Mean	SD	*P* Value	*P* Value
Goniometry																			
Flexion, deg		55.0	4.4	54.3	6.4	55.3	3.7	\.01[b]	55.7	5.9	.24	.04[b]	57.2	5.2	.14	57.1	8.6	.83	≤.99
Extension, deg		151.2	3.9	149.4	18.6	149.9	4.6	.95	152.1	7.6	\.01[b]	≤.99	151.1	3.5	.07	154.2	9.9	\.01[b]	.71
Thigh girth, cm		31.2	2.6	31.4	4.2	31.1	3.3	.94	31.8	2.7	\.01[b]	≤.99	31.1	2.9	.86	31.3	2.3	.07	≤.99
Pedometer, daily steps	1445.7	755.7	1432.8	468.7	829.5	931.266		.58	1086.9	1639.4	.47	≤.99	606.0	309.46	.15	937.7	885.5	.04[b]	≤.99
CMI																			
HVAS, 0-10		6.8	1.2	6.7	1.4	6.7	1.5	.48	7.0	1.2	\.01[b]	≤.99	6.8	1.2	.6	7.1	1.4	\.01[b]	.26
CBPI, 0-10																			
PSS		3.1	1.9	3.3	2.6	3.4	2.3	.69	2.9	1.8	.38	.53	3.7	2.8	.2	3.2	1.9	.09	.04[b]
PIS		3.2	2.2	3.3	2.8	3.4	2.1	.01[b]	3.2	1.9	.78	.02[b]	3.6	2.1	.01[b]	3.3	2.7	.64	.02[b]
COI																			
Stiffness, 0-16		3.4	3.4	3.9	3.9	4.1	3.3	.31	3.5	3.8	.11	.02[b]	4.1	3.2	.31	3.2	3.2	.09	.02[b]
Function, 0-16		3.6	4.1	4.2	4.7	4.1	4.0	.64	4.0	5.1	.91	≤.99	4.4	5.5	.72	3.8	3.8	.92	.35
Gait, 0-20		4.7	5.2	5.1	5.4	5.4	6.1	.29	5.4	4.1	.75	≤.99	5.8	4.3	.02[b]	5.4	5.1	.38	.02[b]
QOL, 0-12		4.5	2.6	4.5	3.6	4.6	2.7	.62	3.8	4.2	.23	.49	4.7	2.9	.06	3.9	3.9	.19	.18
Overall, 0-64		16.4	14.7	17.7	16.9	18.2	13.8	.22	16.8	18.9	.47	.02[b]	18.6	13.8	.14	17.0	17.5	.06	.02[b]
LOAD, 0-52		13.6	10.5	13.3	11.3	14.4	12.7	≤.99	14.3	10.3	.64	≤.99	14.3	10.7	.83	13.3	11.7	.11	.02[b]
Digital thermography, deg																			
DV		24.7	1.9	26.7	20.2	25.2	1.3	.01[b]	24.3	1.2	.75	≤.99	24.4	1.6	.61	23.9	1.6	.32	≤.99
DV max		26.3	1.9	26.1	1.7	25.8	1.0	.06	25.2	1.6	.76	.01[b]	26.7	1.6	.97	26.2	1.5	.21	.03[b]
Lt		28.7	2.7	29.3	2.8	31.6	2.1	\.01[b]	31.1	1.6	\.01[b]	\.01[b]	29.7	2.9	\.01[b]	30.00	2.2	\.01[b]	\.01[b]
Lt max		31.9	3.1	31.2	3.1	34.9	1.0	\.01[b]	34.7	1.0	\.01[b]	\.01[b]	34.9	0.8	\.01[b]	34.3	1.5	\.01[b]	\.01[b]
Synovial fluid																			
IL-1, pg/mL		170.9	120.4	174.9	134.6	72.3	42.4	\.01[b]	98.5	85.9	.04[b]	\.01[b]	—	—	—	—	—	—	—
CRP, mg/mL		0.4	1.0	0.5	1.6	0.3	1.2	\.01[b]	0.2	1.0	.02[b]	≤.99	—	—	—	—	—	—	—
Weightbearing																			
Symmetry index		24.7	20.3	22.6	12.4	18.7	17.1	.06	13.0	13.3	.01[b]	.01[b]	23.9	16.3	.18	10.2	5.4	\.01[b]	.03[b]
Deviation		2.8	3.6	2.1	2.2	2.78	1.987	.3	2.0	1.881	\.05[b]	.36	2.94	2.127	.47	1.21	975.0	\.01[b]	.11

[a]Dashes indicate parameters not measured. CBPI, Canine Brief Pain Inventory; CG, control group; CMI, clinical metrology instrument; COI, Canine Ortho- pedic Index; CRP, C-reactive protein; DV, dorsoventral view; HVAS, Hudson Visual Analog Scale; IL-1, interleukin 1; LOAD, Liverpool Osteoarthritis in Dogs; Lt, lateral view; max, maximal; PCG, platelet concentrate group (treatment); PIS, pain interference score; PSS, pain severity score; QOL, quality of life.
[b]*P* \ .05 (evaluation moment vs treatment day and between groups at each follow-up moment).

had lower mean and maximal thermographic values on the Lt view (*P* \ .01 for both). They also had higher body weight throughout the study (*P* \ .01).

Comparisons by Body Weight

When animals were compared with a weight cutoff set at the mean value of the sample, heavier dogs in the CG had higher PIS (*P* = .04) at the initial evaluation. At 8 days, heavier dogs had lower thermographic mean and maximal values on the DV view (*P* = .03 and *P* = .02, respectively) and lower thigh girth (*P* = .01), as well as bet- ter stiffness (*P* = .03), function (*P* \ .01), gait (*P* = .03), and COI (*P* \ .01). At 15 days, heavier dogs showed higher thigh girth (*P* = .04) and better HVAS (*P* = .04), stiffness, function, gait, QOL, and COI (*P* \ .01). Heavier animals had higher CRP concentrations at 30 days (*P* = .04) and worse HVAS scores (*P* = .02). The same animals had higher thigh girth (*P* \ .01) and IL-1 levels (*P* = .02) at 90 days. In the final evaluation point, heavier animals showed lower mean thermographic values on the DV view (*P* \ .01) and lower joint flexion (*P* = .02) and extension (*P* \ .01). In the PCG, animals with a weight above the cutoff had higher thigh girth (*P* = .02) at the initial evaluation. At 8 days, they had higher mean and maximal temperature val- ues on the DV view (*P* = .02, for both), higher thigh girth (*P* \ .01), and better joint extension (*P* = .01). After 15 days, heavier dogs had lower maximal temperature values on the Lt view (*P* = .01), higher thigh girth (*P* \ .01), and bet- ter joint extension (*P* = .02). At 30 days, these dogs had lower mean and maximal temperature values on the DV view (*P* \ .01 for both) and Lt view (*P* \ .01 for both), in addition to higher thigh girth (*P* \ .01). At the final evalu- ation moment, heavier dogs had lower maximal tempera- ture values on the Lt view (*P* = .01), higher thigh girth (*P* \ .01), and lower IL-1 concentration (*P* \ .01).

Comparisons by Age

When dogs above or below the mean age of the sample were considered, older dogs in the CG had lower maximal values on the thermographic Lt view (*P* = .04) and worse LOAD (*P* = .02), stiffness (*P* \ .01), function (*P* \ .01), gait (*P* \ .01), and COI (*P* \ .01). At 8 days, they showed higher SI (*P* \ .01) and lower maximal values on the thermographic Lt view (*P* = .02), as well as worse LOAD (*P* = .04), stiffness (*P* \ .01), function (*P* \ .01), gait (*P* \ .01), QOL (*P* \ .01), and COI (*P* \ .01). The same was also true at the 15-day evaluation, with these dogs presenting worse LOAD (*P* \ .01), stiffness (*P* \ .01), function (*P* \ .01), gait (*P* \ .01), QOL (*P* \ .01), and COI (*P* \ .01). At the 30-day evaluation, older dogs had higher mean and maxi- mal values on the thermographic DV view (*P* \ .01 and *P* = .02, respectively); higher mean values on the Lt view

TABLE 5
Parameters Evaluated at the Initial Evaluation and at 30, 90, and 180 Days[a]

	30 d							90 d							180 d						
	CG			PCG				CG			PCG				CG			PCG			
Modality	Mean	SD	*P* Value	Mean	SD	*P* Value	*P* Value	Mean	SD	*P* Value	Mean	SD	*P* Value	*P* Value	Mean	SD	*P* Value	Mean	SD	*P* Value	*P* Value
Goniometry																					
Flexion, deg	53.6	2.9	.11	53.0	4.1	.03[b]	\.05[b]	52.7	2.9	.02[b]	48.4	9.5	.01[b]	\.05[b]	51.6	2.2	\.001[b]	50.1	1.7	\.01[b]	.01[b]
Extension, deg	150.8	3.4	.06	151.7	5.9	\.01[b]	≤.99	150.8	2.9	.07	152.1	3.3	\.01[b]	≤.99	151.3	2.9	.17	152.7	3.2	\.01[b]	≤.99
Thigh girth, cm	30.6	2.7	.39	29.7	2.1	.09	≤.99	31.6	2.7	.54	30.3	9.1	.81	≤.99	31.5	2.2	.2	30.7	3.4	.66	≤.99
Pedometer, daily steps	594.5	663.375	.48	1086.9	1639.4	.01[b]	.15	451.9	463.0	.4	1238.0	994.685	.18	.06	434.9	455.8	.2	615.5	331.976	.26	.63
CMI																					
HVAS, 0–10	6.4	1.4	.14	6.9	1.6	\.01[b]	≤.99	6.6	1.7	.22	6.8	1.2	.21	≤.99	6.5	1.4	.04[b]	7.1	1.8	\.01[b]	≤.99
CBPI, 0–10																					
PSS	3.7	2.6	.03[b]	3.3	2.7	\.05[b]	.04[b]	4.1	2.9	.02[b]	3.5	2.2	\.01[b]	.04[b]	3.6	3.1	.02[b]	3.4	2.2	.03[b]	≤.99
PIS	3.8	2.6	.01[b]	3.2	2.7	.06	.04[b]	3.9	2.8	.01[b]	3.9	3.1	\.01[b]	≤.99	3.5	2.4	.01[b]	3.5	3.4	.04[b]	≤.99
Stiffness, 0–16	4.6	4.1	.87	3.7	3.8	.77	.04[b]	4.6	3.9	.33	4.3	3.7	.13	≤.99	4.0	5.7	.82	3.3	3.7	.21	≤.99
Function, 0–16	5.7	5.3	.2	3.7	4.4	.46	.02[b]	5.0	5.2	.21	3.3	2.8	.03[b]	.02[b]	4.0	5.4	≤.99	3.2	3.8	.24	.04[b]
Gait, 0–20	6.9	5.1	.19	3.3	2.7	.55	.02[b]	5.7	5.5	.11	5.4	5.6	.43	.04[b]	4.4	5.4	.87	4.6	6.2	.5	≤.99
QOL, 0–12	5.3	3.3	.39	4.4	4.1	.74	.18	5.1	2.8	.02[b]	4.3	3.8	.23	.19	4.7	2.6	.09	3.6	4.6	.87	.23
Overall, 0–64	22.4	19.1	.04[b]	16.7	18.3	.43	.04[b]	20.1	15.7	.29	19.0	18.4	.04[b]	.04[b]	15.7	14.9	.1	16.1	21.8	.47	≤.99
LOAD, 0–52	16.4	13.1	.22	13.4	12.2	.72	\.01[b]	13.1	12.4	.72	11.8	12.9	.33	.04[b]	13.1	12.4	.88	11.8	12.9	.983	≤.99
Digital thermography, deg																					
DV	25.3	1.5	.36	24.2	1.4	.6	≤.99	26.1	1.2	.04[b]	25.7	1.2	.09	.16	25.6	1.4	.89	25.8	1.1	.06	.47
DV max	25.2	2.1	.88	26.8	1.7	.98	≤.99	27.4	1.4	.14	27.1	1.0	\.05[b]	\.05[b]	26.9	1.4	.74	27.2	1.0	.02[b]	.06
Lt	29.8	2.2	\.01[b]	29.5	2.2	\.01[b]	\.01[b]	28.4	1.8	\.01[b]	27.9	1.3	.03[b]	\.01[b]	27.3	1.8	.21	28.1	1.6	\.01[b]	\.01[b]
Lt max	33.9	1.2	\.01[b]	32.9	2.4	\.01[b]	\.01[b]	30.5	1.9	\.01[b]	30.1	1.5	.06	\.01[b]	29.7	1.9	.13	30.9	1.8	\.01[b]	\.01[b]
Synovial fluid																					
IL-1, pg/mL	122.9	108.9	.05	147.9	129.5	.24	.53	159.6	59.1	.13	206.0	139.7	.7	≤.99	184.2	68.5	.25	167.9	76.2	.34	≤.99
CRP, mg/mL	0.48	0.9	.18	0.27	0.7	.37	≤.99	0.4	0.8	.36	0.23	0.6	.36	≤.99	0.0	0.0	.5	0.0	0.0	.4	≤.99
Weightbearing																					
Symmetry index	18.9	12.2	.04[b]	10.4	10.5	\.01[b]	.01[b]	27.4	12.1	.29	8.9	7.9	\.01[b]	\.05[b]	27.0	27.9	.51	11.8	18.6	.02[b]	.01[b]
Deviation	2.5	1.917	.2	1.71	1.59	.08	.14	2.72	2.27	.29	2.14	1.748	\.05[b]	≤.99	2.61	2.973	.55	2.36	3.954	.45	≤.99

[a]CBPI, Canine Brief Pain Inventory; CG, control group; CMI, clinical metrology instrument; COI, Canine Orthopedic Index; CRP, C-reactive protein; DV, dorsoventral view; HVAS, Hudson Visual Analog Scale; IL-1, interleukin 1; LOAD, Liverpool Osteoarthritis in Dogs; Lt, lateral view; max, maximal; PCG, platelet concentrate group (treatment); PIS, pain interference score; PSS, pain severity score; QOL, quality of life.
[b]*P* \ .05 (evaluation moment vs treatment day [Table 4] and between groups at each follow-up moment).

TABLE 6
Frequency of Radiographic Findings in the Control and Treatment Groups at the Initial and Final Evaluations[a]

Initial					Final					
CG			PCG		CG			PCG		
	No.	%	No.	%	No.	%	*P* Value	No.	%	*P* Value
Irregular wear on the femoral head, making it misshapen and with a loss of its rounded appearance	17	85	20	100	20	100	.08	20	100	≤.99
Flattened or shallow acetabulum with irregular outline	11	55	14	70	20	100	\.01[b]	18	90	\.01[b]
CCO	5	25	12	60	20	100	≤.99	10	50	\.01
New bone formation on the acetabulum and on the femoral head and neck	20	100	17	85	20	100	≤.99	18	90	.08
Angle formed at the cranial effective acetabular rim is worn away	18	90	14	70	20	100	.16	16	80	.10
Subchondral bone sclerosis along the cranial acetabular edge	19	95	19	95	20	100	.32	18	90	.32
CFHO	3	15	5	25	20	100	.18	18	90	.23
Frog leg view										
CCO	2	10	9	45	20	100	\.01[b]	8	40	.71
CFHO	18	90	18	90	20	100	.16	18	90	≤.99

[a]CCO, caudolateral curvilinear osteophyte; CFHO, circumferential femoral head osteophyte; CG, control group; PCG, platelet concentrate group (treatment).
[b]*P* \ .05.

(*P* = .02); and worse joint flexion (*P* = .01), LOAD (*P* \ .01), stiffness (*P* \ .01), function (*P* \ .01), gait (*P* \ .01), QOL (*P* \ .01), and COI (*P* \ .01). At 90 days, the same dogs had worse LOAD (*P* = .04), stiffness (*P* \ .01), function (*P* \ .01), gait (*P* \ .01), QOL (*P* \ .01), and COI (*P* \ .01). At the final evaluation, older dogs had worse deviation (*P* = .03), SI (*P* \ .01), stiffness (*P* \ .01), function (*P* \ .01), gait (*P* \ .01), QOL (*P* \ .01), and COI (*P* \ .01). In the

PCG, older dogs had worse PSS ($P \leq .01$), PIS ($P \leq .01$), stiffness ($P = .04$), function ($P \leq .01$), and COI ($P \leq .01$) at the initial evaluation point. At 8 days, they had lower mean and maximal thermography values on the DV view ($P \leq .01$ for both) and worse joint extension ($P \leq .01$), PIS ($P = .01$), LOAD ($P \leq .01$), function ($P = .01$), gait ($P \leq .01$), and COI ($P = .01$). At 15 days, they had higher maximal temperature values on the Lt view ($P = .04$) and worse PSS ($P \leq .01$) and PIS ($P \leq .01$). At the 30-day evaluation, older dogs had worse PSS ($P = .01$), PIS ($P \leq .01$), LOAD ($P = .04$), and function ($P \leq .01$). At 90 days, they had worse PSS ($P = .04$), PIS ($P = .03$), and gait ($P = .05$). At the final evaluation, these dogs had lower serum CRP levels ($P \leq .01$) and worse PSS, PIS, LOAD, stiffness, function, gait, QOL, and COI ($P \leq .01$ for all).

DISCUSSION

OA is the most commonly diagnosed joint disease in human and veterinary medicine.[2] To our knowledge, this is the first study to describe the effect of a single injection of platelet concentrate on several clinical, imaging, and laboratory signs in a naturally occurring canine model with a long follow-up period.

Previous reports in dogs have pointed out that a single intra-articular injection of platelet products has resulted in clinical improvements for 12 weeks.[1,15] Multiple-injection protocols that provide improvements in ROM, pain, lameness, and kinetics have also been described.[12] We observed significant functional improvements in the PCG, reflected in better limb weightbearing as measured through the SI and deviation from the normal value. These improvements were present up to the last evaluation moment, at 180 days, in line with previous reports that have pointed out the ability of platelet concentrates to improve weightbearing in dogs with OA.[15,19]

It is well-established that OA is a complex low-grade inflammatory process that affects the progression of the disease, with IL-1 considered the most important proinflammatory cytokine responsible for the catabolism in OA.[20,31,36] Although a decrease in IL-1 levels was recorded at 8 days in both groups, concentration in the PCG was still significantly lower than that in the CG. The platelet concentrate was able to significantly reduce the levels of this pivotal cytokine, and the decrease observed in the CG was probably due to the removal of synovial fluid at treatment day, followed by the injection of 0.9% NaCl, similar to the effect of a joint lavage. The anti-inflammatory activity of platelet concentrate has been associated with a positive response to treatment rather than any effect on tissue anabolism or catabolism.[12] This reduction of IL-1 may produce a reduction in inflammatory levels, which in turn are reflected in the temperature values recorded during the thermographic evaluations. Measurements made on the Lt view, in particular, recorded variations throughout the entire follow-up period, similar to what was observed with other evaluation modalities.

Pain is the most relevant clinical sign of OA and a hallmark of the disease.[41] Pain and functional ability are the most important parameters in the evaluation of OA treatment efficacy, with canine studies offering valuable data that may translate to humans.[16,45,56] We used several CMIs to try to capture multiple dimensions of OA, and we observed significant improvements with several of them—in some cases, up to the last evaluation moment. A previous report has described similar long-lasting effects of a single administration of platelet concentrate,[1] but further studies should be done to evaluate if different treatment frequencies can lead to better results. As expected, some improvements were also observed in the CG in the initial evaluations when compared with treatment day probably because of the reduction of inflammatory cytokines within the joint. Still, these effects were not as marked or as long lasting as in PCG.

Radiographic evaluation is a staple of OA monitoring, and CCO and CFHO represent early radiographic signs that predict the development of the clinical signs of hip OA.[33,42] Previous reports have described that a platelet concentrate was able to halt the progression of radiographic signs in treated dogs.[3,15,47] In this study, we observed similar results, with increased frequency of radiographic findings observed in the CG in contrast to what was observed in the PCG, where the frequency of CCO decreased. It was interesting to note that, even though dogs in the PCG showed significant improvements, those that presented CCO in either group had worse CMI scores and measures of limb function and higher thermographic evaluations.

Risk factors for OA include higher body weight and age .8 years.[2] We applied different cutoff values for weight to assess its effect on clinical presentation and in response to treatment. For both groups, increasing body weight generally corresponded to worse clinical and laboratory findings. The exception was SI evaluation above the highest cutoff considered. This may reflect a weight shift from the pelvic to thoracic limbs rather than a real improvement, which should be addressed in further studies. Similar effects were observed for animals above the mean age of the group, with these dogs scoring worse on almost all CMI scores. Since OA is a chronic progressive disease, it was not unexpected to observe that older dogs had worse evaluations, which may be linked with the progression of the disease at its clinical signs.

In humans, platelet concentrates can produce local and transient side effects, such as injection pain and local inflammation, that take 2 to 10 days to resolve.[27,39,40] Similarly, we observed increased lameness in 8 dogs that spontaneously resolved within 48 hours, and this had no effect at the 8-day evaluation. No other side effects were observed. No additional medication was administered to the animals during the follow-up period.

CONCLUSION

To our knowledge, this is the first study to describe the effect of a single injection of platelet concentrate on several clinical, imaging, and laboratory signs in a naturally occurring canine model with a long follow-up period. It provides

important information for the characterization of the effects of this treatment modality of growing interest.

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Quartile 1

Effect of a single intra-articular administration of stanozolol in a naturally occurring canine osteoarthritis model: a randomized trial

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Abstract

To describe the effect of the intra-articular administration of stanozolol in a naturally occurring canine OA model.

Forty canine (N=40) hip joints were randomly assigned to receive stanozolol or saline (control). On treatment day and at 8, 15, 30, 90, and 180 days post-treatment, weight distribution, joint range of motion, thigh girth, digital thermography, radiographic signs, synovial fluid interleukin-1, and C-reactive protein levels were evaluated. Data from four Clinical Metrology Instruments was also gathered. Results were compared with Repeated Measures ANOVA, with a Huynh-Feldt correction, paired-samples t-test, or Wilcoxon signed-rank test, with $p<0.05$.

OA was graded as mild (90%), moderate (5%), and severe (5%). Patients of both sexes, with a mean age of 6.5±2.4 years and a bodyweight of 26.7±5.2kg, were included. No differences were found between groups at treatment day in all considered evaluations. Weight distribution showed significant improvements with stanozolol from 15 days ($p<0.05$) up to 180 days ($p<0.01$). Lower values during thermographic evaluation in both views taken and improved joint extension at 90 ($p=0.02$) and 180 days ($p<0.01$) were observed. Pain and function scores improved up to 180 days. In the control group, radiographic signs progressed, in contrast with stanozolol.

The use of stanozolol was safe and produced significant improvements in weight-bearing, pain score, and clinical evaluations in a naturally occurring canine OA model.

Keywords: Animal model; Canine; Osteoarthritis; Pain; Stanozolol.

Introduction

Osteoarthritis (OA) is a disease spanning all species of mammals. It is particularly important in humans and dogs, being a source of chronic pain and posing a significant burden to societies. Since it has such a significant toll on the quality of life, it implies a considerable cost in healthcare. Its prevalence is expected to rise due to a simultaneous increase in life expectancy and obesity[1–4]. The pathologic process, clinical presentation, and response to treatment in dogs are very similar to those in humans, where degenerative, trauma, and overuse aetiologies occur, making dogs a frequent animal model for the study of OA[5]. The naturally occurring canine model, in particular, provides substantial benefits in comparison to other models. It presents a foreshortened lifespan while maintaining the same life stages of human disease. Also, dogs share many environmental conditions with humans, specifically those that influence human OA. For those reasons, the naturally occurring canine model is easier to study and considered the closest to a gold standard[5–12]. The study of canine OA can provide important insight into the disease in a translational approach under the One Medicine initiative and improve the health and well-being of humans and dogs[11,13].

OA is still an incurable condition, and the medical approach to its treatment aims at slowing disease progression while relieving symptoms, particularly pain, but treatment options are still limited[11,14–16]. Stanozolol is a synthetic derivative of testosterone, and its properties include anabolic/androgenic activity, probably associated with its affinity for androgenic and, at lower doses, glucocorticoid receptors[17]. It has a high androgenic potential, but its long-term use has not induced activity and aggressivity changes in mice[18]. An anti-catabolic effect potentiates stanozolol's anabolic effect at the glucocorticoide receptor level, where it behaves as a competitive antagonist of the catabolic corticosteroids[19]. In vitro human studies and ovine and equine models have described that stanozolol was able to induce fibroblasts, to increase collagen production in a dose-dependent pathway through transforming growth factor-1β synthesis while decreasing nitric oxide production and stimulating the autocrine secretion of insulin-like growth factor-1, which induces osteoblast proliferation and collagen synthesis[20–23]. In humans, an increase of transforming growth factor-1β synthesis is related to a decrease in articular pain[24]. It also demonstrated chondroprotective effects through the downregulation of genes for pro-inflammatory/catabolic cytokines and enzymes associated with OA in equine in vitro chondrocytes[25]. In an ovine surgical model of OA, intra-articular stanozolol was able to preserve the stifle joint's gross anatomy, reducing osteophyte formation, subchondral bone reaction, and promoting articular cartilage regeneration, at 3 and 9 months post-surgery[21]. In dogs, a 0.3mg/kg dose has been described for intra-articular administration, in the management of knee OA, and oral use to treat tracheal collapse[26,27]. Before evaluating multiple administrations, as described in other animal models[21,23], the assessment of a single administration of stanozolol is required to determine treatment safety its effect following intra-articular administration.

This study aims to compare the effect of stanozolol to a control group in the management of OA in a naturally occurring canine model, using several outcome assessment modalities. We hypothesize that stanozolol is able to reduce pain levels improve function in OA joints compared to a control group.

Materials and Methods

This project was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval nº GD/32055/2018/P1, September 25th, 2018), and complies with the ARRIVE reporting guidelines. Written, informed consent was obtained from the Institution responsible for the animals (Guarda Nacional Republicana, Portuguese Gendarmerie). The sample comprised forty (N=40) joints of twenty active Police working dogs with bilateral hip OA. It constituted a convenience sample, similar in size to other reports evaluating OA in canine models28–30. The diagnosis was based on history (difficulty rising,

jumping, and maintaining obedience positions, stiffness, and decreased overall performance), physical examination (pain during joint mobilization, stiffness, and reduced range of motion), and radiographic findings (Orthopedic Foundation for Animals hip scores of mild, moderate or severe). Additional inclusion criteria comprised bodyweight ≥20kg, age >2 years, and a period >6 weeks without receiving any medication or nutritional supplements. All inclusion criteria had to be met for the animal to be included in the study. All animals were submitted to a physical, orthopedic, neurological examination, complete blood count, and serum biochemistry. Cases of suspected or documented orthopaedic, neurological, or concomitant disease were excluded. For this prospective, longitudinal, double-blinded, randomly-controlled study, patients were randomly assigned with the statistical analysis software to a control group (CG, n=20) or a treatment group (SG, n=20). In SG, an intra-articular (IA) administration of stanozolol (Estrombol, Laboratório Fundacion) at a 0.3mg/kg dose was administered, while CG received 2ml of 0.9%NaCl, given IA. Both joints received the same substance, according to the assigned group.

Weight-bearing evaluation

The weight distribution evaluation was performed with a weight distribution platform (Companion Stance Analyzer; LiteCure LLC®, Newark, Delaware, United States). Conducted procedures followed the manufacturer's guidelines and included placing the equipment in the centre of a room, at least 1 meter from the walls, calibrating it at the beginning of each day, and zeroing it before each data collection. The evaluation itself was conducted with the animal placing one foot on each quadrant of the platform. The patient's head was kept facing forward. The left-right symmetry index (SI) was calculated with the following formula: SI=[(WBR-WBL)/((WBR+WBL)x0.5)]x100[31,32], where WBR is the value of weight-bearing for the right pelvic limb, and WBL is the value of weight-bearing for the left pelvic limb. Negative values were made positive. Since normal weight-bearing for the pelvic limb is 20%[33], we also considered the deviation from this value, calculated by subtracting weight-bearing to 20.

Digital thermography evaluation

For collecting digital thermography images, animals were kept for 30 minutes in a controlled temperature room, with the temperature set at 21ºC. Patients were then placed in an upright standing position, as symmetrical as possible. A dorsoventral image was obtained, including the last lumbar to the first coccygeal vertebrae area, at a distance of 60 cm[34]. From the same position, a lateral view was also obtained, with the greater trochanter at the centre, at the same distance. All images were taken with FLIR ThermaCAM E25® model (FLIR Systems, Wilsonville, Oregon, United States). Thermograms were analyzed with free software (Tools, FLIR Systems, Inc), using a rainbow color

pallet. Mean and maximal temperatures were determined by placing boxes of equal size on the hip joint's anatomical area on both views.

Clinical evaluation

With the patient in lateral recumbency, with the affected limb uppermost, thigh girth was determined with a Gullick II measuring tape, at a distance of 70% thigh length, measured from the tip of the greater trochanter, with an extended leg[35]. Hip joint range of motion was then determined with a goniometer at extension and flexion with a flexed stifle[36]. Pedometers (Xiaomi wrist pedometer) were used to measure the patient's activity levels. They were worn around the patient's neck, attached to an adjustable lightweight collar[37], for a week before the first evaluation moment to determine a baseline value and then maintained up to the 30th-day post-treatment. For the 90th and 180th post-treatment days evaluation, the pedometer was placed a week before the evaluation moment. Mean daily counts were considered, calculated by dividing the register number of steps by the number of days considered.

Radiographic evaluation

For the IA administrations and radiographic examination, patients were placed under light sedation through the intravenous administration of a combination of medetomidine (0.01mg/kg) and buthorphanol (0.1mg/kg). A ventrodorsal extended legs and frog-leg views were obtained during radiographic examination. In the ventrodorsal view, the presence of several radiographic findings was considered: misshapen femoral head with a loss of its rounded appearance; a flattened or shallow acetabulum, with an irregular outline; CCO; new bone formation on the acetabulum and femoral head and neck; a worn away angle formed at the cranial effective acetabular rim; subchondral bone sclerosis along the cranial acetabular edge and CFHO[38–41]. In the frog-leg view, the presence of CCO and CFHO was also recorded.

Treatment administration, synovial fluid collection, and evaluation

Patients were positioned in lateral recumbency, with the affected joint uppermost. A small window of 4x4cm area surrounding the greater trochanter was clipped and aseptically prepared. An assistant placed the limb in a neutral, parallel to the table position. A 21-gauge 2.5" length needle was introduced just dorsal to the greater trochanter and perpendicular to the limb's long axis until the joint was reached[42]. Confirmation of correct needle placement was obtained by collecting synovial fluid, and the treatment or saline was administered. The syringes containing the substance to be administrated were prepared by a different researcher and covered to hyde the substance's characteristics and keep the treatment administrator blinded to the treatment. A sample of synovial fluid was saved for the determination of interleukin-1β (IL 1β). C-reactive protein (CRP) concentrations were made using the DuoSet Ancillary Canine IL-1β Reagent kit (R&D Systems,

United Kingdom), read with a FLUOstar OPTIMA (BMG Labtech). Fuji Dri-Chem Slides VC-CRP PS (FUJIFILM Europe GmbH), read with a DRIChem NX500i (FUJIFILM Europe GmbH). Additionally, dogs' trainers completed a copy of HVAS, CBPI, COI, and LOAD after receiving the published instructions for each of them. They were completed in sequence by the same trainer in a quiet room with as much time as needed to answer all items.

After treatment, animals were rested for three consecutive days and resumed their regular activity over five days. Signs of exacerbated pain, persistent stiffness of gait, and changes in posture exhibited by the dogs, were evaluated by the veterinarian on days 1 and 3 after the IA administration. If no complaints were registered, the animal could resume its normal activity[43,44]. Evaluations were conducted on days 0 (treatment day), 8, 15, 30, 90, and 180. An outline of all procedures at each moment is presented in table 1. The same researcher, blinded to the animal's assigned group and identification and moment of evaluation, performed all assessments. For the radiographic and digital thermography evaluation, all personal information was removed before the evaluation. After the study, all patients remained in active Police work.

Statistical analysis

Normality was assessed with a Shapiro-Wilk test. Groups' results were compared in each evaluation moment, and each measured parameter was compared with the result observed on treatment day. Results were compared with a Paired samples t-test, Repeated Measures ANOVA, with a Huynh-Feldt correction, or Wilcoxon signed-ranks test to assess the effect of different parameters on the patients' clinical evolution. A Kaplan-Meier test was performed to evaluate the time to return to baseline values of SI and CMI scores, compared with the Breslow test. With the CBPI, a specific measure of success has been defined and set as a reduction of ≥ 1 in PSS and ≥ 2 in PIS[45]. For that reason, the Kaplan-Meier test was used to evaluate the time for the score to drop below this reduction level in these scores. All results were analysed with IBM SPSS Statistics version 20 (IBM Corporation, New York, USA), and a significance level of $p<0.05$ was set.

Table 1 – Procedures conducted at each moment. Days are counted from treatment day. Legend: CBPI – Canine Brief Pain Inventory; COI – Canine Orthopedic Index; CRP – C-Reactive Protein; HVAS – Hudson Visual Analogue Scale; IL-1 – Interleukin 1; LOAD – Liverpool Osteoarthritis in Dogs; SF – Synovial fluid.

Modality	**Evaluation moment**					
	0 treatment day	**8**	**15**	**30**	**90**	**180**
Stance analysis	X	X	X	X	X	X
Digital Thermography	X	X	X	X	X	X
Pedometer	X	X	X	X	X	X
Goniometry	X	X	X	X	X	X
Thigh girth measurement	X	X	X	X	X	X
Digital radiography	X			X	X	X
Treatment	X					
SF CRP	X	X		X	X	X
SF IL-1	X	X		X	X	X
HVAS	X	X	X	X	X	X
CBPI	X	X	X	X	X	X
COI	X	X	X	X	X	X
LOAD	X	X	X	X	X	X

Normality was assessed with a Shapiro-Wilk test. Groups' results were compared in each evaluation moment, and each measured parameter was compared with the result observed at treatment day. Results were compared with a Paired samples t-test, Repeated Measures ANOVA, with a Huynh-Feldt correction, or Wilcoxon signed-ranks test to assess the effect of different parameters on the patients' clinical evolution. A Kaplan-Meier test was performed to evaluate the time to return to baseline values of SI and CMI scores, compared with the Breslow test. With the CBPI, a specific measure of success has been defined and set as a reduction of ≥1 in PSS and ≥2 in PIS44. For that reason, the Kaplan-Meier test was used to evaluate the time for the score to drop below this reduction level in these scores. All results were analysed with IBM SPSS Statistics version 20 (IBM Corporation, New York, USA), and a significance level of $p<0.05$ was set.

Results

The sample included 40 joints of both intact males (n=22, in 12 CG and 10 in SG) and females (n=18, in 8 CG and 10 in SG) Police working dogs with a mean age of 6.5±2.4 years and bodyweight of 26.7±5.2kg. Dogs were of breeds commonly employed in police forces, similarly distributed between CG and SG: German Shepherd Dogs (n=12, 6 in CG and 6 in SG), Labrador Retriever (n=12, 6 in CG and 6 in SG), Belgian Malinois Shepherd Dogs (n=10, 6 in CG and 4 in SG), and Dutch Shepherd Dog (n=6, 4 in CG and 2 in SG). At the initial evaluation, OA was classified as mild in 36

joints (90%, in 18 CG and 18 in SG), moderate as 2 (5%, all in CG), and severe as 2 (5%, all in SG), according to the Orthopedic Foundation for Animals hip grading scheme46. Levene's test for homogeneity was used to control baseline values, and no differences were found between groups at the initial evaluation. All patients were evaluated in all assessment moments. Increased lameness was observed in four cases of the stanozolol group following administration, which spontaneously resolved within a few days.

Values recorded in stanozolol and control groups for different evaluations made throughout the study, are presented in table 2. Comparing results between groups with repeated measures ANOVA with a Huynh-Feldt correction, significant differences between groups were found concerning deviation ($F(4.4,140.1)=11.2$, $p<0.01$), SI ($F(3.8,121.5)=6.2$, $p<0.01$) , mean temperature on a DV view ($F(3.8,107.8)=4.6$, $p=0.002$), maximal temperature on a DV view ($F(3.4,95.1)=3.7$, $p=0.011$), mean temperature on a Lt view ($F(5,150)=37.1$, $p<0.001$), maximal temperature on a Lt view ($F(3.9,118.2)=123.7$, $p<0.001$), thigh girth ($F(5,170)=6.7$, $p<0.001$) joint extension ($F(3.6,107.5)=171.3$, $p<0.001$), joint flexion ($F(5,170)=15.9$, $p<0.001$) and IL-1 synovial concentration ($F(1.8,64.5)=7.4$, $p=0.002$). Evolution of SI is presented in figure 1 and Kaplan Meier curves are presented in figure 2.

Significant differences were also observed with different CMI, specifically PSS ($F(3.8,124.1)=2.6$, $p=0.044$), PIS ($F(3.7,117.6)=3.9$, $p=0.007$), LOAD ($F(2.5,81.3)=3.3$, $p=0.03$), Function ($F(2.9,93.9)=2.8$, $p=0.048$) and Gait ($F(5,160)=2.6$, $p=0.026$). Kaplan Meier curves for LOAD are presented in figure 3.

The frequency of different radiographic findings at the initial and final evaluations is presented in table 3. The time to return to baseline values for SI and CMIs, calculated with Kaplan-Meier estimators, is shown in table 4.

Table 2 – Mean values (±standard deviation) of different parameters evaluated throughout the study. CBPI – Canine Brief Pain Inventory; CRP – C-reactive protein; COI – Canine Orthopedic Index; DV – dorsoventral view; HVAS – Hudson Visual Analogue Scale; IL-1 – Interleukin 1; LOAD – Liverpool Osteoarthritis in Dogs; LT – lateral view; PIS – Pain Interference Score; PSS – Pain Severity Score; QOL – Quality of Life. * indicates significance when comparing both groups at each follow-up moment.

Parameters		Treatment day				8 days					15 days				
		Control		Stanozolol		Control		Stanozolol		p	Control		Stanozolol		p
		mean	SD	mean	SD	mean	SD	mean	SD		mean	SD	mean	SD	
Goniometry	Flexion (°, mean±SD)	55,0	4.4	56.6	3.7	55.3	3.7	55.9	4.5	1,00	57.2	5.2	56.2	3.2	1,00
	Extension (°, mean±SD)	151.2	3.9	156.9	6,0	149.9	4.6	156.9	6,0	<0.01	151.1	3.5	115.1	6.4	1,00
	Thigh girth (cm, mean±SD)	31.2	2.6	29.1	1.9	31.1	3.3	29.1	2.1	1,00	31.1	2.9	29.8	2,0	1,00
	Pedometer (daily steps±SD)	1445.7	755.7	910.9	811.2	829.5	931.3	1165.2	684.5	1,00	606,0	309.5	1043.2	733.1	0.43
CMI	HVAS (0-10)	6.8	1.2	6.7	1.3	6.7	1.5	6.6	1.4	1,00	6.8	1.2	7.1	0.8	1,00
	CBPI - PSS (0-10)	3.1	1.9	2.9	1.5	3.4	2.3	3.1	2.3	0.53	3.7	2.8	1.9	2.1	0.04*
	CBPI - PIS (0-10)	3.2	2.2	2.3	1.7	3.4	2.1	2.9	1.9	0.02*	3.6	2.1	1.9	1.2	0.01*
	COI - Stiffness (0-16)	3.4	3.4	4,0	2.8	4.1	3.3	2.3	2.3	0.56	4.1	3.2	1.5	1.9	0.02*
	COI - Function (0-16)	3.6	4.1	4,0	3.6	4.1	4,0	1.8	2.1	<0.01*	4.4	5.5	0.9	1.4	<0.01*
	COI - Gait (0-20)	4.7	5.2	5.2	3.9	5.4	6.1	3.1	3.4	1,00	5.8	4.3	1.8	2.9	0.02*
	COI - QOL (0-12)	4.5	2.6	4.3	2.5	4.6	2.7	4,0	2.2	1,00	4.7	2.9	3.3	2.3	1,00
	COI - Overall score (0-64)	16.4	14.7	17.5	12.4	18.2	13.8	11.2	9,0	0.7	18.6	13.8	7.5	7.4	0.29
	LOAD (0-52)	13.6	10.5	8.2	5.2	14.4	12.7	11.1	7.2	0.17	14.3	10.7	11.1	7.2	0.02*
Digital Thermography	DV (°, mean±SD)	24.7	1.9	25.1	1.9	25.2	1.3	23.7	1.9	0.04*	24.4	1.6	24.2	1.4	1,00
	DV max (°, mean±SD)	26.3	1.9	25.9	1.9	25.8	1,0	25.5	1.9	1,00	26.7	1.6	26.1	2.6	1,00
	Lt (°, mean±SD)	28.7	2.7	25.8	2.2	31.6	2.1	30.1	2,0	<0.01*	29.7	2.9	29.4	2.4	<0.01*
	Lt max (°, mean±SD)	31.9	3.1	27.6	2.1	34.9	1,0	34.7	1.2	<0.01*	34.9	0.8	34.8	1.1	<0.01*
Synovial fluid	IL-1 (pg/mL, mean±SD)	170.9	120.4	155,0	145.5	72.3	42.4	92.8	81.9	<0.01*	-	-	-	-	-
	CRP (mg/mL, mean±SD)	0.4	1,0	0.3	0.3	0.3	1.2	0.2	0.3	0.35	-	-	-	-	-
Weight-bearing	Symmetry Index (mean±SD)	24.7	20.3	24.1	13.9	18.7	17.1	21.6	16.4	1,00	23.9	16.3	24.7	18.3	1,00
	Deviation (mean±SD)	2.8	3.6	4.25	3.5	2.78	1.987	2.65	1.8	0.71	2.94	2.127	2.31	1.9	<0.05*

Table 2 (cont.) – Mean values (±standard deviation) of different parameters evaluated throughout the study. CBPI – Canine Brief Pain Inventory; CRP – C-reactive protein; COI – Canine Orthopedic Index; DV – dorsoventral view; HVAS – Hudson Visual Analogue Scale; IL-1 – Interleukin 1; LOAD – Liverpool Osteoarthritis in Dogs; LT – lateral view; PIS – Pain Interference Score; PSS – Pain Severity Score; QOL – Quality of Life. * indicates significance when comparing both groups at each follow-up moment.

Parameters		30 days					90 days					180 days				
		Control		Stanozolol		p	Control		Stanozolol		p	Control		Stanozolol		p
		mean	SD	mean	SD		mean	SD	mean	SD		mean	SD	mean	SD	
Goniometry	Flexion (°, mean±SD)	53.6	2.9	55.4	5.3	1,00	52.7	2.9	55.8	5.5	0.02*	51.6	2.2	50.9	1.8	<0.01*
	Extension (°, mean±SD)	150.8	3.4	153.2	4.6	0.07	150.8	2.9	154.8	2.9	0.59	151.3	2.9	155,0	3,0	0.04*
	Thigh girth (cm, mean±SD)	30.6	2.7	28.9	1.4	1,00	31.6	2.7	32.5	2.7	1,00	31.5	2.2	29.8	1.9	1,00
	Pedometer (daily steps±SD)	594.5	663.4	869.2	1091.5	0.58	451.9	463.0	440,0	455.3	0.36	434.9	455.8	588.33	788.3	0.14
CMI	HVAS (0-10)	6.4	1.4	7.1	1.3	1,00	6.6	1.7	6.6	1.3	1,00	6.5	1.4	6.9	1.2	1,00
	CBPI - PSS (0-10)	3.7	2.6	2.4	1.9	<0.05*	4.1	2.9	2.9	1.9	0.04*	3.6	3.1	2.6	1.8	0.02*
	CBPI - PIS (0-10)	3.8	2.6	2.4	1.8	<0.01*	3.9	2.8	2.4	1.8	0.02*	3.5	2.4	2.5	1.7	1,00
	COI - Stiffness (0-16)	4.6	4.1	1.8	2.2	0.03*	4.6	3.9	2.1	2.2	0.58	4,0	5.7	1.5	2.5	0.41
	COI - Function (0-16)	5.7	5.3	0.9	1.6	<0.01*	5,0	5.2	1.6	1.8	<0.01*	4,0	5.4	1.2	2.1	<0.01*
	COI - Gait (0-20)	6.9	5.1	2.2	3,0	<0.02*	5.7	5.5	3.2	4.4	<0.05*	4.4	5.4	2.5	3.7	<0.05*
	COI - QOL (0-12)	5.3	3.3	2.8	2,0	1,00	5.1	2.8	2.7	2.1	1,00	4.7	2.6	2.4	1.2	1,00
	COI - Overall score (0-64)	22.4	19.1	7.6	8.2	0.13	20.1	15.7	9.6	9.2	0.16	15.7	14.9	7.6	9.2	0.14
	LOAD (0-52)	16.4	13.1	6.4	6.5	<0.01*	13.1	12.4	7.1	6.9	<0.01*	13.1	12.4	7.6	7.1	<0.01*
Digital Thermography	DV (°, mean±SD)	25.3	1.5	25.2	2.9	1,00	26.1	1.2	25.6	1.1	1,00	25.6	1.4	25.8	1.5	1,00
	DV max (°, mean±SD)	25.2	2.1	26.7	2.8	1,00	27.4	1.4	26.9	1.3	0.04*	26.9	1.4	25.9	1.5	0.02*
	Lt (°, mean±SD)	29.8	2.2	29.9	2.2	<0.01*	28.4	1.8	28.7	1.9	<0.01*	27.3	1.8	28.3	2.1	<0.01*
	Lt max (°, mean±SD)	33.9	1.2	34.5	0.9	<0.01*	30.5	1.9	31.1	2.2	<0.01*	29.7	1.9	30.1	2.3	<0.01*
Synovial fluid	IL-1 (pg/mL, mean±SD)	122.9	108.9	122.6	96.4	0.58	159.6	59.1	139.8	57.2	1,00	184.2	68.5	165.5	64.2	1,00
	CRP (mg/mL, mean±SD)	0.48	0.9	0.7	2,0	1,00	0.4	0.8	0.3	0.7	1,00	0,0	0,0	0.1	0.4	1,00
Weight-bearing	Symmetry Index (mean±SD)	18.9	12.2	5.6	7.2	<0.01*	27.4	12.1	11,0	6.9	<0.01*	27.0	27.9	6.9	7.3	0.01*
	Deviation (mean±SD)	2.5	1.917	1.31	1.2	0.03*	2.72	2.27	1.85	2.8	0.7	2.61	2.973	2.3	3.2	<0.01*

Table 3 – Frequency of radiographic findings in the Control and Treatment Groups, at the initial and final evaluations

Radiographic finding	T0				180d				
	Control		Stanozolol		Control		Stanozolol		
	Absolut	%	Absolut	%	Absolut	%	Absolut	%	p
Irregular wear on the femoral head. making it misshapen and with a loss of its rounded appearance	17	85%	20	100%	20	100%	18	90%	1,00
Flattened or shallow acetabulum. with irregular outline	11	55%	10	50%	20	100%	16	80%	<0.05*
Caudolateral curvilinear osteophyte (CCO)	5	25%	5	25%	20	100%	15	75%	<0.05*
New bone formation on the acetabulum and on femoral head and neck	20	100%	16	80%	20	100%	18	90%	0.16
The angle formed at the cranial effective acetabular rim is worn away	18	90%	15	75%	20	100%	18	90%	0.32
Subchondral bone sclerosis along the cranial acetabular edge	19	95%	20	100%	20	100%	18	90%	1,00
Circumferential femoral head osteophyte (CFHO)	3	15%	9	45%	20	100%	10	50%	0.71

Table 4 – Time to return to baseline values for SI and CMIs, calculated with Kaplan-Meier estimators and compared with the Breslow test. * indicates significance.

Parameters	Breslow test	Treatment			
		Control		Stanozolol	
		mean±SD	95% CI	mean±SD	95% CI
Simmetry Index	0.022*	47.0±11.8	23.8±70.2	94.2±15.9	62.9±125.4
HVAS	0.000*	48.7±12.4	25.4±73.9	129.8±13.4	103.5±156.1
PSS	0.089	63.2±17.2	29.6±96.8	94.6±16.4	62.5±126.7
PIS	0.000*	8.4±0.4	7.7±9.0	109.6±17.3	75.8±143.2
LOAD	0.000*	40.7±10.6	19.9±61.4	123.8±14.2	95.9±151.6
Stiffness	0.019*	64.7±16.9	31.4±97.9	111.2±15.9	80.6±142.9
Function	0.003*	65.4±13.4	39.2±91.6	124.5±15.4	94.2±154.8
Gait	0.028*	52.7±14.6	23.9±81.4	103.6±15.7	72.8±134.4
QOL	0.656	60.9±15.0	31.4±90.4	66.2±17.5	31.8±100.6
COI	0.122	52.7±13.4	26.5±78.9	78.1±14.0	50.6±105.6

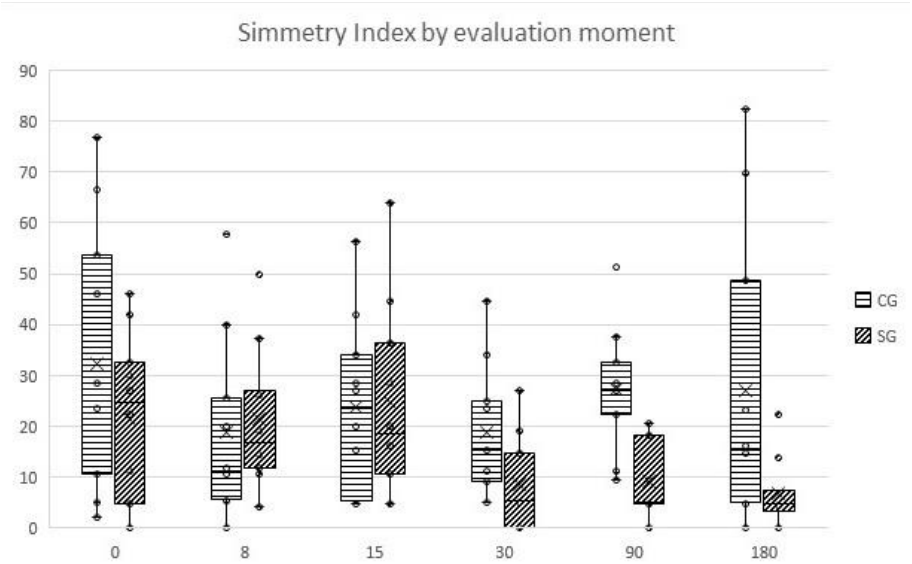

Figure 1 - Overall evolution of Symmetry Index in the control group and treatment group. Box plots represent the median, 25th and 75th percentiles, and whiskers represent 10th and 90th percentiles.

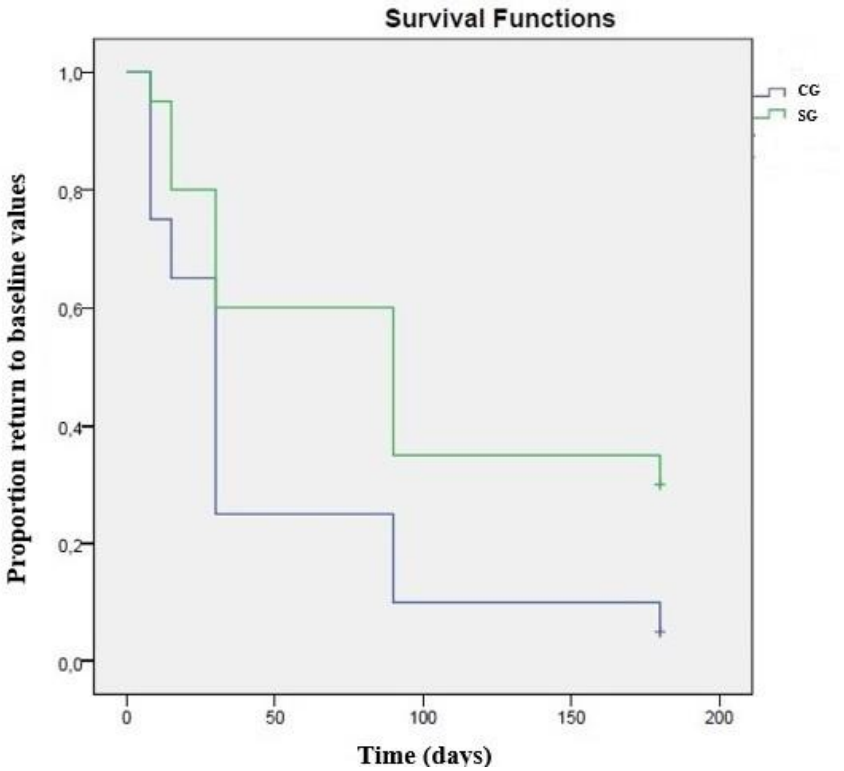

Figure 2 - Kaplan-Meier curve demonstrating a significant difference between stanozolol group and control group in time for Symmetry index (SI) to return to baseline values.

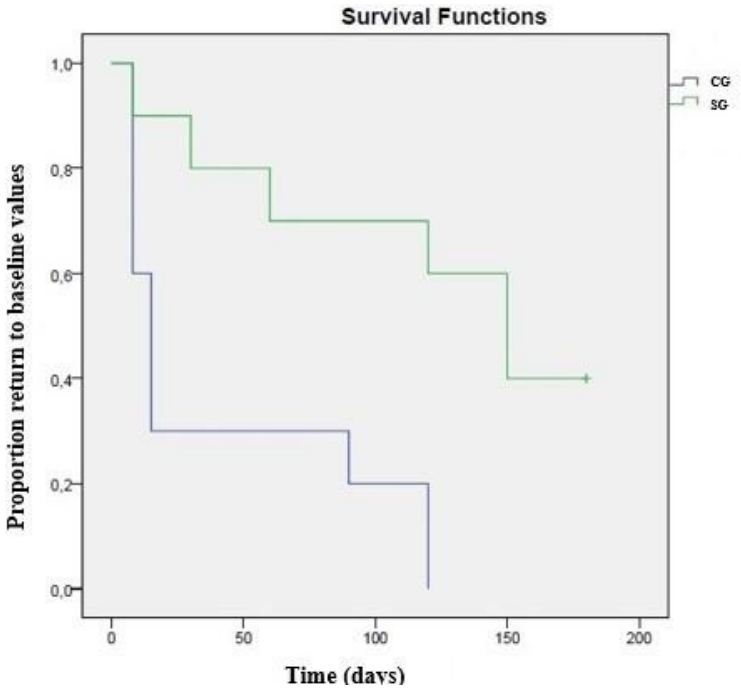

Figure 3 - Kaplan-Meier curve demonstrating a significant difference between stanozolol group and control group in time for Liverpool Osteoarthritis in Dogs (LOAD) to return to baseline values.

Discussion

This study describes the effect of a single injection of stanozolol, showing that stanozolol had a significant impact on OA joints, improving weight distribution, pain, and function scores compared to the control group. Different animal models have been used to study its effects. In horses with naturally occurring OA, a positive response to treatment has been described in 82.5% of cases22. The positive effect of stanozolol in this naturally occurring canine model was observed from 15-30 days up to the last evaluation moment, at 180 days after treatment, when considering the functional assessment based on weight distribution. Interestingly, this effect was observed even with a single administration, while in the remaining animal models, multiple administrations were carried out21–23. This effect is observable in the Kaplan Maier test results for SI, with results of the stanozolol group taking significantly longer to return to baseline values. SI is commonly used to assess lameness, but their calculation with pressure-sensitive walkways has some limitations in OA patients47. While it is still unknown if the same limitations apply to the static evaluation of weight-bearing, we looked at different weight-distribution compensation mechanisms by calculating SI and deviation values. It was reasonable to expected improvements in SG only after a relatively large period after the intra-articular administration since stanozolol acts by inducing transforming growth factor 1β synthesis. A further possible stanozolol mechanism of action may be related to its induction in aromatase

expression48. It has been demonstrated that the human articular cartilage expresses aromatase and that reduced expression of aromatase could facilitate the development of OA49,50. Aromatase inhibitor therapy in humans to address other medical conditions might be associated with common musculoskeletal symptoms and with substantial functional disability51.

Pain is a hallmark of OA, and canine studies offer valuable data that may translate to humans52–54. Results show that a single intra-articular stanozolol administration significantly improved pain and function scores compared with the control group, raging until the 90-day evaluation moment and, in some cases, until the last evaluation moment. A significant difference was also observed with the Kaplan Meier test for the majority of the considered scores. Through the same period, control group scores worsened, as would be expected as the disease progresses. It is interesting to note that some patients in the control group still recorded better scores in follow-up evaluations. This may be due to OA's natural course, with patients sometimes showing spontaneous improvements through time, only to see symptoms reappear in the future. An additional possibility is based on the fact that placebo saline injections can produce an effect in functional improvements that can last up to 6-month 55. Even though this is possible and may be reflected in some patients' scores, the control group as a whole showed the expected progression of the disease. Additional clinical improvements were observed in the stanozolol group, with improved range of motion during joint extension. A consistent finding with the thermographic evaluation was that higher values were registered in the control group throughout the study, particularly in the last evaluation moments. Digital thermography can assess inflammatory pain and identify osteoarthritic patients56,57. Our results seem to support this finding, with higher temperature values determined with this technique corresponding to patients with worse functional evaluation and clinical signs. During digital thermography of dogs, the coat's type and color must be taken into account58,59. All of the breeds included in this study had short hair, some had a double coat, and breeds had similar distribution between groups.

IL-1 is commonly pointed out as a major proinflammatory cytokine responsible for the catabolism in OA in several species, dogs, horses, and humans included1,60,61. Therapeutic approaches targeting IL-1 have been developed and shown a positive effect in animal models62. The evaluation of synovial fluid can add important information regarding disease burden and progression63,64. A previous report has described an improvement in synovial fluid characteristics of animals treated with intra-articular stanozolol22. We only observed significant changes at eight days, with both groups showing a reduction from the values recorded at the initial evaluation, but the stanozolol group had higher values. Visual inspection of patients' synovial fluid in the control group at the 8-day evaluation point showed an easily noticeable increased turbidity. The amount of turbidity grossly relates to the amount of inflammation65. The stanozolol administration may cause a transient

increase in joint inflammation, which may also account for functional improvements, measured with weight distribution, were only observed after this period. Also, since stanozolol acts by inducing transforming growth factor 1β synthesis and reducing nitric oxide, it may not significantly impact IL-1 levels. It is also important to keep in mind that exercise influences inflammatory arthropathies parameters, and increase joint loading adds to secondary inflammation in OA joints66,67. As these animals were working dogs, physical activity may also play a role in this finding. The injection of 0.9% NaCl, used in as the control, added to the removal of synovial fluid for analysis, thus removing pro-inflammatory cytokines, may have had a similar effect to that of a joint lavage, and therefore account for the lower IL-1 levels observed in the control group at 8 days.

Radiographic evaluation is a staple of OA monitoring, and CCO and CFHO represent early radiographic signs that predict the development of the clinical signs of hip OA38,68–70. There is a low relationship between radiographic changes, clinical signs, and limb function71. As expected, radiographic signs in the control group progress throughout the follow-up period, representing the natural evolution of OA. In the stanozolol group, the majority of considered radiographic signs did not progress, and some improved. This effect has been described in an ovine surgical induce model, with stanozolol reducing subchondral bone reaction and promoting articular cartilage regeneration21. Although the effect has been previously described, future studies have confirmed these changes as no histological samples were collected in this study.

Studies regarding the use of stanozolol in human OA are not performed due to its potential anabolic effects72,73. The dose demonstrated to produce anabolic effects is 10mg twice a week, given through intramuscular administration74. In this model, we used the described 0.3mg/kg dose for intra-articular use in dogs26,27. Even if the administered dose may have approached the 10mg level in some patients, a single administration was used, thus not exceeding the dose needed to produce the anabolic effect. In a study aimed to determine the best intra-articular dose of stanozolol in horses, multiple administrations at the highest dose tested (5mg) also did not produce any side effects23. It is known that after intra-articular administration of stanozolol, it passes rapidly from the joint space to systemic circulation, with maximal plasma concentration registered at 6 hours post-administration. It is then eliminated rapidly and detected in plasm for no more than 36h post local administration75. In an ovine model, no weight gain was attributed to the anabolic effect of stanozolol21. We also did not recorded significant increases in body weight, which could be attributed to stanozolol. In mice treated with a long-term, high-dose stanozolol regime did not produce significant changes in activity patterns and aggressiveness18. No event of aggressiveness or personality changes were reported in treated animals.

Side-effects of intra-articular stanozolol have been previously reported in horses. They include a transient post-injection swelling in the treated joint, which disappeared after a few days without intervention22. Similarly, we observed increased lameness in four cases, which spontaneously resolved within a few days. No additional medication was administered to the animals during the follow-up period. The study presents some limitations, namely the fact that the majority of animals had mild OA. It would be of interest to include a larger proportion of animals representing the remaining hip grades. Including a larger number of patients also is of interest. It is also important to determine the biological significance of the changes observed. This assessment was made with the Kaplan-Meier test, but the determination of what constitutes a meaningful improvement has not been yet made for some of the evaluations performed. For that reason, we evaluated how long did it take for the assessment to return or drop below the value of the initial presentation, as it was the point which motivated the need for medical assistance. As this study was a clinical treatment experiment, no joint histological samples were collected and analyzed. For that reason, the effect of stanozolol on actual disease progression could not be determined on this animal model, and only radiographic progression was evaluated. Further studies should also consider this drug's intra-articular effects, including cytotoxicity, different dose evaluations, and administration frequencies, similar to what is described in other animal models.

Conclusions

This study describes the effect of a single injection of stanozolol in a naturally occurring canine model, with a long follow-up period. The use of stanozolol was safe and produced significant improvements in weight-bearing, pain score, and clinical evaluations.

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RESEARCH ARTICLE Open Access

Effect of a single intra-articular high molecular weight hyaluronan in a naturally occurring canine osteoarthritis model: a randomized controlled trial

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Abstract

Background: Osteoarthritis (OA) is a complex joint disease and chronic pain source, affecting a patient's quality of life and posing a financial burden. As the dog is considered a nearly ideal species for translation research of human OA and the most used model for research, exploring spontaneous dog OA under the One Health/One Medicine concept can improve both humans and dogs' health and well-being.

Methods: In a clinical treatment experiment, forty (*N*=40) joints were selected and randomly assigned to a control group (CG), which received 0.9% NaCl or a treatment (HG), which received Hylan G-F 20. Evaluations were performed on treatment day (T0), 8, 15, 30, 90, and 180 days post-treatment. They consisted of four different Clinical Metrology Instruments (CMI), evaluation of weight distribution, joint range of motion, thigh girth, radiographic and digital thermography imaging, synovial fluid interleukin-1 (IL-1), and C-reactive protein concentrations. Results were compared with repeated measures ANOVA, with a Huynh-Feldt correction, Paired samples *T*-test, or Wilcoxon signed-ranks test, with $p<0.05$.

Results: Patients had a mean age of 6.5±2.4 years and a bodyweight of 26.6±5.2kg, and joints graded as mild (*n*=28, 70%), moderate (*n*=6, 15%), and severe OA (*n*=6, 15%). No differences were found between groups at T0. Symmetry index and deviation showed significant improvements in HG from 30 days ($p<0.01$) up to 180 days ($p=0.01$). Several CMI scores, particularly pain scores, improved from 90 to 180 days. Radiographic signs progressed in both groups. In both groups, increasing body weight and age corresponded to worse clinical presentation. IA hyaluronan administration produced increased lameness in six cases, which resolved spontaneously.

Conclusions: This study characterizes the response to treatment with Hylan G-F 20, which can produce significant functional and pain level improvements in patients with OA, even those with factors related to worse response to treatment.

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Background

Osteoarthritis (OA) is a highly prevalent disease world- wide, which affects all mammals and a leading cause of disability. It can negatively impact both the population's physical and mental well-being, with substantial health- care resources and costs associated with managing the dis-ease [1, 2]. The dog is an ideal species to study human OA, with the advantages of being anatomically, biochem- ically, genomically, and molecularly similar to humans, with clinical progression and treatment similarities [3]. At the same time, they have a foreshortened lifespan but with human equivalent life and disease stages while sharing many environmental variations that influence human OA. The study of spontaneous canine OA and its treatment can add to the knowledge of the treatment of the human disease as well, under the One Medicine initiative [4, 5].

OA is an incurable condition, and its management fo- cuses on alleviating symptoms, particularly pain. An add- itional goal is to improve overall joint function while slowing down disease progression [5, 6]. Hyaluronan, the high molecular glycosaminoglycan, is synthesized by chondrocytes and synovial fibroblasts [7]. It forms the backbone of proteoglycans aggregates interwoven with collagen to create hyaline cartilage's unique structure [8]. Information from animal models shows that endogenous hyaluronan is cleaved by free radicals in OA. Its quantity and quality are affected in OA joints, more severely in clinically affected patients, supporting its exogenous ad- ministration [9]. Even though its mechanism of action is not entirely understood and clinical trials have provided contradictory results, hyaluronan treatment aims to re- duce pain and improve function by supplementing syn- ovial fluid viscosity and elasticity [10]. Additional anti- inflammatory, anti-nociceptive, and chondroprotective properties have been suggested, through the enhancement of cartilage synthesis, blunting response to IL-1, protec- tion from the damage of oxygen free radicals, and protec- tion of chondrocytes from apoptosis [7, 11].

Human reports show that intra-articular hyaluronan, given once weekly for 3 weeks, increased mobility and reduces pain and the need for nonsteroidal anti- inflammatory drugs to control pain [12]. A systematic review concluded that there is a lack of standardization regarding intra- articular hyaluronan administrations for hip OA, with no consensus on its efficacy [13]. Although it is not clear if any formulation has a superior disease- modifying effect [14], high molecular weight products seem to produce better results, particularly in patients with mild radiographic disease [15]. A recent report showed that both single or 1- 3 weekly injections of Hylan G-F 20 at 1 year following the first injection for knee OA are efficacious and generally well tolerated for long-term use [16]. Many studies performed in canine experimental OA models have failed to demonstrate clear benefits of hyaluronan supplementation [17]. In a canine surgical model, IA hyaluronan provided clinically significant improvement in pain, function, lameness, and kinetics compared to pre-treatment and saline control, without preventing OA's progression [18]. In a rabbit model, hyaluronan administration produced a more nor- mal cartilage after immobilization [19]. In dogs with nat- urally occurring OA, treatment groups have significantly better results than a control group by the 6th week post- treatment [20].

Multiple agents influence OA catabolism, but interleu- kin 1 (IL-1) is commonly pointed out as the major pro- inflammatory cytokine [21]. C-reactive protein (CRP) is an acute-phase protein produced during inflammatory reactions or tissue injury from an early stage [22].

Radiographic examination is a staple in OA's assess- ment, and the ventrodorsal (VD) hip extended view is the most commonly used projection. An additional use- ful projection is the ventrodorsal flexed view, also called frog-legged view (FL), specifically in the evaluation of the circumferential femoral head osteophyte (CFHO) and caudolateral curvilinear osteophyte (CCO), early radiographic signs related to the development of the clinical symptoms [23]. Digital thermal imaging relies on the between physiologic functions generated heat and its relation with skin temperature control, being reliable in assessing inflammatory arthritis pain and osteoarthritic subjects [24]. Functional evaluation is also paramount in determining response to treatment in OA, and stance analysis has been reported as a sensitive evaluation for detecting lameness in dogs [25]. It evaluates weight dis- tribution since patients commonly bear less weight on a painful limb [26]. An additional functional evaluation in- cludes determining activity levels and mobility impair- ments since they are associated with musculoskeletal pain [27]. Pedometers are capable of measuring ambula- tory activity with acceptable accuracy [28]. Clinical examination of patients commonly includes evaluating muscle masses, muscular atrophy being a consistent finding in OA patients, and determining the joint range of motion (ROM, flexion, and extension), which can present restrictions [29].

Pain is a hallmark of OA, and canine studies offer valuable data that may translate to humans [30, 31]. For pain evaluation and its impact on patients' lives, several clinical metrology instruments (CMI) have been devel- oped. The Liverpool Osteoarthritis in Dogs (LOAD) and the Canine Brief Pain Inventory (CBPI) are the most commonly used [27], with the CBPI being divided into a pain severity score (PSS) and a pain interference score (PIS) [32]. The Canine Orthopedic Index (COI, divided into four scores: stiffness, gait, function, and quality of life (QOL) and the Hudson Visual Analogue Scale (HVAS), developed to assess the degree of lameness in

dogs, are additional validated evaluation tools [33, 34]. Digital thermal imaging is a technique that has recently gained attention. It relies on heat generated during physiologic functions and its relation with skin temperature control [35]. It has been used to assess in- flammatory arthritis pain and differentiate normal from osteoarthritis subjects [24].

This study aimed to describe the effect of a high mo- lecular weight hyaluronan product (Hylan G-F 20) in OA management in a naturally occurring canine model. We hypothesize that a single administration will reduce clinical signs of OA compared with a control group.

Methods

The study protocol was approved by the ethical review committee of the Universidade de Évora (ORBEA, ap- proval n° GD/32055/2018/P1, September 25, 2018) and complies with the ARRIVE guidelines. Written informed consent was obtained from the Institution responsible for the animals. Twenty patients with naturally occurring bilateral hip OA, constituting a convenience sample, were signaled from a population of active police working dogs, comprising forty (*N*=40) hips joints. The diagnosis was made based on history, physical, orthopedic, neuro- logical, and radiographic examinations. Additional inclu- sion criteria included a bodyweight of ≥ 20kg, age ≥2 years, and they should not have received any medication or nutritional supplements for at least 6 weeks. Patients with other suspected or documented orthopedic or con- comitant disease and not tolerant of data collection were excluded.

Treatment administration

In a double-blinded study, patients were randomly assigned using the statistical analysis software to two groups, 10 dogs per group, and treated bilaterally: a con- trol group (CG, *n*=20), which received an intra-articular (IA) administration of 2ml of 0.9%NaCl, and a treatment group (HG, *n*=20), which received a single IA adminis- tration of 2ml of Hylan G-F 20 (Synvisc®, Sanofi, Portugal). Radiographic examinations and IA adminis- trations were conducted under light sedation, using a combination of medetomidine (0.01mg/kg) and buthor- phanol (0.1mg/kg), both given intravenously simultan- eously. The procedure for intra-articular administrations to the hip joint has been described before [36]. The patient was positioned in lateral recumbency, with the affected joint uppermost, to access the joint of interest. A window of 4 × 4cm surrounding the greater trochan- ter was clipped and aseptically prepared. An assistant then positioned the limb in a neutral and parallel to the table position. The joint space was accessed using a 21- gauge with 2.5″ length needle, introduced just dorsal to the greater trochanter and perpendicular to the limb's long axis. Correct needle placement was confirmed by collecting synovial fluid (immediately collected and processed for future analysis), and the treatment or sa- line were administered. Patients were rested for three consecutive days following treatment, after which nor-mal activity was resumed over 5 days. One and 3 days after the IA procedure, animals were examined by the assisting veterinarian for signs of exacerbated pain, per- sistent stiffness of gait, and changes in posture exhibited. Evaluations were conducted on days 0 (treatment day),8, 15, 30, 90, and 180 by the same researcher. An outline of procedures and evaluations conducted in each evalu- ation moment is presented in Table 1.

Evaluation of weight-bearing distribution

The weight distribution evaluation was performed with aweight distribution platform (Companion Stance Ana- lyser; LiteCure LLC®, Newark, DE, USA). According tothe manufacturer's guidelines, the equipment was placed in the center of a room, at least 1 m from the walls. Itwas calibrated at the beginning of each day and zeroed before each data collection. After this procedure, animals were placed with one foot in each quadrant of the plat- form, using gentle restraint when required. A left-right symmetry index (SI) was calculated with the following formula: $SI=[(WBR-WBL)/((WBR+WBL) \times 0.5)]\times 100$ (WBR is the weight-bearing of the right limb, and WBL is the weight-bearing of the left limb). Negative values were made positive [37]. We additionally considered a deviation from normal 20% weight-bearing for a pelvic limb, calculated by subtracting WB to 20.

Digital thermography imaging

Digital thermography evaluation was conducted in a room with a controlled temperature, set at 21°C. Previous to col- lecting the images, animals were allowed to walk around the room for 30 min. They were then placed in an upright standing position, and a dorsoventral thermographicimage was obtained, including the area from the last lum- bar vertebra to the first coccygeal vertebra, at a distance of 60cm [38], FLIR ThermaCAM E25® model (FLIR Systems, Wilsonville, OR, USA). Images were analyzed using the free software Tools (FLIR Systems, Inc), with a rainbow color pallet. Boxes of equal size were placed on the hip joint's anatomical area on both views to determine mean and maximal temperatures.

Radiographic evaluation

In the VD radiographic projection [23], seven radio- graphic signs were assessed: irregular wear on the fem- oral head, making it misshapen and with a loss of its rounded appearance; a flattened or shallow acetabulum, with irregular outline; CCO; new bone formation on the acetabulum and femoral head and neck; a worn away

Table 1 Outline of procedures and evaluations conducted in each evaluation moment. Days are counted from treatment day

Modality	Evaluation moment									
0 Treatment day	8	15	30	90	180					
Treatment				X						
Functional assessment										
Stance analysis				X		X	X	X	X	X
Pedometer				X		X	X	X	X	X
Goniometry				X		X	X	X	X	X
Thigh girth measurement				X		X	X	X	X	X
Imaging										
Digital Thermography				X		X	X	X	X	X
Digital radiography				X				X	X	X
Clinical Metrology Instruments										
HVAS				X		X	X	X	X	X
CBPI				X		X	X	X	X	X
COI				X		X	X	X	X	X
LOAD				X		X	X	X	X	X
Laboratorial evaluation										
SF CRP				X		X	X		X	X
SF IL-1				X		X	X		X	X

CBPI, Canine Brief Pain Inventory; *COI*, Canine Orthopedic Index; *CRP*, C- reactive protein; *HVAS*, Hudson Visual Analogue Scale; *IL-1*, interleukin 1; *LOAD*, Liverpool Osteoarthritis in Dogs; *SF*, synovial fluid

angle formed at the cranial effective acetabular rim; subchondral bone sclerosis along the cranial acetabular edge; and CFHO.

Clinical and laboratorial findings

Thigh girth was determined with a Gullick II measuring tape, at a distance of 70% thigh length, measured from the greater trochanter's tip, with an extended leg [39]. Hip joint ROM was obtained with a goniometer at ex- tension and flexion with a flexed stifle [40]. Pedometers were worn around the patient's neck, attached to an ad- justable lightweight collar [41]. Pedometers were worn for 1 week before the first evaluation moment to set a baseline value. For each of the following evaluations, ani-mals worn the pedometer for a week before that evalu- ation moment. A mean daily count was calculated by dividing the registered number of steps by the number of considered days. In each evaluation moment, trainers completed a copy of HVAS, CBPI, COI, and LOAD after receiving the published instructions for each of them. They were completed sequentially by the same handler, in a quiet room, with as much time as needed to answer all items. From the synovial sample collected, IL 1ß and CRP concentrations were determined with the DuoSet Ancillary Canine IL-1ß Reagent kit (R&D Systems, UK), read with a FLUOstar OPTIMA (BMG Labtech), and Fuji Dri-Chem Slides VC-CRP PS (FUJIFILM Europe GmbH), read with a DRIChem NX500i (FUJIFILM Eur- ope GmbH), respectively.

Data analysis

Normality was assessed with a Shapiro-Wilk test. Results were compared between groups in each of the evaluation moments. To evaluate the effect of different parameterson patients' clinical evolution, results were compared by sex, body weight, age, and different radiographic findings with repeated measures ANOVA, with a Huynh-Feldt correction, paired samples *T*-test, or Wilcoxon signed-ranks test. A Kaplan-Meier test was performed to evalu- ate the time to return to baseline values of SI and CMI scores, compared with the Breslow test. All results were analyzed with IBM SPSS Statistics version 20, and a sig- nificance level of $p<0.05$ was set.

Results

This study sample comprised 40 joints of active police working dogs, with a mean age of 6.5±2.4 years, a mean bodyweight of 26.6±5.2kg, and of both sexes (13 males and 7 females). Dogs were of breeds commonly employed in police forces, similarly distributed between CG and TH: German Shepherd Dogs (n=6, 3 in CG and 3 in TH), Labrador Retriever (n=6, 3 in CG and 3 in TH), Belgian Malinois Shepherd Dogs (n=5, 3 in CG and2 in TH), and Dutch Shepherd Dog (n=3, 2 in CG and 1in TH). At the initial evaluation, joints were graded with the OFA hip grading scheme as mild (n=28, 70%), mod- erate (n=6, 15%), and severe (n=6, 15%). No differences were found between groups at the initial evaluation. In- creased lameness was observed in 6 joints HG, which spontaneously resolved within 48-72h.

Clinical and CMI results

Values recorded for different evaluations in each group at T0 are presented in Table 2. Comparing results between groups with repeated measures ANOVA with a Huynh- Feldt correction, significant differences between groups were found concerning deviation ($F(5, 160)=3.7$, $p=0.004$), SI ($F(3.6, 114.4)=3.6$, $p=0.011$), mean temperature on a DV view ($F(3.9, 103.1)=4.8$, $p=0.001$), maximal temperature on a DV view ($F(3.9, 101.7)=4.4$, $p=0.003$), mean temperature on a Lt view ($F(5, 140)=36.3$, $p<0.001$), maximal temperature on a Lt view ($F(4.8, 133.3)=86.7$, $p<0.001$), joint flexion ($F(4.2, 130.5)=18.4$, $p<0.001$), and IL-1 synovial concentration ($F(2.4, 85.8)=5.3$, $p=0.004$). Significant differences were also observed with different CMI, specifically PSS ($F(5, 140)=2.8$, $p=0.021$), PIS ($F(2.7, 75.1)=3.4$, $p=0.026$), Function ($F(5, 140)=2.6$, $p=0.026$), Gait ($F(5, 140)=2.3$, $p=0.044$), and COI ($F(5, 140=2.2$, $p<0.05$). The evolution of SI in CG and HG is presented in Fig. 1. Re- sults of the Kaplan-Meier test are presented in Table 3.

Kaplan-Meier curves for stiffness score and PIS are presented in Figs. 2 and 3, respectively.

Radiographic evaluations

The frequency of different radiographic findings at the initial and final evaluations is presented in Table 4. Cases without CFHO on a VD view in the CG, on thefirst assessment, had a better joint extension at the 8-day evaluation ($p<0.01$) and better HVAS ($p=0.02$), PSS ($p= 0.01$), and PIS scores ($p=0.03$). At 15 days, they had a higher mean thermographic evaluation on a Lt view ($p= 0.02$), better PSS ($p=0.02$), and PIS scores ($p<0.05$). The higher mean thermographic evaluation on a Lt view was also observed at 30 days ($p=0.01$). At 90 days, these joints had better HVAS scores ($p=0.02$). At the final evaluation, they had higher maximal thermographic evaluation on a Lt view ($p=0.04$) and better PSS ($p=0.05$) and PIS scores ($p<0.03$). In the HG, cases without CFHO had higher thigh girth ($p=0.03$). At 8 days, they had higher body weight ($p<0.01$), lower deviation ($p<0.01$), lower mean and maximal thermographic evaluation on a DV ($p=0.02$ and p=0.04, respectively) and mean on a Lt view ($p<0.02$), and higher thigh girth ($p=0.01$). At 15 days, these joints had lower deviation ($p=0.03$), lower mean, and maximal thermographic evaluation on a DV ($p=0.03$ for both) and maximal on a Lt view ($p<0.05$). At 30 days, they had a higher thigh girth ($p<0.01$). At the 90-day evaluation moment, they had better deviation ($p=0.02$), a finding again observed at 180 days ($p<0.05$).

Evaluations by sex

In the CG, female dogs had significantly lower body weight in all evaluation moments ($p=0.01$). At the initial evaluation, females had higher values in all thermo- graphic evaluations ($p<0.01$) and lower PIS scores ($p= 0.04$). At 8 days, the same was true regarding thermo- graphic evaluation ($p<0.01$), except maximal value on a Lt view and higher joint extension values ($p<0.01$). At 15 days, females still showed higher joint extension ($p= 0.04$) and lower PIS scores ($p=0.03$). At the 30 days' evaluation, females showed higher thermographic max- imal values on an LT view max ($p<0.01$). At 90 days, fe- male dogs had lower thigh girth ($p=0.03$) and better PSS and PIS scores ($p=0.01$). In the final evaluation moment, female dogs had higher extension values ($p=0.02$) and better HVAS ($p=0.02$), PSS ($p<0.01$), PIS ($p<0.01$), stiff-
ness ($p=0.02$), function ($p=0.02$), gait ($p<0.01$), QOL ($p= 0.02$), and COI ($p=0.01$) scores. In the HG, at the initial evaluation, females had lower pedometer counts ($p= 0.02$), better deviation ($p=0.02$) and SI ($p<0.05$), higher mean and maximal values on a Lt view ($p=0.02$ and $p< 0.01$, respectively), and lower thigh girth ($p<0.01$). At the 8-day evaluation moment, females had higher mean and maximal thermographic values on the DV view and mean value on a Lt view ($p<0.01$ for all) and lower thigh girth ($p<0.01$). At 15 days, females had lower pedometer counts ($p=0.04$), still had mean and maximal thermo- graphic values on the DV, and mean value on a Lt view ($p<0.01$ for all), lower thigh girth ($p<0.01$), and worse joint extension ($p=0.02$). At 30 days, females still had mean and maximal thermographic values on the DV and mean value on a Lt view ($p<0.01$, $p=0.01$, and $p<0.05$, respectively), in addition to lower thigh girth ($p<0.01$). At the 90 days' evaluation moment, females had worse SI ($p=0.03$), higher maximal thermography evaluation on a lateral ($p<0.05$), and synovial fluid CRP ($p=0.02$). At the final evaluation moment, females had better joint flexion ($p=0.04$) and serum higher CRP ($p=0.02$). They also had lower body weight throughout the study ($p<0,01$).

Evaluations by bodyweight

Comparing animals with a weight cut-off set at the sample's mean value at 8 days, lighter subjects had higher thermographic mean and maximal values on a DV ($p=0.03$ and $p=0.02$, respectively), higher thigh girth ($p=0.01$), and worse stiffness ($p=0.03$), function ($p< 0.01$), gait ($p=0.03$), and COI scores ($p<0.01$). At 15 days, lighter cases showed lower thigh girth ($p=0.04$) and worse HVAS ($p<0.05$), stiffness, function, gait QOL, and COI scores ($p<0.01$). Lighter animals had lower PCR concentrations at 30 days ($p=0.04$) and bet-ter HVAS scores ($p=0.02$). The same animals had lower thigh girth ($p<0.01$) and IL-1 levels ($p=0.02$) at 90 days. In the final evaluation point, lighter animals showed higher mean thermographic values on a DV view ($p< 0.01$) and higher joint flexion ($p=0.02$) and extension ($p<0.01$). In HG, animals below the threshold had a higher mean value on a Lt view ($p=0.03$), lower thigh girth ($p<0.01$), and worse joint extension ($p<0.05$) on the initial evaluation. At 8 days, they had lower pedom- eter counts ($p<0.01$), worse deviation ($p=0.03$), higher mean temperature values on a Lt view ($p<0.01$), lower thigh girth ($p<0.01$), and worse function score ($p=0.02$). After 15 days, lighter subjects had lower pedometer counts ($p=0.04$), higher mean and maximal temperature values on the DV view ($p=0.01$ for both), as mean value on a Lt view ($p=0.02$), lower thigh girth ($p<0.01$), worse joint extension ($p=0.02$), and function score ($p<0.01$). At 30 days, these cases had lower thigh girth ($p<0.01$), worse joint extension ($p<0.01$), function, and QOL scores ($p=0.03$). At the 90-day evaluation, animals below the cut-off had worse flexion ($p=0.03$), higher synovial CRP concentration ($p=0.04$), and worse func-tion score ($p=0.03$). At the final evaluation moment, lighter subjects had worse deviation ($p<0.01$), higher mean and maximal temperature values on the Lt view ($p=0.01$), and worse function score ($p=0.02$).

Table 2 Mean values (±standard deviation) of different parameters evaluated at the initial evaluation and throughout the study

Modality		Treatment day					8 days					15 days				
		CG		HG		p	CG		HG		p	CG		HG		p
		Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Goniometry	Flexion (°, mean±SD)	55.0	4.4	54.9	4.1		55.3	3.7	55.9	3.8	0.1	57.2	5.2	58.6	4.7	0.3
Extension (°, mean±SD)		151.2	3.9	149.8	8.6		149.9	4.6	149.8	8.6	1.00	151.1	3.5	150.2	5.5	
	Thigh girth (cm, mean±SD)	31.2	2.6	30.4	3.3		31.1	3.3	29.3	2.8	1.0	31.1	2.9	31.3	3.2	1.0
	Pedometer (daily steps±SD)	1445.7	755.7	1107.0	998.8		829.5	931.3	782.0	842.9	1.0	606.0	309.5	845.0	472.0	1.0
CMI	HVAS (0–10)	6.8	1.2	6.6	1.4		6.7	1.5	6.7	1.3	1.0	6.8	1.2	6.8	1.4	1.0
CBPI—PSS (0–10)		3.1	1.9	3.3	2.6		3.4	2.3	2.8	1.5	0.04*	3.7	2.8	2.6	1.6	0.04*
CBPI—PIS (0–10)		3.2	2.2	3.4	2.3		3.4	2.1	3.2	1.9	0.02*	3.6	2.1	3.3	2.7	0.02*
COI—Stiffness (0–16)		3.4	3.4	17.0	10.5		4.1	3.3	4.2	3.2	0.88	4.1	3.2	3.8	2.6	0.38
COI—Function (0–16)		3.6	4.1	3.4	2.9		4.1	4.0	4.4	3.2	0.06	4.4	5.5	4.3	3.2	0.37
COI—Gait (0–20)		4.7	5.2	4.6	3.5		5.4	6.1	6.6	4.2	0.46	5.8	4.3	7.0	4.3	0.86
COI—QOL (0–12)		4.5	2.6	7.4	4.7		4.6	2.7	4.4	2.5	0.40	4.7	2.9	4.4	2.2	0.22
COI—Overall score (0–64)		16.4	14.7	4.5	3.1		18.2	13.8	19.7	12.0	0.75	18.6	13.8	19.6	11.1	0.19
LOAD (0–52)		13.6	10.5	19.9	12.7		14.4	12.7	16.2	9.4	0.73	14.3	10.7	16.6	9.4	0.18
Digital thermography	DV (°, mean±SD)	24.7	1.9	25.8	1.4		25.2	1.3	24.9	1.3	0.02*	24.4	1.6	24.4	1.5	1.0
DV max (°, mean±SD)		26.3	1.9	26.6	1.6		25.8	1.0	26.2	1.2	1.0	26.7	1.6	25.8	1.4	1.0
Lt (°, mean±SD)		28.7	2.7	26.9	2.1		31.6	2.1	31.6	2.6	<0.01*	29.7	2.9	30.8	2.5	<0.01*
Lt max (°, mean±SD)		31.9	3.1	30.4	3.3		34.9	1.0	33.8	2.8	<0.01*	34.9	0.8	34.3	0.7	<0.01*
Synovial fluid	IL-1 (pg/mL, mean±SD)	170.9	120.4	182.4	157.4		72.3	42.4	141.2	138.1	0.01*	-	-	-	-	-
CRP (mg/mL, mean±SD)		0.4	1.0	0.2	0.3		0.3	1.2	0.2	0.0	0.2	-	-	-	-	-
Weight bearing	Symmetry index (mean±SD)	24.7	20.3	21.7	24.9		18.7	17.1	36.4	37.5	<0.01*	23.9	16.3	23.9	23.7	0.4
Deviation (mean±SD)		2.8	3.6	3.8	3.5		2.78	1.987	4.1	3.6	1.0	2.94	2.127	2.5	2.7	1.0

Modality		30 days					90 days					180 days				
		CG		HG		p	CG		HG		p	CG		HG		p
		mean	SD	mean	SD		mean	SD	mean	SD		mean	SD	mean	SD	
Goniometry	Flexion (°, mean±SD)	53.6	2.9	56.3	4.5	1.0	52.7	2.9	51.7	2.6	0.03*	51.6	2.2	49.1	5.0	<0.01*
Extension (°, mean±SD)		150.8	3.4	152.0	6.0		150.8	2.9	152.0	4.7		151.3	2.9	150.7	11.5	
	Thigh girth (cm, mean±SD)	30.6	2.7	29.3	2.2	0.4	31.6	2.7	31.1	5.5	1.0	31.5	2.2	31.6	4.2	1.0
	Pedometer (daily steps±SD)	594.5	663.4	760.0	292.0	1.0	451.9	463.0	635.0	43.0	1.0	434.9	455.8	652.0	90.9	1.0
CMI	HVAS (0–10)	6.4	1.4	6.9	1.5	0.56	6.6	1.7	6.5	1.2	0.85	6.5	1.4	6.5	1.6	0.61
CBPI—PSS (0-10)		3.7	2.6	3.0	2.6	<0.05*	4.1	2.9	2.9	2.1	0.04*	3.6	3.1	2.9	2.4	0.04*
CBPI—PIS (0-10)		3.8	2.6	3.2	2.7	0.02*	3.9	2.8	3.2	2.7	0.03*	3.5	2.4	3.2	2.7	<0.05*

Table 2 Mean values (±standard deviation) of different parameters evaluated at the initial evaluation and throughout the study *(Continued)*

Modality				Treatment day				8 days					15 days			
				CG		HG		CG		HG		*p*	CG		HG	*p*
				Mean	SD	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD
	COI—Stiffness (0–16)	4.6	4.1	3.5	3.8	0.11	4.6	3.9	4.0	4.0	0.49	4.0	5.7	4.3	4.3	0.81
	COI—Function (0–16)	5.7	5.3	4.3	3.9	0.02*	5.0	5.2	4.1	4.1	0.02*	4.0	5.4	3.3	3.8	<0.05*
	COI—Gait (0–20)	6.9	5.1	5.2	5.1	0.03*	5.7	5.5	5.0	4.9	<0.05*	4.4	5.4	6.1	5.8	0.36
	COI—QOL (0–12)	5.3	3.3	4.0	2.4	0.11	5.1	2.8	4.4	2.7	0.84	4.7	2.6	4.2	2.9	0.79
	COI—Overall score (0–64)	22.4	19.1	5.2	5.1	<0.01*	20.1	15.7	5.0	4.9	0.01*	15.7	14.9	19.7	17.3	0.59
	LOAD (0–52)	16.4	13.1	15.0	9.7	0.09	13.1	12.4	15.3	10.8	0.56	13.1	12.4	16.4	11.8	0.99
Digital termography	DV (°, mean±SD)	25.3	1.5	26.3	2.2	1.0	26.1	1.2	26.5	0.9	0.7	25.6	1.4	25.6	26.0	1.0
	DV max (°, mean±SD)	25.2	2.1	27.6	2.0	<0.05*	27.4	1.4	27.5	1.2	0.2	26.9	1.4	27.4	0.9	0.3
	Lt (°, mean±SD)	29.8	2.2	31.6	1.8	<0.01*	28.4	1.8	29.9	1.7	<0.01*	27.3	1.8	29.9	1.7	<0.01*
	Lt max (°, mean±SD)	33.9	1.2	34.6	0.7	<0.01*	30.5	1.9	31.8	1.7	<0.01*	29.7	1.9	30.9	2.0	<0.01*
Synovial fluid	IL-1 (pg/mL, mean±SD)	122.9	108.9	124.2	86.9	0.2	159.6	59.1	159.6	59.1	0.6	184.2	68.5	152.3	83.7	1.0
	CRP (mg/mL, mean±SD)	0.48	0.9	0.3	0.1	0.2	0.4	0.8	0.4	0.2	0.1	0.0	0.0	0.3	0.2	1.0
Weight bearing	Symmetry index (mean±SD)	18.9	12.2	14.5	15.0	<0.01*	27.4	12.1	7.6	7.5	<0.05*	27.0	27.9	5.2	3.9	0.01*
	Deviation (mean±SD)	2.5	1.9	1.9	1.8	<0.01*	2.72	2.27	1.3	1.3	<0.05*	2.61	2.973	2.6	2.9	0.01*

CBPI, Canine Brief Pain Inventory; *CRP*, C-reactive protein; *COI*, Canine Orthopedic Index; *DV*, dorsoventral view; *HVAS*, Hudson Visual Analogue Scale; *IL-1*, interleukin 1; *LOAD*, Liverpool Osteoarthritis in Dogs; *LT*, lateral view; *PIS*, Pain Interference Score; *PSS*, Pain Severity Score; *QOL*, quality of life

*Significance when comparing the value registered by a group at an evaluation moment with T0, and comparing both group at each follow-up moment

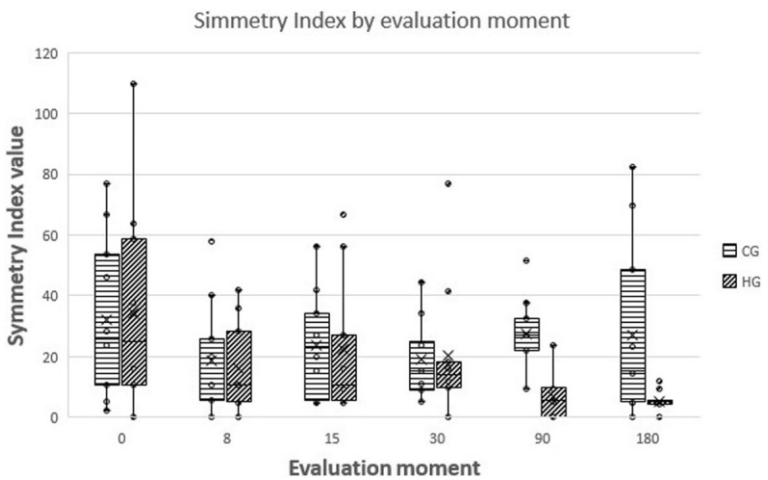

Fig. 1 Evolution of symmetry index in the control group (CG) and treatment group (HG) throughout the follow-up period. Box plots represent median and 25th and 75th percentiles, and whiskers represent 10th and 90th percentiles

Evaluations by age

Considering cases above or below the mean age of the sample, in the CG, younger subjects had higher maximal values on the thermographic Lt view (p=0.04) and better LOAD (p=0.02), stiffness (p<0.01), function (p<0.01), gait (p<0.01), and COI (p<0.01) scores. At 8 days, they showed lower SI (p<0.01), higher maximal values on the thermographic Lt view (p=0.02), and better LOAD (p= 0.04), stiffness (p<0.01), function (p<0.01), gait (p<0.01), QOL (p<0.01), and COI (p<0.01) scores. The same was also true at the 15-day evaluation, with these cases presenting better LOAD (p<0.01), stiffness (p<0.01), function (p<0.01), gait (p<0.01), QOL (p<0.01), and COI (p<0.01) scores. At the 30-day evaluation, younger subjects had lower mean and maximal values on the thermographic DV (p<0.01 and p=0.02, respectively) and Lt view (p=0.02, for the mean value), better joint flexion (p=0.01), and better LOAD (p<0.01), stiffness (p<0.01), function (p<0.01), gait (p<0.01), QOL (p<0.01), and COI (p<0.01) scores. At 90 days, the same cases had better LOAD (p=0.04), stiffness (p<0.01), function (p<0.01), gait (p<0.01), QOL (p<0.01), and COI (p<0.01) scores. At the final evaluation, younger subjects had better deviation and SI (p=0.03 and p<0.01, respectively), and stiffness

Table 3 Time to return to baseline values for weight bearing distributions (symmetry index and deviation) and CMIs, calculated with Kaplan-Meier estimators and compared with the Breslow test

Variable	Breslow test	Group			
		CG		HG	
		mean±SD	95% CI	mean±SD	95% CI
Simmetry Index	<0.01*	47.0±11.8	23.8±70.2	104.1±15.1	15.1±74.5
Deviation	<0.01*	44.8±12.1	21.1±68.5	96.2±16.3	64.2±128.1
HVAS	<0.01*	48.7±12.4	25.4±73.9	117.0±13.2	91.1±142.9
PSS	<0.01*	63.2±17.2	29.6±96.8	142.6±11.9	119.1±166.0
PIS	<0.01*	8.4±0.4	7.7±9.0	114.0±16.0	82.6±145.4
LOAD	<0.01*	40.7±10.6	19.9±61.4	141.8±11.6	119.2±164.4
Stiffness	0.03*	64.7±16.9	31.4±97.9	129.8±13.9	102.6±157.0
Function	<0.01*	65.4±13.4	39.2±91.6	168.0±6.6	155.1±180.8
Gait	<0.01*	52.7±14.6	23.9±81.4	115.5±13.1	89.9±141.1
QOL	<0.01*	60.9±15.0	31.4±90.4	125.6±12.2	101.6±149.6
COI	0.06	52.7±13.4	26.5±78.9	93.1±16.7	60.3±125.9

COI, Canine Orthopedic Index; *HVAS*, Hudson Visual Analogue Scale; *LOAD*, Liverpool Osteoarthritis in Dogs; *PIS*, Pain Interference Score; *PSS*, Pain Severity Score; *QOL*, quality of life
*Significance

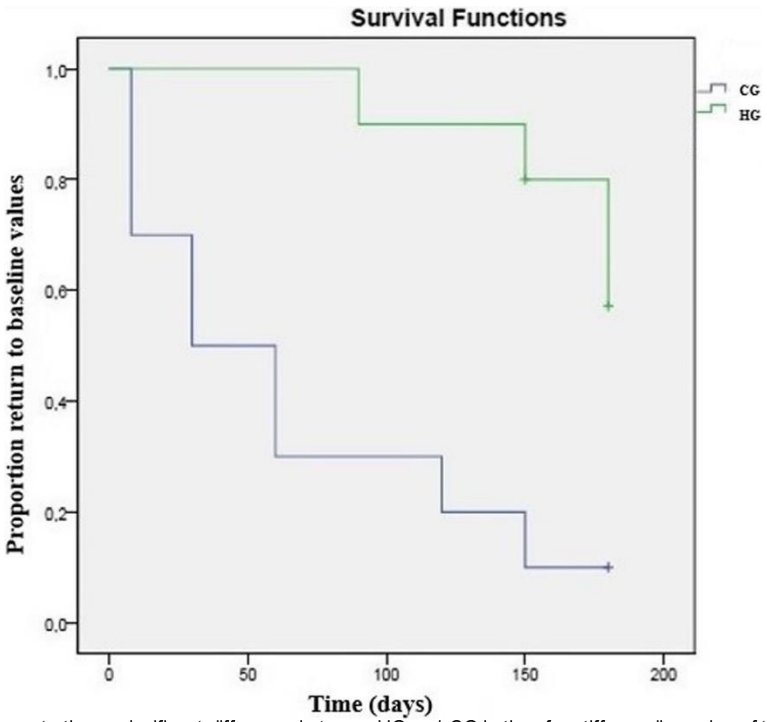

Fig. 2 Kaplan-Meier curves demonstrating a significant difference between HG and CG in time for stiffness dimension of the Canine Orthopedic Index to return to baseline values (p≤0.01)

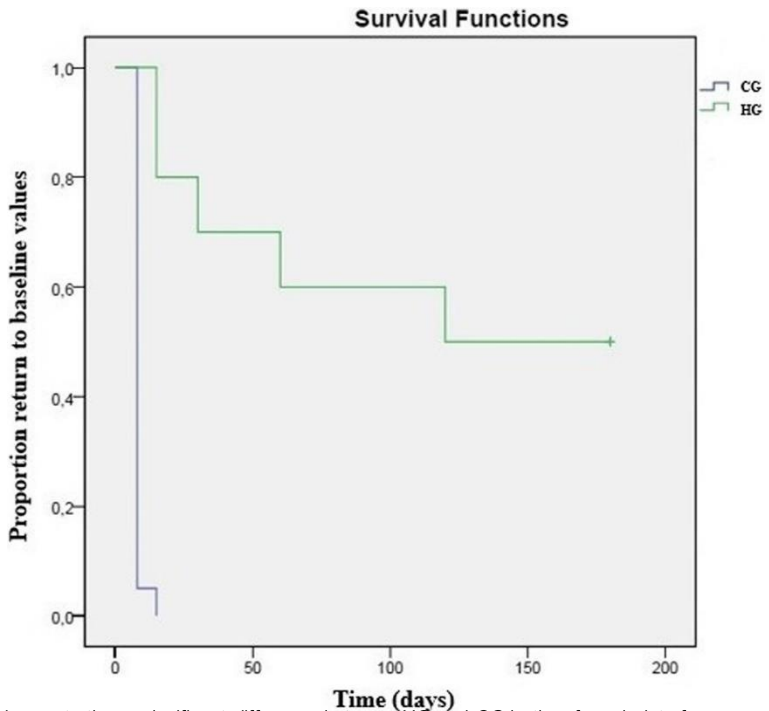

Fig. 3 Kaplan-Meier curves demonstrating a significant difference between HG and CG in time for pain interference score of the Canine Brief Pain Inventory to return to baseline values (*p*≤0.01)

Table 4 Frequency of radiographic findings in the control and treatment groups, at the initial and final evaluations

Radiographic finding	T0				180d					
	CG		HG		CG			HG		
	Absolut	%	Absolut	%	Absolut	%	p	Absolut	%	p
Irregular wear on the femoral head, making it misshapen and with a loss of its rounded appearance	18	90%	17	85%	20	100%	0.08	20	100%	0.16
Flattened or shallow acetabulum, with irregular outline	9	45%	11	55%	20	100%	< 0.01*	20	100%	< 0.01*
Caudolateral curvilinear osteophyte (CCO)	5	25%	5	25%	20	100%	1.00	20	100%	0.48
New bone formation on the acetabulum and on femoral head and neck	16	80%	20	100%	20	100%	1.00	20	100%	< 0.05*
The angle formed at the cranial effective acetabular rim is worn aw ay	12	60%	18	90%	20	100%	0.16	20	100%	< 0.05*
Subchondral bone sclerosis along the cranial acetabular edge	20	100%	19	95%	20	100%	0.32	20	100%	1.0
Circumferential femoral head osteophyte (CFHO)	3	15%	3	15%	20	100%	0.18	20	100%	< 0.01*

(p<0.01), function (p<0.01), gait (p<0.01), QOL (p<0.01), and COI (p<0.01) scores. In the HG, younger subjectshad higher pedometer counts (p<0.01), lower mean and maximal values on the thermographic DV (p<0.01 forboth) and Lt view (p<0.02, for the mean value), higher thigh girth (p=0.04), and worse HVAS (p=0.02), PSS, PIS, LOAD, stiffness, and function scores (p<0.01 for all) at the initial evaluation. The same was observed at 8 days for mean and maximal values on the thermographic DV (p<0.01 for both), lower joint flexion (p<0.05), and worse HVAS, PSS, PIS, LOAD, stiffness, function, and QOL scores (p<0.01 for all). At 15 days, they had thigh girth (p=0.03) and worse HVAS, PSS, PIS, LOAD, stiff- ness, and gait scores (p<0.01 for all). After 30 days, these joints had lower mean and maximal values on the thermographic DV (p<0.01 for both) and Lt view (p<
0.01 for both), better joint flexion (p=0.01), and better HVAS, PSS, PIS, stiffness, function, and QOL scores (p<
0.01 for all). At the 90-day evaluation, again, they had lower mean and maximal values on the thermographicon the Lt view (p<0.01 for both), and better HVAS, PSS, PIS, LOAD, stiffness, function, QOL, and COI scores (p<0.01 for all). At the final evaluation, they had devi- ation and SI (p<0.05 and p=0.03, respectively), and better HVAS, PSS, PIS, stiffness, gait, and QOL scores (p<0.01 for all).

Discussion

Osteoarthritis is the most commonly diagnosed joint disease in human and veterinary medicine, with limited treatment options. In addition to the anatomical and biochemical similarities between dogs and humans, they also share an environment and lifestyle. For those reasons, the study of animal OA could be beneficial for both species [2, 5]. To our knowledge, this is the first study to describe the effect of a single injection of high-density hyaluronan (G-F 20) on several clinical, imaging, and laboratorial signs in a naturally occurring canine model, with a long follow-up period. Dog OA, particu- larly naturally occurring OA, resembles closely human OA regarding anatomy, disease heterogeneity, and pro- gression [42].

Many studies performed in canine experimental OA models have failed to demonstrate clear benefits of hyaluronan supplementation [17]. IA hyaluronan provided clinically significant improvement in animals with stifle OA in pain, function, lameness, and kinetics compared to pre-treatment and saline control in a canine surgical model. Maximum benefits were noted at 4-8 weeks andgradually tapered down by a 6-month evaluation time point [18]. In dogs with naturally occurring OA, treat- ment groups have significantly better results than a con-trol group by the 6th week post-treatment but accompanied by exercise restrictions, leading to im- provements in the control group [20]. In this study, we have observed significant improvements in the HG with several evaluation modalities, which, in some cases,lasted up to the last evaluation moment, at 180 days post-treatment. These include functional improvements measured by the evaluation of weight-bearing, to im- provements in other dimensions of OA, as measured with the CMIs, but particularly with the two scores of the CBPI. In addition to group improvements in HG, in- dividual CMI scores also improved in most animals from the first evaluation post-treatment, but particularly after
15 days. This improvement is observable with the Kaplan-Meier test results for SI, with results in HG tak- ing significantly longer to return to baseline values. Itwas also noticeable with different CMI scores and di- mensions. Although clear anatomical similarities exist, some care must be taken when extrapolating dogs to humans. The dog, being a quadruped, supports 60% of

body weight in the thoracic limbs and 40% in the pelvic limbs, which differs from the biped posture of humans, which can affect OA's progression [43, 44].

A proposed direct analgesic effect for hyaluronan has been suggested in animal models by action over the opi- oid receptor [45]. An additional proposed mechanism of action for hyaluronan is producing endogenous hyaluro- nan production by the exogenous administration, basedon in vitro and in vivo studies [46]. This last mechanism may be supported because the product is rapidly cleared from the joint, and maximal clinical improvement doesnot occur for several weeks, between 60 and 90 days, while persisting for much longer [47]. Our results partly support these findings, with the difference that signifi- cant improvements were reached sooner and lasted lon- ger. Although we did not measure the amount and the persistence of the exogenous hyaluronan within the joint, the visual examination of SF in HG at the 8-day evaluation showed a clear SF, with increased viscositythat of Hylan G-F 20.

OA is a low-grade inflammatory disease, and IL-1 is themost important pro-inflammatory cytokine responsible for the catabolism in OA, affecting the disease's progression [48], and the histopathology and pathogenesis of dog OA closely resembles that of human OA [5]. IA hyaluronan in- hibits degenerative cartilage changes in animal models duemainly to its pro-inflammatory cytokines and degradative en-zymes [49]. Low molecular weight hyaluronan seems to bemost effective in reducing the release of cytokines [50]. Al-though a decrease in IL-1 levels was recorded in both groups,at 8 days, its concentration in CG was significantly lower. At this moment, this is probably due to the removal of synovialfluid at treatment day, followed by the injection of 0.9% NaCl, similar to the effect of a joint lavage, which may bemore effective than the administration of hyaluronan in re-ducing IL-1 levels. Still, IL-1 concentration levels remained lower than those at the initial evaluation in both groups. Asthis study was a clinical treatment experiment, no joint histo- logical samples were collected, which would help evaluate differences between Hylan G-F 20 and 0.9% NaCl injection. The reduction of IL-1 may reduce inflammatory levels, which are reflected in the temperature values re- corded during the thermographic evaluations. Mea- surements made on the Lt view, in particular, recorded variations throughout the entire follow-up period, with lower levels being recorded in CG.

Pain is the most relevant clinical sign of OA, and its evaluation is paramount to determine OA treatment effi- cacy so that data may be translated to human medicine [30]. There is strong evidence that humans and animals' type of pain is analogous, as they share neurophysiologic similarities [51]. However, painful experiences in OA are complex, involving several dimensions [52]. While ex- tremely useful in a clinical setting, CMIs can be susceptible to the caregiver placebo effect, associated with the variability in emotional and cognitive compo- nents of pain perception. On the other hand, the animal itself will not show a significant placebo effect, and the ability to perform daily activities will likely reflect a lower level of pain [53, 54]. We used several CMIs, to try to capture multiple dimensions of OA. As a whole, individual CMI scores in CG tended to worsen through time, while HG scores tended to improve. Still, some an- imals in CG showed improvements. While some patients with OA may spontaneously improve, a more plausible explanation is related to removing cytokine-loaded SF at treatment day, followed by the injection of 0.9% NaCl, similar to the effect of a joint lavage. Placebo saline in- jections have shown functional improvements that can last up to a 6-month follow-up [55].

Radiographic evaluation is a staple of OA monitoring. CCO and CFHO represent early radiographic signs that predict the development of hip OA clinical signs [23]. Previous reports have described that hyaluronan could not prevent OA progression based on radiographic as- sessment [18]. However, it decreased signs of pain and improved joint function after the onset of OA [56]. Our results support these findings. In CG, several radio- graphic findings progressed throughout the follow-up period, as expected in the disease's natural evolution. This was also observed in HG, even though some radio- graphic findings did only change at 180 days. Still, des- pite the evolution of radiographic findings, patients in HG showed better clinical, functional, and pain findings than CG. Also, in the 8-30 days' evaluation period, no significant differences were observed in HG between ani- mals with and without CCO and CFHO at the initial evaluation.

OA risk factors are well characterized and include hav- ing a higher bodyweight or being of older age [2]. To as- sess these factors' influence in response to treatment, we applied different cut-off values for weight. In both groups, increasing body weight generally correspondedto worse CMI. In HG, heavier patients had SI evaluation and deviation, even though the group still had better re- sults than CG throughout the study. Previous reports in- dicated that larger dogs achieved improvements of 30% or more at 12 weeks [47]. We described improvements earlier, even in heavier patients. Male dogs also scored worse in considered CMI, but this may be related to the fact that male dogs were significantly heavier than fe- males in all considered moments.

Regarding age, similar results were observed. Consid- ering animals above the sample's mean age, these pa- tients scoring worse on almost all CMI scores had lower pedometer counts and higher thermography values. Since OA is a chronic, progressive disease, it was not unexpected to see older patients record worse

evaluations, which may be linked with the disease's progression at its clinical signs. While expected, the difference in treatment results is quite pronounced, more than the effect of increased body weight.

IA hyaluronan administration has been described as producing mild heat, swelling, and/or erythema post-injection, which resolved spontaneously within a week [18]. These adverse effects are well tolerated and usually restricted to the injected joint [57, 58]. Similarly, we observed increased lameness in six cases, reflecting on the 8-day SI and deviation evaluations, when significantly worse scores were kept at HG. This spontaneously resolved by the 15-day evaluation. No additional medication was administered to the animals during the follow-up period. Considering the obtained results, Hylan G-F 20 may be a good therapeutic option for managing canine hip OA. Its administration was able to reduce pain levels and improve joint function compared to a control group. Due to the close resemblance of canine and human OA, it is possible that the same recommendation can be made for human hip OA. Still, as some differences in weight bearing exist between the two species, futures studies should enroll a greater number of animals and assess if similar results are observed in humans.

Conclusions

This study describes the effect of a single injection high molecular weight hyaluronan product in a naturally occurring canine model, with a long follow-up period. It provides important information for the characterization of the response to treatment, showing that Hylan G-F 20 can produce significant functional and pain level improvements in patients with OA, even those with factors related to worse response to treatment. For that reason, Hylan G-F 20 can be considered as a good therapeutic option for OA management, even in more advanced cases.

Abbreviations

CBPI: Canine Brief Pain Inventory; CMI: Clinical Metrology instruments; COI: Canine Orthopedic Index; CRP: C-reactive protein; HVAS: Hudson Visual Analogue Scale; IL-1: Interleukin 1; LOAD: Liverpool Osteoarthritis in Dogs; OA: Osteoarthritis; PIS: Pain Interference Score; PSS: Pain Severity Score; QOL: Quality of life

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Authors' contributions

JCA designed the protocol, conducted treatments, and prepared the manuscript. PJ and AS selected patients and conducted treatments. CL and LMC revised the protocol and prepared the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

This project was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval n° GD/32055/2018/P1, September 25, 2018) and complies with ARRIVE guidelines. All methods were carried out in accordance with relevant guidelines and regulations. Written, informed consent was obtained from the Institution responsible for the animals (Guarda Nacional Republicana, Portuguese Gendarmerie) through dispatch of the Doctrine and Training Commander n°327/16, dated September 16, 2016.

Consent for publication

Not applicable.

Competing interests

FujiFilm Europe GmbH provided the CRP tests used in this study, the Stance Analyser was provided by Companion, LiteCure LLC®, and the digital thermography camera was provided by Specman, Lda®.

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RESEARCH ARTICLE

The intra-articular administration of triamcinolone hexacetonide in the treatment of osteoarthritis. Its effects in a naturally occurring canine osteoarthritis model

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Abstract

Objective

To evaluate the effect of an intra-articular (IA) administration of triamcinolone hexacetonide, compared with saline.

Patients and methods

Forty (N = 40) hip joints were randomly assigned to a treatment group (THG, n = 20, receiving IA triamcinolone hexacetonide) and a control group (CG, n = 20, receiving IA saline). On treatment day (T0), and at 8, 15, 30, 90 and 180 days post-treatment, weight distribution, joint range of motion, thigh girth, digital thermography, radiographic signs, synovial fluid interleukin-1 and C-reactive protein levels were evaluated. Data from four Clinical Metrology Instruments was also gathered. Results were compared Repeated Measures ANOVA, with a Huynh-Feldt correction, Paired Samples T-Test or Wilcoxon Signed Ranks Test. A Kaplan-Meier test was performed to compare both groups, with $p<0.05$.

Results

Joints were graded as mild (65%), moderate (20%) and severe (15%). Patients of both sexes, with a mean age of 6.5±2.4 years and bodyweight of 26.7±5.2kg, were included. No differences were found between groups at T0. Comparing THG to CG, weight distribution showed significant improvements in THG from 8 ($p = 0.05$) up to 90 days ($p = 0.01$). THG showed lower values during thermographic evaluation in the Lt view ($p<0.01$). Pain and function scores also improved from 30 to 180 days. Increasing body weight, age, and presence of caudolateral curvilinear osteophyte corresponded to worse response to treatment.

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Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: The CRP tests were provided by FujiFilm Europe GmbH. The Stance Analyser used in this study was provided by Companion, LiteCure LLC. The digital thermography camera was provided by Specman, Lda. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

Results of the Kaplan Meier test showed significant differences between groups, with THG performing better considering several evaluations and scores.

Conclusion

THG recorded significant improvements in weight-bearing and in with the considered CMIs, particularly pain scores. Lower thermographic values were registered in THG up to the last evaluation day. Age, sex, and radiographic findings did significantly influenced response to treatment.

Introduction

Osteoarthritis (OA) is a disease transversal to all mammals and a source of chronic pain. For that reason, it represents a considerable burden to societies, representing a large investment in healthcare, while reducing productivity and quality of life [1–3]. Since OA is symptomatic only in the affected joint while, at the same time, lacking obvious extra-articular manifesta- tions, it is well suited to administer local therapy by intra-articular (IA) injection [4, 5]. The changes that occur in slowly progressive spontaneous dog OA closely match those of human OA, while maintaining the same life stages that go by at a faster progression rate, and sharing many of the same environmental conditions. For those reasons, the naturally occurring canine model is considered the closest to a gold standard [6–11].

The medical approach to OA aims at slowing disease progression while relieving symptoms, particularly pain [9, 12]. IA corticosteroids have been used for several decades to palliate pain and inflammation associated with OA and of joint's surrounding tissues [13, 14]. Its use should be especially considered in patients with moderate to severe pain, nonresponding to oral analgesic/non-steroidal anti-inflammatory drugs. A human systematic review has deemed triamcinolone more effective than betamethasone and methylprednisolone [15]. Triamcinolone hexacetonide (TH), in particular, is described as able to provide pain relief and improved mobility for prolonged periods [16, 17]. In a canine model of OA, animals treated with IA TH showed a significant reduction of osteophyte size compared with a control group. At the histological level, TH significantly reduced the severity of OA structural changes of cartilage and had no deleterious effects on normal cartilage [18]. By effect is obtained through a dose-dependent reduction in the cartilage proteolytic enzyme stromelysin, interleukin 1β, and the oncogenes c-fos and c-myc, which are involved in the metalloproteinases synthesis [19]. In human patients, the mean duration of effect of TH in patients with OA is around seven months [20].

Since pain and functional limitations are the most relevant clinical signs of OA, clinical tri- als and studies need to assess these parameters in order to evaluate patients and assessment of response to treatment [21–23]. Clinical metrology instruments (CMI) represent a patient-centred approach, and the most commonly used are the Canine Brief Pain Inventory (CBPI, divided in a pain severity score—PSS, and a pain interference score—PIS) and the Liverpool Osteoarthritis in Dogs (LOAD). Further validated CMIs include the Hudson Visual Analogue Scale (HVAS), and the Canine Orthopaedic Index (COI, divided into four scores: stiffness, gait, function and quality of life—QOL). As a whole, CMIs complement the evaluation of the multidimensional experience that is OA related pain [9, 23–31]. Typically, OA pain is local- ized and related to movement or weight-bearing of the affected joints, and affected patients commonly bear less weight on a painful limb. Evaluating weight distribution through stance analysis is a sensitive evaluation canine lameness [22, 23, 32]. Additional functional

evaluations aim at assessing activity levels and mobility impairments [26]. Pedometry is a simple and inexpensive method to assess mobility levels, which can measure ambulatory activity with an acceptable level of accuracy [33]. Additional clinical measurements include the examination of muscle masses and evaluation of the joint range of motion, which are consistently reduced and restricted in OA patients [34–37].

Imaging plays a key role in the assessment of patients with joint disease and, in cases of hip OA, the ventrodorsal (VD) hip extended view is the most common pelvic radiographic projection. The ventrodorsal flexed view, also called frog-legged view (FL), useful for further evaluation of the presence of the circumferential femoral head osteophyte (CFHO) and caudolateral curvilinear osteophyte (CCO), radiographic findings related with the development of clinical signs [38–43]. By correlating changes in temperature patterns with various disease, degenerative or injury processes, digital thermography can provide a reproducible diagnostic tool [44–46]. This diagnosis modality can differentiate normal from osteoarthritis subjects [47, 48].

Since OA is a low-grade inflammatory disease, the analysis of synovial fluid (SF) can add additional information regarding the disease's characterization [1]. Interleukin 1 (IL-1) is the most important proinflammatory catabolic cytokine in OA, with a highly potent capability of inducting cartilage degradation and relation with lameness duration [49–51]. C-reactive protein (CRP) can be produced at the level of the inflamed tissues, and its shifts occur from a very early stage [52, 53]. It has been highly associated with knee OA in humans [54].

The goal of this study is to compare the effect of triamcinolone hexacetonide to a control group in the management of OA in a naturally occurring canine model, using several outcome assessment modalities. We hypothesize the intra-articular administration of triamcinolone hexacetonide will be able to reduce the clinical signs of OA, compared to a control group.

Materials and methods

The study protocol was approved by the ethical review committee of the Universidade de Évora (ORBEA, approval n° GD/32055/2018/P1, September 25th, 2018), and complies with the ARRIVE guidelines. Written, informed consent was obtained from the Institution responsible for the animals. Twenty dogs were selected based on medical history records, physical, orthopaedic, neurological and radiographic examinations compatible with hip OA, and the sample comprised forty (N = 40) joints of twenty active police working dogs with bilateral hip OA. To be included in the study, patients should be over two years, have a bodyweight over 20kg and should not have received any medication or nutritional supplement for over six weeks before enrolment in the study. Patients with any other documented or suspected orthopaedic or neurological disease, or additional concomitant disease (ruled out through physical examination, complete blood count, and serum chemistry profile), were excluded.

In a double-blinded study, dogs with affect joints were randomly assigned to a control group (CG, n = 20 joints) or a treatment group (THG, n = 20 joints). Evaluations were conducted on days 0 (treatment day), 8, 15, 30, 90 and 180. Days were counted from treatment day (day 0). An outline of all procedures on each evaluation day is presented in Table 1. The same researcher performed all evaluations.

On treatment day, patients in CG received an IA administration of 2ml of 0.9%NaCl. On the same day, patients in THG received an IA administration of 20mg in a volume of 1 ml of triamcinolone hexacetonide (Bluxam, Riemser Pharma). IA administrations and radiographic examination were conducted under light sedation, induced with a combination of medetomidine (0.01mg/kg) and buthorphanol (0.1mg/kg), given intravenously. After all procedures were conducted, sedation was reversed with atipamezole (100–150μg/kg), administered intramuscularly. Both a VD extended legs and FL views were obtained. On the VD view, the

Table 1. Procedures conducted in each day. Days are counted from treatment day.

Procedure	Day					
	0	8	15	30	90	180
Treatment	X					
Digital Thermography	X	X	X	X	X	X
Digital radiography	X			X	X	X
Stance analysis	X	X	X	X	X	X
Pedometer	X	X	X	X	X	X
Goniometry	X	X	X	X	X	X
Thigh girth measurement	X	X	X	X	X	X
Clinical Metrology Instruments	X	X	X	X	X	X
Synovial fluid CRP	X	X		X	X	X
Synovial fluid IL-1	X	X		X	X	X

CMI—Clinical Metrology Instruments; CRP—C-Reactive Protein; IL-1—Interleukin 1.

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presence of the following findings was assessed; an irregular wear on the femoral head, making it misshapen and with a loss of its rounded appearance; a flattened or shallow acetabulum, with irregular outline; CCO; new bone formation on the acetabulum and femoral head and neck; a worn away angle formed at the cranial effective acetabular rim; subchondral bone sclerosis along the cranial acetabular edge; and CFHO [43, 55, 56]. For the IA administration, patients were positioned in lateral recumbency, with the joint of interest uppermost. A window of 4x4cm in the area surrounding the greater trochanter was clipped and aseptically prepared. The limb was then placed in a neutral, parallel to the table position and a 21-gauge with 2.5" length needle was introduced just dorsal to the greater trochanter, perpendicular to the long axis of the limb until the joint was reached [57]. Confirmation of correct needle placement was obtained through the collection of SF. As much SF as possible was aspirated and kept for the posterior determination of IL-1β and CRP concentrations, and the treatment or saline was administered.

Evaluation of weight distribution was carried out with a weight distribution platform (Companion Stance Analyzer; LiteCure LLC[1], Newark, Delaware, United States), following manufacturer's guidelines. The equipment was placed in the centre of a room, at least 1 meter from the walls. It was also calibrated at the beginning of each day and zeroed before each data collection. For the evaluation, patients were led to stand on the platform, with one foot on each quadrant, with their heads facing forward. The left-right symmetry index was calculated with the following formula: symmetry index = $[(WB_R-WB_L)/((WB_R+WB_L)x0.5)]x100$ [28, 58], where WB_R is the value of weight-bearing for the right pelvic limb, and WB_L is the value of weight-bearing for the left pelvic limb. Negative values were made positive. Additionally, we considered a deviation from the normal 20% weight-bearing for a pelvic limb [59], calculated by subtracting WB to 20.

Pedometers were worn around the patient's neck, attached to an adjustable lightweight col- lar, to measure ambulatory activity and mobility levels [60]. They were worn for a week before the first evaluation time, to order to establish a baseline value, and before each evaluation time. Mean daily counts were considered, calculated by dividing the register number of steps by the number of considered days. In a quiet room, with as much time as needed to answer all items, trainers completed a copy of HVAS, CBPI, COI and LOAD, in sequence by the same handler at all evaluation days.

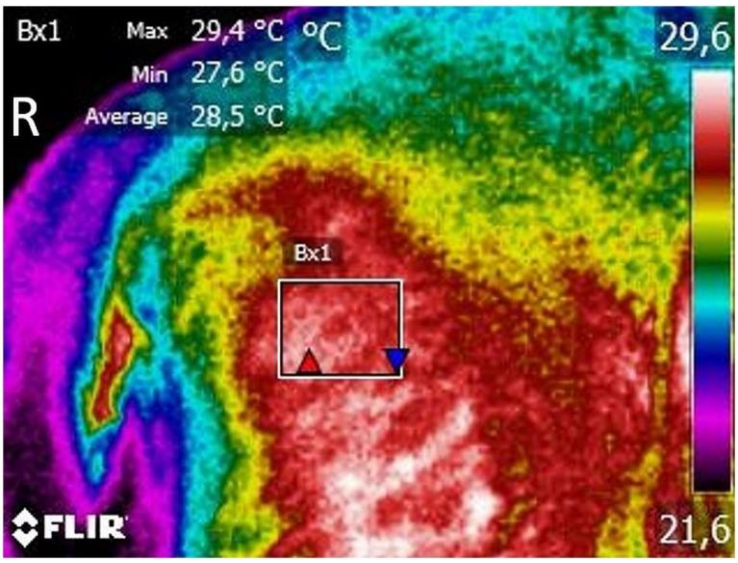

Fig 1. A lateral view of a dog with moderate osteoarthritis, with the greater trochanter in the centre, at a distance of 60 cm. The range of temperature was set at 15–40˚C and emissivity at 0.98. Thermographic images were analyzed with a Rainbow HC colour pallet. A temperature box is placed on the anatomical area of the hip joint.

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For the digital thermography evaluation, animals were kept for 30 minutes in a room with controlled temperature, at 21˚C. During this period, they were allowed to walk around the room calmly. A dorsoventral image was obtained with patients in a symmetrical upright standing, including the area from the last lumbar to the first coccygeal vertebrae, at a distance of 60 cm [61]. A lateral view was also obtained, with the greater trochanter in the centre, at the same distance. All images were taken with a FLIR ThermaCAM E25[1] model (FLIR Systems, Wilsonville, Oregon, United States). The posterior analysis was conducted with free software (Tools, FLIR Systems, Inc), using a rainbow colour pallet. Temperature boxes were placed on the anatomical area of the hip joint, to determine mean and maximal temperatures (Fig 1).

Both thigh girth and joint range of motion were determined with the patient in lateral recumbency. A Gullick II measuring tape was used to evaluate thigh girth, at a distance of 70% thigh length, measured from the tip of the greater trochanter, with an extended leg [62]. Hip joint ROM was then determined with a goniometer at extension and flexion with a flexed stifle [63].

Determination of IL-1β and CRP concentrations were made using Fuji Dri-Chem Slides VC-CRP PS (FUJIFILM Europe GmbH), read with a DRIChem NX500i (FUJIFILM Europe GmbH), and a DuoSet Ancillary Canine IL-1β Reagent kit (R&D Systems, United Kingdom), read with a FLUOstar OPTIMA (BMG Labtech).

After treatment, animals were rested for three consecutive days and resumed their regular activity over five days. On days 1 and 3 after the procedure, the veterinarian examined all patients in order to determine existing signs of exacerbated pain, persistent stiffness of gait and changes in posture. If no complaints were registered, the animal could resume its normal activity [64, 65].

Normality was assessed with a Shapiro-Wilk test. Different group's results were compared in each evaluation day, and each measured parameter was compared with the result observed at treatment day. To assess the effect of different parameters on the patients' clinical evolution, results were compared by sex, age and different cut off values for body weight with Repeated

Measures ANOVA, with a Huynh-Feldt correction, Paired Samples T-Test, or Wilcoxon Signed Ranks Test. A Kaplan-Meier test was performed to evaluate the time to return to baseline values of symmetry index and CMI scores, compared with the Breslow test. All results were analyzed with IBM SPSS Statistics version 20, and a significance level of $p < 0.05$ was set.

Results

The sample included 40 hip joints (n = 20 left and n = 20 right) of active police working dogs with a mean age of 6.5±2.4 years and bodyweight of 26.7±5.2kg. Both sexes (male n = 28, female n = 12) and four breeds were represented: German Shepherd Dogs (n = 8), Belgian Malinois Shepherd Dogs (n = 6), and Dutch Shepherd Dog (n = 6). At T0, 26 joints were classified as mild (65%), 8 as moderate (20%) and 6 as severe (15%), according to the Orthopedic Foundation for Animals hip grading scheme. No differences were found between groups at the initial evaluation. After the initial rest period, all animals resumed normal activity, with similar workload and movement compared to that before treatment.

Clinical and laboratorial findings

Values recorded for different assessments at the initial evaluation, and its variations throughout the study, for THG and CG, are presented in Table 2. Comparing results between groups with repeated measures ANOVA with a Huynh-Feldt correction, significant differences between groups were found concerning body weight ($F(2.8,140) = 4.2$, $p < 0.01$), deviation ($F(4.8,109) = 2.8$, $p = 0.02$), symmetry index ($F(2.8,77.8) = 7.5$, $p < 0.01$), mean temperature on a DV view ($F(3.9,93.9) = 6.6$, $p < 0.01$), maximal temperature on a DV view ($F(3.6,86.2) = 6.9$, $p < 0.01$), mean temperature on a Lt view ($F(4.5,113.2) = 26.7$, $p < 0.01$), maximal temperature on a Lt view ($F(4.1,101.6) = 96.2$, $p < 0.01$), joint flexion ($F(4.9,146.7) = 19.5$, $p < 0.01$), IL-1 synovial concentration ($F(1.9,58.3) = 4.9$, $p = 0.02$). Significant differences were observed between groups regarding CMI scores, specifically PSS ($F(5,120) = 2.4$, $p < 0.05$), PIS ($F(5,120) = 2.6$, $p = 0.03$) and function ($F(2.8,69.2) = 2.4$, $p = 0.04$). Evolution of the symmetry index in CG and THG is presented in Fig 2. Results of the Kaplan Meier test are presented in Table 3. Kaplan Meier curves for symmetry index and function score are presented in Figs 3 and 4, respectively. A dorsoventral digital thermography view is presented in Fig 5.

Radiographic findings

Frequency of different radiographic findings observed in CG and THG, at the initial evaluation, are presented in Table 4. In THG, an increase in the frequency of flattened or shallow acetabulum, with irregular outline was observed at 90 and 180 day ($p < 0.05$). Increased new bone formation on the acetabulum and femoral head and neck was also observed at 90 day ($p < 0.05$), as the frequency of CCO at 180 day ($p < 0.05$).

In CG, an increase in the frequency of flattened or shallow acetabulum, with irregular out- line, an increase was observed at 90 ($p < 0.01$) and 180 day ($p < 0.01$), compared with the initial evaluation day. In the THG, patients without CCO had higher LOAD ($p < 0.05$), PSS ($p = 0.02$), PIS ($p = 0.01$), stiffness ($p = 0.01$), function ($p < 0.05$), gait ($p < 0.01$), QOL ($p < 0.01$) and COI scores ($p < 0.01$), and lower HVAS scores ($p < 0.01$). At 15 day, they had lower mean and maximal thermographic evaluations on a DV ($p = 0.01$ for both) and mean on a Lt view ($p = 0.04$), and higher LOAD ($p < 0.01$), stiffness ($p = 0.04$), function ($p < 0.01$), gait ($p < 0.01$), QOL ($p < 0.01$) and COI scores ($p < 0.01$). Again at the 30 and 90 day evaluations, those without CCO at the initial evaluation had higher HVAS ($p < 0.05$ and $p < 0.01$, respectively), and lower PSS ($p < 0.01$ for both), PIS ($p < 0.01$ and $p = 0.02$, respectively), LOAD ($p < 0.01$ for both), stiffness ($p = 0.04$ and $p = 0.02$, respectively), function ($p < 0.01$ and $p = 0.04$, respectively), gait

Table 2. Mean values (±standard deviation) of different parameters evaluated at the initial evaluation and throughout the study.

Modality		Treatment day				8 days							15 days								
		CG		THG		CG			THG			P	CG			THG			P		
		mean	SD	mean	SD	mean	SD	p	mean	SD	p		mean	SD	p	mean	SD	p			
Goniometry	Flexion (˚. mean±SD)	55.0	4.4	57.0	4.1	55.3	3.7	<0.01[}]	55.3	4.7	0.09	1.0	57.2	5.2	0.14	58.0	5.3	0.53	1.0		
	Extension (˚. mean±SD)	151.2	3.9	148.0	8.8	149.9	4.6	0.95	149.4	4.5	0.41		151.1	3.5	0.07	151.3	5.1	<0.05[}]	1.0		
	Thigh girth (cm. mean ±SD)	31.2	2.6	30.4	3.4	31.1	3.3	0.94	30.8	3.1	1.0	0.49	31.1	2.9	0.86	32.6	3.5	<0.01[}]	0.58		
	Pedometer (daily steps ±SD)	1445.7	755.7	1539.6	397.3	829.5	931.3	0.58	989.8	1554.6	0.85		606.0	309.5	0.15	1215.1	793.8	0.03[}]	1.0		
CMI	HVAS (0–10)	6.8	1.2	5.7	1.9	6.7	1.5	0.48	6.4	1.7	0.19	0.16	6.8	1.2	0.6	6.3	1.6	0.72	0.16		
	CBPI—PSS (0–10)	3.1	1.9	4.2	2.8	3.4	2.3	0.69	2.9	1.8	0.03[}]	<0.05[}]	3.7	2.8	0.2	3.2	1.9	0.04[}]	0.04[}]		
	CBPI—PIS (0–10)	3.2	2.2	4.8	3.3	3.4	2.1	0.01[}]	3.3	2.1	0.04[}]	0.02[}]	3.6	2.1	0.01[}]	3.5	2.1	0.03[}]	0.01[}]		
	COI—Stiffness (0–16)	3.4	3.4	6.8	4.2	4.1	3.3	0.31	5.5	3.6	0.38	0.51	4.1	3.2	0.31	5.1	4.0	0.46	10.19		
	COI—Function (0–16)	3.6	4.1	6.3	5.7	4.1	4.0	0.64	4.1	4.0	0.21	<0.05[}]	4.4	5.5	0.72	3.8	3.9	0.75	0.03[}]		
	COI—Gait (0–20)	4.7	5.2	10.5	5.9	5.4	6.1	0.29	7.3	4.7	0.18	0.21	5.8	4.3	0.02[}]	6.8	5.6	<0.05[}]	0.19		
	COI—QOL (0–12)	4.5	2.6	6.2	3.9	4.6	2.7	0.62	5.5	3.0	0.28	0.85	4.7	2.9	0.06	4.4	3.6	0.56	0.09		
	COI—Overall score (0–64)	16.4	14.7	29.8	19.1	18.2	13.8	0.22	23.8	14.6	0.19	0.48	18.6	13.8	0.14	21.0	16.7	0.83	0.19		
	LOAD (0–52)	13.6	10.5	23.2	14.1	14.4	12.7	1.0	18.3	12.0	0.98	0.33	14.3	10.7	0.83	18.0	12.3	0.88	0.14		
Digital Thermography	DV (˚. mean±SD)	24.7	1.9	25.3	0.6	25.2	1.3	0.01[}]	25.3	0.6	0.08	<0.01[}]	24.4	1.6	0.61	23.4	2.5	0.03[}]	1.0		
	DV max (˚. mean±SD)	26.3	1.9	26.4	1.6	25.8	1.0	0.06	24.9	1.6	0.24	<0.01[}]	26.7	1.6	0.97	24.6	2.5	0.03[}]	1.0		
	Lt (˚. mean±SD)	28.7	2.7	26.5	1.9	31.6	2.1	<0.01[}]	30.5	2.8	<0.01[}]	<0.01[}]	29.7	2.9	<0.01[}]	28.5	4.0	0.02[}]	<0.01[}]		
	Lt max (˚. mean±SD)	31.9	3.1	30.4	4.1	34.9	1.0	<0.01[}]	34.6	1.1	<0.01[}]	<0.01[}]	34.9	0.8	<0.01[}]	34.0	1.9	<0.01[}]	<0.01[}]		
Synovial fluid	IL-1 (pg/mL. mean±SD)	170.9	120.4	208.5	95.2	72.3	42.4	<0.01[}]	98.4	80.8	0.04[}]	0.04[}]	-	-	-	-	-	-	-		
	CRP (mg/mL. mean±SD)	0.4	1.0	2.5	3.5	0.3	1.2	<0.01[}]	0.0	0.0	0.18	1.0	-	-	-	-	-	-	-		
Weight-bearing	Symmetry Index (mean ±SD)	24.7	20.3	53.9	50.4	18.7	17.1	0.06	19.2	18.1	<0.05[}]	<0.05[}]	23.9	16.3	0.18	18.9	10.9	0.01[}]	0.03[}]		
	Deviation (mean±SD)	2.8	3.6	4.7	4.4	2.78	1.987	0.3	2.1	1.9	0.08	0.43	2.94	2.127	0.47	2.2	1.6	<0.05[}]	0.02[}]		

Modality		30 days							90 days							180 days						
		CG			THG			P	CG			THG			P	CG			THG			P
		mean	SD	P	mean	SD	P		mean	SD	p	mean	SD	p		mean	SD	P	mean	SD	P	
Goniometry	Flexion (˚. mean±SD)	53.6	2.9	0.11	51.8	3.9	<0.01[}]	<0.01[}]	52.7	2.9	0.02[}]	52.0	3.8	<0.01[}]	<0.01[}]	51.6	2.2	0.00[}]	49.3	4.3	<0.01[}]	<0.01[}]
	Extension (˚. mean±SD)	150.8	3.4	0.06	152.3	3.7	0.09	1.0	150.8	2.9	0.07	151.9	3.0	<0.01[}]	0.23	151.3	2.9	0.17	150.2	3.7	0.26	1.0
	Thigh girth (cm. mean ±SD)	30.6	2.7	0.39	29.5	3.1	0.25	0.44	31.6	2.7	0.54	31.5	3.7	0.09	0.43	31.5	2.2	0.2	30.2	3.7	0.96	0.32
	Pedometer (daily steps ±SD)	594.5	663.4	0.48	747.9	548.2	0.65	0.15	451.9	463.0	0.4	410.8	497.4	0.15	0.08	434.9	455.8	0.2	376.0	263.3	<0.01[}]	0.63
CMI	HVAS (0–10)	6.4	1.4	0.14	6.3	1.9	0.64	0.17	6.6	1.7	0.22	6.5	1.3	0.84	0.21	6.5	1.4	0.04[}]	6.4	1.6	0.13	0.12
	CBPI—PSS (0–10)	3.7	2.6	0.03[}]	3.8	2.6	0.02[}]	0.01[}]	4.1	2.9	0.02[}]	3.2	2.1	0.57	0.32	3.6	3.1	0.02[}]	3.6	2.5	0.98	0.23
	CBPI—PIS (0–10)	3.8	2.6	0.01[}]	5.7	5.3	0.04[}]	<0.05[}]	3.9	2.8	0.01[}]	3.1	2.4	0.07	0.33	3.5	2.4	0.01[}]	4.0	3.0	0.63	0.22
	COI—Stiffness (0–16)	4.6	4.1	0.87	5.3	4.0	0.78	0.48	4.6	3.9	0.33	4.9	3.6	0.50	0.39	4.0	5.7	0.82	4.8	4.4	0.10	0.48
	COI—Function (0–16)	5.7	5.3	0.2	5.7	5.3	0.79	<0.05[}]	5.0	5.2	0.21	4.9	4.4	0.75	<0.01[}]	4.0	5.4	1.0	3.3	3.8	0.39	0.01[}]
	COI—Gait (0–20)	6.9	5.1	0.19	7.8	6.8	0.69	0.19	5.7	5.5	0.11	6.7	4.6	0.18	0.16	4.4	5.4	0.87	6.5	5.8	0.01[}]	0.46
	COI—QOL (0–12)	5.3	3.3	0.39	5.0	3.7	<0.01[}]	0.57	5.1	2.8	0.02[}]	4.3	2.7	0.49	0.59	4.7	2.6	0.09	4.4	3.2	0.03[}]	0.25
	COI—Overall score (0–64)	22.4	19.1	0.04[}]	22.9	19.7	0.75	0.26	20.1	15.7	0.29	20.3	14.5	0.53	0.14	15.7	14.9	0.1	20.9	18.9	0.09	0.21
	LOAD (0–52)	16.4	13.1	0.22	18.1	13.5	0.47	0.88	13.1	12.4	0.72	15.1	9.4	0.07	0.17	13.1	12.4	0.88	16.0	12.0	0.03[}]	0.07
Digital Thermography	DV (˚. mean±SD)	25.3	1.5	0.36	24.4	0.8	0.36	1.0	26.1	1.2	0.04[}]	26.3	1.6	0.02[}]	0.68	25.6	1.4	0.89	25.1	0.9	0.57	1.0
	DV max (˚. mean±SD)	25.2	2.1	0.88	25.9	0.7	0.31	1.0	27.4	1.4	0.14[}]	27.6	1.1	<0.01[}]	0.02[}]	26.9	1.4	0.74	26.5	0.9	0.78	1.0
	Lt (˚. mean±SD)	29.8	2.2	<0.01[}]	29.5	2.3	<0.01[}]	<0.01[}]	28.4	1.8	<0.01[}]	29.0	2.6	<0.01[}]	<0.01[}]	27.3	1.8	0.21	28.7	2.3	<0.01[}]	<0.01[}]
	Lt max (˚. mean±SD)	33.9	1.2	<0.01[}]	33.7	1.6	<0.01[}]	<0.01[}]	30.5	1.9	<0.01[}]	31.4	2.6	<0.01[}]	<0.01[}]	29.7	1.9	0.13	31.2	2.3	<0.01[}]	<0.01[}]

(*Continued*)

Table 2. (Continued)

Synovial fluid	IL-1 (pg/mL. mean±SD)	122.9	108.9	0.05	186.6	104.5	0.24	1.0	159.6	59.1	0.13	169.1	55.3	0.36	1.0	184.2	68.5	0.25	145.1	33.5	0.07	1.0
	CRP (mg/mL. mean±SD)	0.48	0.9	0.18	0.1	0.2	0.43	1.0	0.4	0.8	0.36	0.0	0.0	0.42	1.0	0.0	0.0	0.5	0.0	0.0	0.18	1.0
Weight-bearing	Symmetry Index (mean ±SD)	18.9	12.2	0.04‡	13.3	8.6	<0.01‡	<0.01‡	27.4	12.1	0.29	14.0	13.5	0.02‡	0.02	27.0	27.9	0.51	20.7	22.7	0.01‡	0.22
	Deviation (mean±SD)	2.5	1.917	0.2	1.8	2.3	0.02‡	<0.01‡	2.72	2.27	0.29	2.3	2.1	0.04‡	<0.01‡	2.61	2.973	0.55	2.0	2.8	0.04‡	0.75

CBPI—Canine Brief Pain Inventory; CRP—C-reactive protein; COI—Canine Orthopedic Index; DV—dorsoventral view; HVAS—Hudson Visual Analogue Scale; IL-1 –Interleukin 1; LOAD— Liverpool Osteoarthritis in Dogs; LT—lateral view; PIS—Pain Interference Score; PSS—Pain Severity Score; QOL—Quality of Life.

‡ indicates significance when comparing the value registered by a group at an evaluation day with T0 (p), and comparing both groups at each follow-up day (P).

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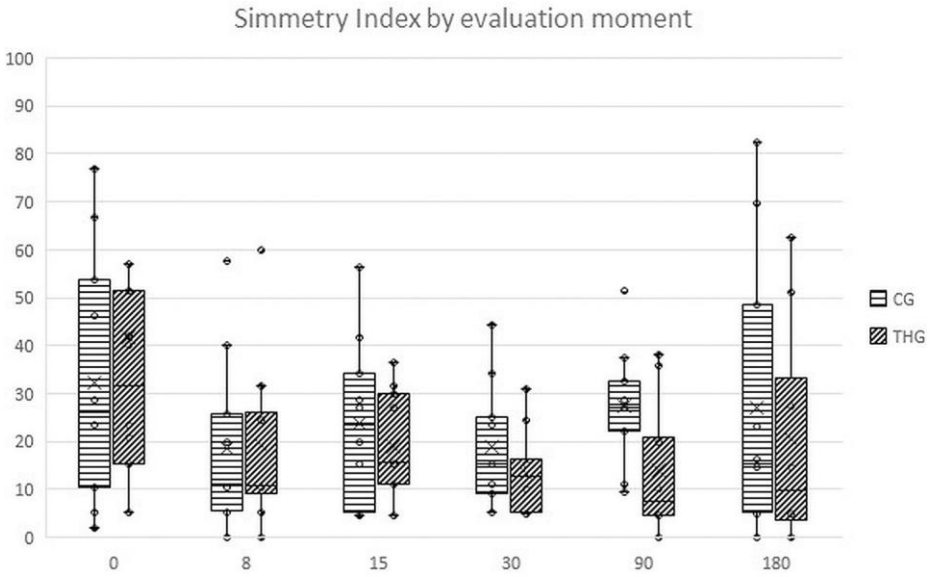

Fig 2. Overall evolution of symmetry index in the control group (CG) and treatment group (THG). Box plots represent the median, 25th and 75th percentiles, and whiskers represent 10th and 90th percentiles.

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($p < 0.01$ and $p = 0.04$, respectively), QOL ($p < 0.01$ for both) and COI scores $p < 0.01$ and $p = 0.02$, respectively). At the final evaluation, they had only higher HVAS score ($p = 0.03$). In CG, animals without CCO at the initial evaluation did now show significant differences with those that did. However, at the 30-day evaluation, they had higher mean thermographic evaluation on a Lt view ($p = 0.04$), better joint flexion ($p = 0.03$), lower IL-1 and higher CRP concentration levels ($p = 0.04$ for both). On the 90 day evaluation, animals without CCO had lower maximal thermographic evaluation on a Lt view ($p < 0.05$) and lower CRP values t 180 days ($p = 0.02$).

Table 3. Time to return to baseline values for weight-bearing distributions (symmetry index and deviation) and CMIs, calculated with Kaplan-Meier estimators and compared with the Breslow test.

Variable	Breslow test	Treatment			
		CG		THG	
		mean±SD	95% CI	mean±SD	95% CI
Symmetry Index	0.003†	47.0±11.8	23.8±70.2	96.0±12.8	70.9±121.1
Deviation	0.022†	44.8±12.1	21.1±68.5	81.8±14.7	52.9±110.6
HVAS	0.269	48.7±12.4	25.4±73.9	66.1±14.2	38.3±93.9
PSS	0.065	63.2±17.2	29.6±96.8	90.2±17.6	55.7±124.7
PIS	0.000†	8.4±0.4	7.7±9.0	118.6±16.3	86.7±150.5
LOAD	0.000†	40.7±10.6	19.9±61.4	124.3±15.9	93.1±155.5
Stiffness	0.004†	64.7±16.9	31.4±97.9	130.8±11.6	108.1±153.5
Function	0.046†	65.4±13.4	39.2±91.6	112.6±15.6	81.9±143.2
Gait	0.001†	52.7±14.6	23.9±81.4	117.0±15.1	87.5±146.5
QOL	0.044†	60.9±15.0	31.4±90.4	119.3±17.5	85.0±153.6
COI	0.146	52.7±13.4	26.5±78.9	85.6±15.9	54.4±116.9

COI—Canine Orthopedic Index; HVAS—Hudson Visual Analogue Scale; LOAD—Liverpool Osteoarthritis in Dogs; PIS—Pain Interference Score; PSS—Pain Severity Score; QOL—Quality of Life.

† indicates significance.

https://doi.org/10.1371/journal.pone.0245553.t003

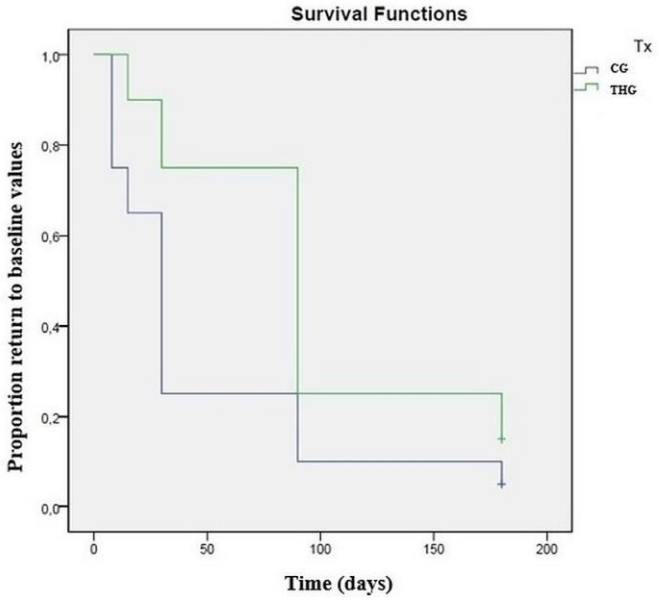

Fig 3. Kaplan-Meier curve demonstrating a significant difference between the control group (CG) and triamcinolone hexacetonide group (THG) in time for the symmetry index to return to baseline values (p = 0.003).

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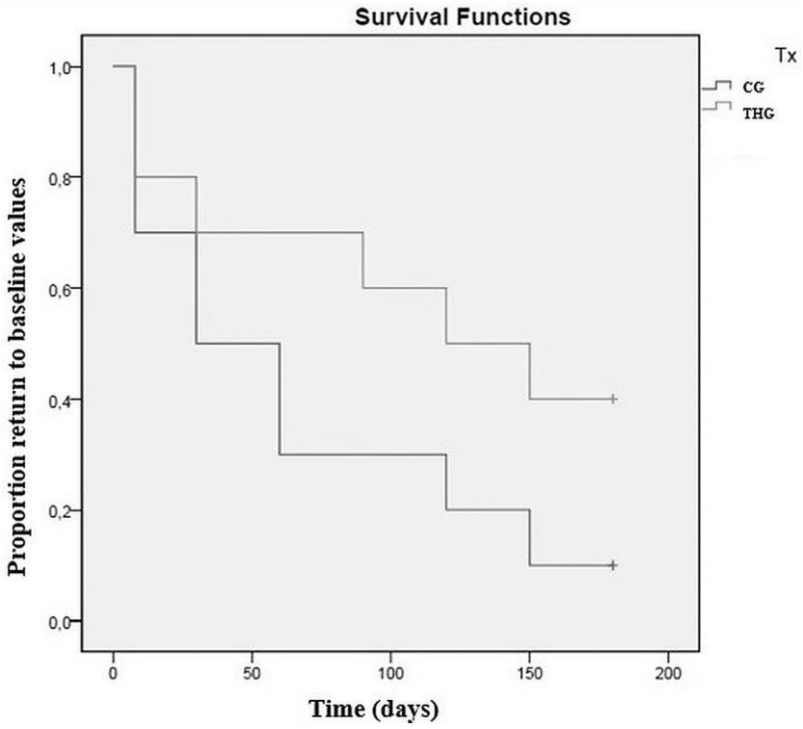

Fig 4. Kaplan-Meier curve demonstrating a significant difference between the control group (CG) and triamcinolone hexacetonide group (THG) in time for function score to return to baseline values (p = 0.046).

https://doi.org/10.1371/journal.pone.0245553.g004

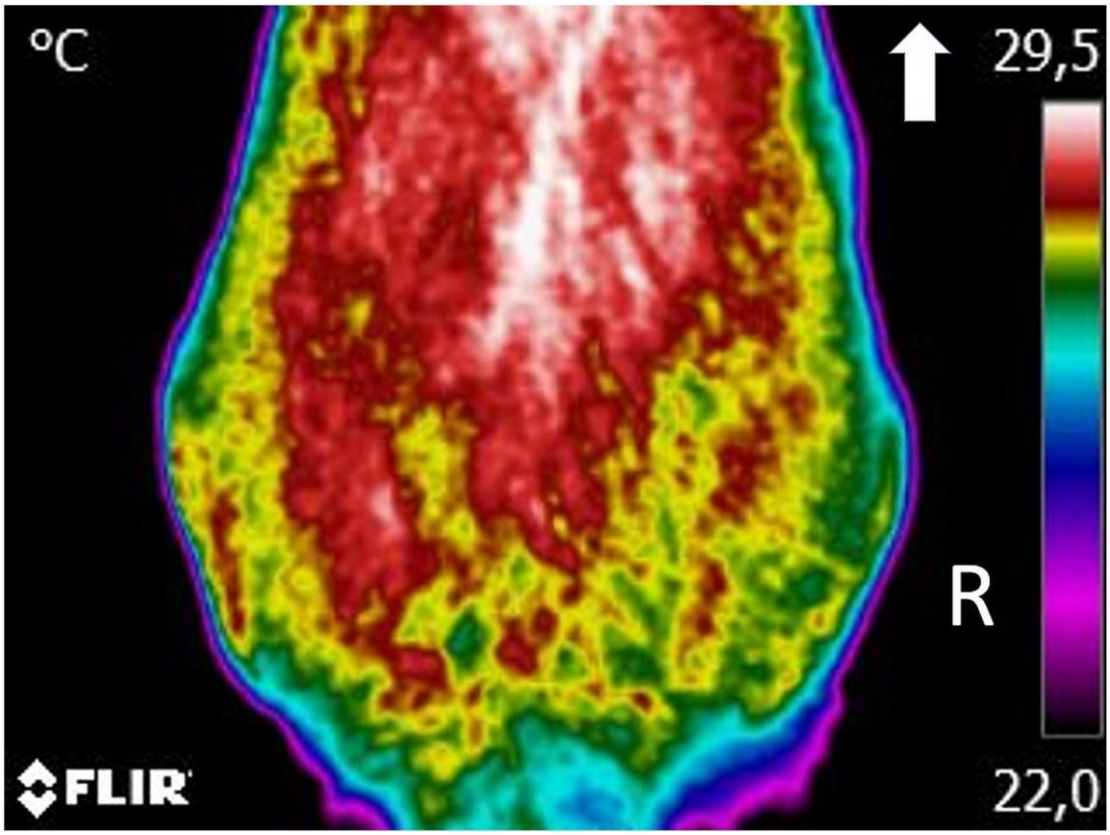

Fig 5. A dorsoventral view of a dog with moderate osteoarthritis, including the area from the last lumbar vertebra to the first coccygeal vertebra at a minimum, at a distance of 60 cm. Arrow indicates cranial direction. The range of temperature was set at 15–40˚C and emissivity at 0.98. Thermographic images were analyzed with a Rainbow HC colour pallet.

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In the THG, patients without CFHO at the initial evaluation had lower pedometer counts ($p < 0.01$) and lower HVAS ($p = 0.04$) and higher PIS scores ($p = 0.03$) on that day. At 8 day, they had higher body weight ($p = 0.02$) and higher deviation ($p = 0.01$) and symmetry index ($p < 0.05$). At 15 day, they had higher body weight ($p = 0.04$), lower pedometer count ($p < 0.01$)

and higher LOAD score ($p < 0.05$). Again at 30 day, these patients showed higher deviation

($p = 0.04$) and symmetry index ($p < 0.01$). At 90 day, they had higher deviation ($p = 0.03$), sym- metry index ($p < 0.01$) and higher synovial IL-1 concentration ($p = 0.01$). At the final

Table 4. Frequency of radiographic findings in the Control (CG) and Treatment Groups (THG) in a ventrodorsal and frog-leg views, at the initial evaluation.

Radiographic finding	THG				CG			
	Present		Absent		Present		Absent	
Irregular wear on the femoral head, making it misshapen and with a loss of its rounded appearance	20	100%	0	0%	17	85%	3	15%
Flattened or shallow acetabulum, with irregular outline	16	80%	4	20%	11	55%	9	45%
Caudolateral curvilinear osteophyte (CCO)	8	40%	12	60%	5	25%	15	75%
New bone formation on the acetabulum and on femoral head and neck	17	85%	3	15%	20	100%	0	0%
The angle formed at the cranial effective acetabular rim is worn away	18	90%	2	10%	18	90%	2	10%
Subchondral bone sclerosis along the cranial acetabular edge	20	100%	0	0%	19	95%	1	5%
Circumferential femoral head osteophyte (CFHO)	8	40%	12	60%	3	15%	17	85%

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evaluation day, they had lower pedometer counts ($p = 0.02$). In CG, joints without CFHO at the initial evaluation had higher joint extension ($p < 0.01$) and HVAS ($p = 0.02$), lower PSS ($p = 0.01$) and PIS scores ($p = 0.03$) at the 8 day evaluation. At 15 day, they had higher mean thermographic values on a Lt view ($p = 0.02$), lower PSS ($p = 0.02$) and PIS scores ($p < 0.05$). This higher mean thermographic values on a Lt view was again observed at 30 day ($p = 0.01$) and higher HVAS scores ($p = 0.02$) at 90 day. At the final evaluation, they had higher maximal thermographic values on a Lt view ($p = 0.04$), and lower PSS ($p = 0.05$) and PIS scores ($p < 0.03$).

Comparisons by sex

In the THG, females had lower symmetry index ($p < 0.01$) and lower synovial CRP concentration ($p < 0.01$). At the 8 day evaluation day, females were lighter than males ($p = 0.04$) and had higher synovial IL-1 levels ($p = 0.04$). Additional differences were observed at 15 days, with female dogs having lower mean and maximal temperatures on a DV ($p < 0.01$ for both) and Lt views ($p = 0.04$ and $p < 0.01$, respectively). At 30 days, females had higher pedometer counts ($p < 0.01$) and, at 90 days, lower mean and maximal temperatures on a DV view ($p < 0.05$ and $p < 0.01$, respectively). At the final evaluation day, no differences were observed between sexes. Female dogs of CG had significantly lower body weight throughout the study ($p = 0.01$). In the first evaluation, they also showed higher values in all thermographic evaluations ($p < 0.01$) and lower PIS scores ($p = 0.04$). Again at 8 days, higher thermographic evaluations were recorded ($p < 0.01$), except maximal value on a Lt view, as higher joint extension values ($p < 0.01$). A higher joint extension was again observed in female dogs at 15 days ($p = 0.04$), with lower PIS scores ($p = 0.03$). At the 30 days evaluation females again showed higher thermographic maximal values on an LT view max ($p < 0.01$). Female dogs at 90 days had lower thigh girth ($p = 0.03$) and lower PSS and PIS scores ($p = 0.01$). At the final evaluation day, they had higher extension values ($p = 0.02$), higher HVAS ($p = 0.02$), and lower PSS ($p < 0.01$) e PIS ($p < 0.01$), stiffness ($p = 0.02$), function ($p = 0.02$), gait ($p < 0.01$), QOL ($p = 0.02$) and COI ($p = 0.01$) scores.

Comparisons by bodyweight

Comparing animals in THG with a weight below the mean value of the sample, had higher deviation ($p = 0.03$), symmetry index ($p = 0.04$), mean and maximal values on thermography DV view ($p < 0.01$ and $p = 0.02$, respectively), lower thigh girth ($p < 0.01$) and lower synovial IL-1 levels ($p = 0.02$). At 8 days, they had lower thigh girth ($p < 0.01$) and lower PSS ($p = 0.01$), PIS ($p < 0.01$) and stiffness scores ($p = 0.01$). After 15 days, lighter patients had higher symmetry index ($p = 0.03$) and lower thigh girth ($p < 0.01$). At 30 days, these patients had higher pedometer counts ($p = 0.01$) and lower thigh girth ($p < 0.01$). At the 90-day evaluation, they had higher mean and maximal values on thermography Lt view ($p = 0.02$ and $p < 0.01$, respectively), lower thigh girth ($p < 0.01$) and higher joint flexion ($p = 0.03$). 30 At the final evaluation day, lighter animals had lower mean and maximal values on thermography a DV ($p < 0.01$ for both) but higher on a Lt view ($p < 0.01$ for both), lower thigh girth ($p < 0.01$) and higher joint flexion ($p = 0.03$). In CG, lighter patients registered had lower PIS scores ($p = 0.04$) at the initial evaluation. These patients, at the 8 day evaluation, had higher thermographic mean and maximal values on a DV ($p = 0.03$ and $p = 0.02$, respectively), lower thigh girth ($p = 0.01$), and higher stiffness ($p = 0.03$), function ($p < 0.01$), gait ($p = 0.03$) and COI scores ($p < 0.01$). Significant differences were again observed at 15 days, with lighter patients showing lower thigh girth ($p = 0.04$) and HVAS ($p < 0.05$), and higher stiffness, function, gait QOL e COI scores ($p < 0.01$). They also had lower CRP concentrations at 30 days ($p = 0.04$) and higher HVAS

scores ($p = 0.02$). At 90 days, they had lower thigh girth ($p < 0.01$) and IL-1 levels ($p = 0.02$) at 90 days. At the final day of evaluation, lighter animals showed higher mean thermographic values on a DV view ($p < 0.01$), and higher joint flexion ($p = 0.02$) and extension ($p < 0.01$).

Comparisons by age

Considering patients above or below the mean age in the THG, at the initial evaluation younger patients had higher mean and maximal temperature on a Lt view ($p < 0.01$ for both), and lower PSS ($p = 0.01$), stiffness ($p = 0.04$), function ($p = 0.02$), gait ($p = 0.02$), QOL ($p = 0.04$), and COI scores (0.01). After treatment, at 8 days, they had lower deviation ($p = 0.03$) and symmetry index ($p = 0.04$), higher mean temperature on a Lt view ($p < 0.01$), lower synovial IL-1 concentration ($p = 0.04$), and lower PIS ($p < 0.02$) and function scores ($p = 0.03$). At 15 days, they had higher mean and maximal temperature on a DV view ($p < 0.01$ for both) and Lt view ($p < 0.01$ for both), and lower PIS ($p < 0.01$), stiffness ($p = 0.03$), gait ($p < 0.01$) and COI score ($p = 0.04$). After 30 days, younger animals had higher mean and maximal temperature on a Lt view ($p = 0.01$ for both), higher HVAS ($p < 0.01$), and lower PSS ($p < 0.01$), PIS ($p < 0.01$), LOAD ($p < 0.01$), stiffness ($p < 0.01$) and QOL scores ($p < 0.01$). Differences regarding CMI scores were observed again at 90 days, with the same patients having higher HVAS ($p < 0.01$), and lower PSS ($p = 0.01$), PIS ($p < 0.01$) and QOL scores ($p = 0.01$). At the final evaluation, patients bellow the mean age value had higher pedometer counts ($p < 0.01$), lower deviation ($p = 0.02$) and SI ($p < 0.01$), higher HVAS ($p < 0.01$), and lower PSS ($p < 0.01$), PIS ($p < 0.01$), LOAD ($p < 0.01$), stiffness ($p < 0.01$), function ($p = 0.04$), gait ($p < 0.01$), QOL ($p < 0.01$) and COI scores ($p < 0.01$). In the CG at the initial evaluation, younger patients had higher maximal values on the thermographic Lt view ($p = 0.04$), lower LOAD ($p = 0.02$), stiffness ($p < 0.01$), function ($p < 0.01$), gait ($p < 0.01$) and COI ($p < 0.01$) scores. After 8 days, they showed lower SI ($p < 0.01$), higher maximal values on the thermographic Lt view ($p = 0.02$) and lower LOAD ($p = 0.04$), stiffness ($p < 0.01$), function ($p < 0.01$), gait ($p < 0.01$), QOL ($p < 0.01$) and COI ($p < 0.01$) scores. Again at 15 days, younger patients presented lower LOAD ($p < 0.01$), stiffness ($p < 0.01$), function ($p < 0.01$), gait ($p < 0.01$), QOL ($p < 0.01$) and COI ($p < 0.01$) scores. At the 30 day evaluation, they again presented improved evaluations in several parameters, with lower mean and maximal values on the thermographic DV ($p < 0.01$ and $p = 0.02$, respectively) Lt view ($p = 0.02$, for the mean value), higher joint flexion ($p = 0.01$) and lower LOAD ($p < 0.01$), stiffness ($p < 0.01$), function ($p < 0.01$), gait ($p < 0.01$), QOL ($p < 0.01$) and COI ($p < 0.01$) scores. Better CMI scores was again observed at 90 days, specifically lower LOAD ($p = 0.04$), stiffness ($p < 0.01$), function ($p < 0.01$), gait ($p < 0.01$), QOL ($p < 0.01$) and COI ($p < 0.01$) scores. At the final evaluation, patients bellow the sample mean age had lower deviation and SI ($p = 0.03$ and $p < 0.01$, respectively), and stiffness ($p < 0.01$), function ($p < 0.01$), gait ($p < 0.01$), QOL ($p < 0.01$) and COI ($p < 0.01$) scores.

Discussion

OA is a leading cause of disability around the world, impacting the physical and mental well-being of populations, posing a substantial toll on healthcare and financial resources [66]. To our knowledge, this is the first study to describe the effect of a single injection of triamcinolone hexacetonide on several clinical, imaging and laboratorial signs in a naturally occurring canine osteoarthritis model, with a long follow up period.

There are some reports evaluating the effect of IA TH in humans. A 2-year follow-up study showed that TH has long-term safety, with no deleterious effects being observed deriving from IA administration [67–69]. Also, patients treated had significant increases in ROM and improvements in pain [67]. These improvements are noticeable with the results of the Kaplan

Maier test for symmetry index, with results in SG taking significantly longer to return to baseline values. It was also observable with different scores, as function or stiffness. Comparing TH to a saline injection, TH (40mg) had higher effectiveness than the placebo group in the four weeks in terms of pain in movement, pain scale, and ultra-sound measurement of synovial hypertrophy [70]. Treatment with 20mg or 40mg of TH produced equal relapse after six months in patients with chronic polyarthritis and when treating medium-sized joints. With that in mind, since no difference in outcome was found between the compared doses, the authors advised that lower dose should be preferred, reducing pharmaceutical costs and metabolic side effects [71, 72]. As a whole, these reports present the overall safety and effectiveness of IA TH in the management of OA, measured with multiple validated CMI and other clinical evaluations. With this animal model, a single IA TH administration was able to significantly reduce weight-bearing changes in affected joints up to the 90-day evaluation compared with a control group. Besides changes in different CMI scores, particularly pain scores calculated with the CBPI, were observed. This is of particular interest, since pain is a hallmark of OA, and its characterization produces valuable data that may translate to humans [21, 73, 74]. Individual CMI scores in THG improved for a majority of animals were observed with several of the considered questionnaires, in many cases up to the last evaluation. A significant difference was also observed with the Kaplan Meier test for the majority of the considered scores. In contrast, patients in the CG had worse scores (meaning lower HVAS scores and higher values in the remaining considered CMIs scores) throughout the follow-up period, particularly with as time progressed. Our results are in line with previously described effects for TH, but since in dogs OA progresses faster while maintaining the same stages [9], it is possible that in humans results may be observed for a more extended period. Additionally, since patients who composed this sample are active working dogs, their musculoskeletal structures are under increased stress and effort [75], leading to an earlier decline in initially observed improvements. It would also be of interest to have intermediary follow-ups between the 90 and 180-day evaluations, in order to further precise the duration of treatment efficacy. Some patients in the CG also showed some improvements, particularly at the 8 and 15-day evaluations. It may be due to the natural evolution of osteoarthritis, with the possibility for spontaneous improvements in some stages of the disease. An additional possibility is related to the removal of cytokine loaded synovial fluid at the time of the evaluation and the posterior administration of saline, which can lead to an effect similar to a joint lavage. In fact, placebo saline injections have shown an effect in functional improvements that can last up to a 6-month follow-up [76].

IL-1 is commonly pointed out as the most important proinflammatory cytokine responsible for the catabolic events in OA [1, 49, 50]. Corticosteroids are, of the medications available for the treatment of OA, the ones with most potent anti-inflammatory activity, specifically through the downregulation of the synthesis of inflammatory mediators such as IL-1β, TNF-α and COX-2 in the synovial fluid [64, 77–79]. In this study, significant differences between CG and THG were only observed at the 8-day evaluation and, even though IL-1 levels were lower in both groups compared to the initial evaluation, levels in CG were lower. This shows that TH can reduce IL-1 levels, which can be partly responsible for its ability to improve OA clinical signs, but the removal of synovial fluid and the administration of saline is also able to do so. Despite this effect, IL-1 levels cannot be the sole responsible for OA clinical signs, since, despite lower IL-1 levels of CG, this group still had worse clinical signs.

Radiographic evaluation is a staple of OA monitoring. Previous OA animal studies have demonstrated a decrease in disease progression or a protective role of corticosteroids injections, based on histological and biochemical findings [18, 80–84]. A recent systematic review of canine models of OA induction concludes that the reports regarding its IA use appear to be unanimously positive, with lower doses with sustained joint concentrations having a protective

effect [85]. In CG, the natural progression of the disease was observed as expected, and radiographic signs progressed throughout the follow-up period. In the THG, radiographic signs also progressed, particularly in the more advanced follow-up days. Although radiographic signs progressed, they seemed to be less severe in THG. We only characterized radiographic sings as present or absent, so this eventual protective role of IA TH may not be entirely recorded. The evaluation of CCO and CFHO is of particular clinical interest, as they represent early radiographic signs that predict the development of the clinical [42, 43, 86, 87]. Our results support this finding since animals with CCO or CFHO in both views showed worse clinical signs at the initial evaluations. Also, animals with these radiographic findings showed a worse response to treatment, despite THG having a positive evolution compared with CG. Digital thermography can assess inflammatory pain in osteoarthritic patients [47, 48]. Our findings showed mixed results regarding the thermographic evaluation. While, in some evaluation days, animals with higher temperatures recorded in different thermography evaluations corresponded to those patients with worse clinical signs, in other days, it did not. This may indicate that other characteristics may play an important role, e.g. the amount of muscle masses surrounding the joint, in this case, represented by thigh girth, or variations in body weight. Documented risk factors for OA include higher bodyweight and increasing age [2]. To evaluate the effect of these factors, we considered results with a cut-off for weight and compared younger to older patients. Considering bodyweight at different cut-off points, heavier patients in both groups generally showed worse clinical signs, particularly with different CMI scores. This effect of body weight may also be responsible for the fact that females also had better CMI scores than males since they were also lighter. Similarly, patients with age above the mean sample age showed worse evaluations during the follow-up period and worse response to treatment in THG. This may reflect that older patients may have a more degenerate joint with more advanced OA-induced changes, and therefore worse clinical signs, with a reduced ability to show improvements in response to therapy.

Side effects of IA procedures are mainly related to discomfort from the procedure itself, localized pain post-injection and flushing. IA corticosteroids can also cause synovitis, in a reactive reaction called a steroid flare, with a described prevalence of 2–6% [4, 78, 88, 89]. More rare reported side effects include crystal-induced synovitis, calcification and steroid arthropathy [90]. We observed increased lameness in four patients, which spontaneously resolved within 48 hours. No additional medication was administered to the animals during the follow-up period. When compared to other therapeutic options, IA TH may be a more cost-effective option due to its lower cost [91].

Conclusions

To our knowledge, this is the first study to describe the effect of a single injection of triamcinolone hexacetonide in a naturally occurring canine model, with a long follow up period. THG recorded significant improvements in weight-bearing up to the 90-day follow-up. Improvements were also observed with the considered CMIs, particularly pain scores. Lower thermographic values were registered in THG up to the last evaluation day. Age, sex, and radiographic findings did significantly influenced response to treatment.

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6. A THERAPEUTIC PROTOCOL FOR THE INTRA-ARTICULAR TREATMENT OF PATIENTS WITH HIP OA USING THE DOG AS AN ANIMAL MODEL

Intra-articular triamcinolone hexacetonide, stanozolol, hylan G-F 20 and a platelet concentrate for the control and treatment of osteoarthritis in a naturally occurring canine osteoarthritis model: a randomized controlled study – Published in Scientific Reports – Impact factor 3.998, Quartile 1.

Comparison of efficacy if the intra-articular injection of triamcinolone hexacetonide, stanozolol, hylan G-F 20 and a platelet concentrate in police working dogs with bilateral hip osteoarthritis – Published in Frontiers in Veterinary Science – Impact factor 2.140, Quartile 1.

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Intraarticular triamcinolone hexacetonide, stanozolol, Hylan G-F 20 and platelet concentrate in a naturally occurring canine osteoarthritis model

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Osteoarthritis (OA) is a disease transversal to all mammals, a source of chronic pain and disability, a huge burden to societies, with a significant toll in healthcare cost, while reducing productivity and quality of life. The dog is considered a useful model for the translational study of the disease, closely matching human OA, with the advantage of a faster disease progression while maintaining the same life stages. In a prospective, longitudinal, double-blinded, negative controlled study, one hundred (N = 100) hip joints were selected and randomly assigned to five groups: control group (CG, n = 20, receiving a saline injection), triamcinolone hexacetonide group (THG, n = 20), platelet concentrate group (PCG, n = 20), stanozolol group (SG, n = 20) and hylan G-F 20 group (HG). Evaluations were conducted on days 0 (To, treatment day), 8, 15, 30, 60, 90, 120, 150 and 180 days post-treatment, consisting of weight distribution analysis and data from four Clinical Metrology Instruments (CMI). Kaplan–Meier estimators were generated and compared with the Breslow test. Cox proportional hazard regression analysis was used to investigate the influence of variables of interest on treatment survival. All results were analyzed with IBM SPSS Statistics version 20 and a significance level of $p < 0.05$ was set. Sample included joints of 100 pelvic limbs (of patients with a mean age of 6.5 ± 2.4 years and body weight of 26.7 ± 5.2 kg. Joints were graded as mild (n = 70), moderate (n = 20) and severe (n = 10) OA. No differences were found between groups at To. Kaplan–Meier analysis showed that all treatments produced longer periods with better results in the various evaluations compared to CG. Patients in HG and PCG took longer to return to baseline values and scores. A higher impact on pain interference was observed in THG, with a 95% improvement over CG. PCG and HG experienced 57–81% improvements in functional evaluation and impairments due to OA, and may be a better options for these cases. This study documented the efficacy of several approaches to relieve OA clinical signs. These approaches varied in intensity and duration. HG and PCG where the groups were more significant improvements were observed throughout the follow-up periods, with lower variation in results.

Osteoarthritis (OA) is a disease transversal to all mammals[1]. Being a source of chronic pain and disability, it represents a huge burden to societies, with a significant toll in healthcare cost, while reducing productivity and quality of life[2,3]. Its prevalence is expected to rise, due to a simultaneous increase in life expectancy and obesity[4]. The pathologic process, clinical presentation and response to treatment are very similar in humans and dogs, making the dog a frequent animal model for the study of osteoarthritis[5]. In fact, the changes that occur in slowly progressive spontaneous dog OA closely match those of human OA, with the added advantage of a faster disease progression while maintaining a juvenile, adolescent, adult and geriatric life stages. In addition, companion

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animals share many of the same environmental conditions that their human counterparts. For those reasons the natural occurring canine model is considered an useful model of human OA, and exploring spontaneous canine OA can help improve human and dog health[6–11].

The medical approach to OA aims at slowing disease progression while relieving symptoms, particularly pain, and improving overall function[9,12]. Imaging plays a key role in the assessment of patients with joint disease. In the case of hip OA, the ventrodorsal (VD) hip extended view is the most commonly performed radiographic view[13,14]. This view is a valuable tool for evaluating the presence of hip OA[15]. Affected patients commonly bear less weight on an affected limb, since OA pain is related to movement or weight-bearing impairments of the affected joints. Evaluating weight distribution through stance analysis is a sensitive evaluation of lameness in dogs[16–19]. Weight distribution and off-loading or limb favouring at the stance are commonly used subjective assessments during the orthopaedic examination[20]. Dogs with OA may not be overtly lame at a walk or a trot but exhibit subtle shifts in body weight distribution at a stance due to pain[18,21]. Stance analysis has been reported as sensitive for detecting lameness in dogs, proposed to be an equivalent or superior measurement of pain associ- ated with hip OA than vertical impulse and peak vertical force VI and PVF[18]. OA pain is a multi-dimensional experience, which encompasses more than just a functional aspect, and treatment interventions must address this reality[17,22,23]. Clinical metrology instruments (CMI) represent a patient-centred approach, and the most commonly used ones to evaluate dogs are the Canine Brief Pain Inventory (CBPI, divided in a pain severity score—PSS, and a pain interference score—PIS) and the Liverpool Osteoarthritis in Dogs (LOAD). In addition, the Hudson Visual Analogue Scale (HVAS), developed to assess the degree of lameness in dogs, and the Canine Orthopaedic Index (COI, divided in four scores: stiffness, gait, function and quality of life—QOL) are further validated CMIs which can complement the evaluation of the multi-dimensional, not directly measured experi- ence that is OA related pain[9,17,19,24–30].

IA therapies several advantages over systemic medications, as safety, especially when certain comorbidities are present, and bioavailability[31]. IA corticosteroids have been used for several decades to palliate pain and inflammation associated with OA and surrounding tissues[32,33]. Triamcinolone hexacetonide (TH), in particular, is described as able to provide pain relief and improved mobility for prolonged periods[34,35]. Autologous platelets are a regenerative treatment modality for OA, used with the aim to stimulate the natural healing cascade and regeneration of tissues, through a supraphysiologic release of growth factors directly at the treatment site[36–39]. Stanozolol is a synthetic derivative of testosterone, and its properties include anabolic/androgenic activity[40]. When administered IA, it is able to induce fibroblasts to increase collagen production, decrease nitric oxide production and induce osteoblast proliferation and collagen synthesis[41–44]. It also has a chondroprotective and cartilage regeneration effect, while reducing osteophyte formation and subchondral bone reaction[42,45]. Even though hyaluronan's mechanism of action is not completely known and clinical trials have provided contradic- tory results, the aim of its use in the treatment of patients with OA is to reduce pain and improve function by supplementing the viscosity and elasticity of synovial fluid[46,47]. Additional anti-inflammatory, anti-nociceptive and chondroprotective properties have been suggested[48,49]. High molecular weight products seem to produce better results, particularly in patients with mild radiographic disease[50,51].

In order to assess long-term outcomes and to identify factors associated with poorer outcome, we compared the effect of the intraarticular administration triamcinolone hexacetonide, hylan G-F 20, stanozolol and a platelet concentrate in the management of OA in a natural occurring canine model. We hypothesize that the different treatments will be able to reduce the clinical signs of OA, compared to a control group.

Results

The sample included 100 pelvic limbs (n = 50 left and n = 50 right) of fifty active Police working dogs, with a mean age of 6.5 ± 2.4 years and body weight of 26.7 ± 5.2 kg, representing both sexes (male n = 60, female n = 40). They were housed in kennels of the Portuguese Gendarmerie Canine Unit, similar in size. All dogs remained in active work during and after the study, and engaged in search and rescue, product detection and use of force mission. Active work and training were conducted on a daily basis, with their individual handlers. At T0, 70 joints were classified as mild, 20 as moderate and 10 as severe, according to the Orthopedic Foundation for Animals hip grading scheme. Values and scores of each evaluation in all groups at T0 are presented in Table 1. No differences were found between groups at the initial evaluation ($p = 0.22$ for SI, $p = 0.075$ for deviation, $p = 0.12$ for HVAS, $p = 0.23$ for PSS, $p = 0.22$ for PIS, $p = 0.07$ for LOAD, $p = 0.48$ for stiffness, $p = 0.10$ for function, $p = 0.46$ for gait, $p = 0.25$ for QOL and $p = 0.21$ for COI).

All patients were followed up to the last evaluation moment (180 days) and, during this period, no additional treatment or medications was administered. Results of the Kaplan–Meier estimators with each evaluation method are presented in Table 2. All treatments showed longer periods with better results in the various evaluations compared to CG. Patients in HG and PCG, in particular, took longer to return to baseline values and scores. Results of the Cox proportional hazard regression are presented in Table 3. Increased lameness was observed in 16 patients in PCG, 8 in SG, 6 in HG and 4 in THG, which spontaneously resolved within 48–72 h.

Discussion

Osteoarthritis is a leading cause of disability around the world, which affects both the physical and mental well-being of populations. It poses a huge toll on healthcare resources and productivity52. Despite extensive research, still has limited treatment options available9,12. To our knowledge, this is the first prospective, negative controlled, double-blinded study to compare the effect of commonly used and novel IA treatments for the management of OA, in a naturally occurring canine model, with a long follow up period. Human reports on the effect of IA TH describe its long term safety, with improvements in joint range of motion and pain compared with a saline injection, with no differences between treatment with 40 mg or 20 mg

	CG	THG	HG	SG	PCG
Weight (kg. mean ± SD)	28.2 ± 6.2	26.5 ± 6.5	26.7 ± 3.5	27.1 ± 3.2	25.1 ± 9.6
Deviation (mean ± SD)	3.6 ± 2.7	4.7 ± 4.4	3.8 ± 3.5	4.3 ± 3.5	4.1 ± 2.2
Symmetry Index (mean ± SD)	24.8 ± 26.5	23.9 ± 50.4	21.7 ± 24.9	24.1 ± 13.9	22.6 ± 12.4
HVAS (0–10)	6.8 ± 1.2	5.7 ± 1.9	6.6 ± 1.4	6.7 ± 1.3	6.7 ± 1.4
PSS (0–10)	3.1 ± 1.9	4.2 ± 2.8	3.3 ± 2.6	2.9 ± 1.5	3.3 ± 2.6
PIS (0–10)	2.8 ± 1.7	4.8 ± 3.3	3.4 ± 2.3	2.3 ± 1.7	3.3 ± 2.8
LOAD (0–52)	13.6 ± 10.5	23.2 ± 14.1	17.0 ± 10.5	8.2 ± 5.2	13.3 ± 11.3
Stiffness (0–16)	3.4 ± 3.4	6.8 ± 4.2	3.4 ± 2.9	4.0 ± 2.8	3.9 ± 3.9
Function (0–16)	3.6 ± 4.1	6.3 ± 5.7	4.6 ± 3.5	4.0 ± 3.6	4.2 ± 4.7
Gait (0–20)	5.3 ± 3.9	10.5 ± 5.9	7.4 ± 4.7	5.2 ± 3.9	5.1 ± 5.4
QOL (0–12)	4.3 ± 2.5	6.2 ± 3.9	4.5 ± 3.1	4.3 ± 2.5	4.5 ± 3.6
COI (0–64)	17.6 ± 12.4	19.8 ± 19.1	19.9 ± 127	17.5 ± 12.4	17.7 ± 16.9

Table 1. Mean values (± standard deviation) at initial evaluation of evaluation conducted for control and treatment groups. *CG* Control group, *THG* Triamcinolone hexacetonide group, *HG* Hylan G-F 20 group, *SG* Stanozolol group, *PCG* Platelet concentrate group, *HVAS* Hudson Visual Analogue Scale, *PSS* Pain Severity Score, *PIS* Pain Interference Score, *LOAD* Liverpool Osteoarthritis in Dogs, *QOL* Quality of Life, *COI* Canine Orthopedic Index.

		Treatment									
		CG		THG		SG		HG		PCG	
Variable	Breslow test	Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI
Symmetry Index	0.000*	47.0 ± 11.8	23.8–70.2	96.0 ± 12.8	70.9–121.1	94.2 ± 15.9	62.9–125.4	104.1 ± 15.1	15.1–74.5	159.0 ± 10.3	138.9–179.1
Deviation	0.000*	44.8 ± 12.1	21.1–68.5	81.8 ± 14.7	52.9–110.6	55.6 ± 11.8	32.3–78.8	96.2 ± 16.3	64.2–128.1	138.0 ± 12.5	113.4–162.6
HVAS	0.000*	48.7 ± 12.4	25.4–73.9	66.1 ± 14.2	38.3–93.9	129.8 ± 13.4	103.5–156.1	117.0 ± 13.2	91.1–142.9	144.0 ± 11.6	121.2–166.8
PSS	0.015*	63.2 ± 17.2	29.6–96.8	90.2 ± 17.6	55.7–124.7	94.6 ± 16.4	62.5–126.7	142.6 ± 11.9	119.1–166.0	150.5 ± 9.7	130.9–169.1
PIS	0.000*	8.4 ± 0.4	7.7–9.0	118.6 ± 16.3	86.7–150.5	109.6 ± 17.3	75.8–143.2	114.0 ± 16.0	82.6–145.4	135.0 ± 10.6	114.2–155.8
LOAD	0.000*	40.7 ± 10.6	19.9–61.4	124.3 ± 15.9	93.1–155.5	123.8 ± 14.2	95.9–151.6	141.8 ± 11.6	119.2–164.4	120.0 ± 12.8	94.9–145.0
Stiffness	0.009*	64.7 ± 16.9	31.4–97.9	130.8 ± 11.6	108.1–153.5	111.2 ± 15.9	80.6–142.9	129.8 ± 13.9	102.6–157.0	141.0 ± 16.9	119.9–162.1
Function	0.000*	65.4 ± 13.4	39.2–91.6	112.6 ± 15.6	81.9–143.2	124.5 ± 15.4	94.2–154.8	168.0 ± 6.6	155.1–180.8	135.0 ± 9.4	116.5–153.5
Gait	0.001*	52.7 ± 14.6	23.9–81.4	117.0 ± 15.1	87.5–146.5	103.6 ± 15.7	72.8–134.4	115.5 ± 13.1	89.9–141.1	123.0 ± 12.5	98.5–147.5
QOL	0.004*	60.9 ± 15.0	31.4–90.4	119.3 ± 17.5	85.0–153.6	66.2 ± 17.5	31.8–100.6	125.6 ± 12.2	101.6–149.6	120.8 ± 13.1	95.1–146.5
COI	0.011*	52.7 ± 13.4	26.5–78.9	85.6 ± 15.9	54.4–116.9	78.1 ± 14.0	50.6–105.6	93.1 ± 16.7	60.3–125.9	138.0 ± 10.8	116.9–159.1

Table 2. Survival probability calculated with Kaplan–Meier estimators and compared with the Breslow test. See Table 1 for legend. *Indicates significance.

of TH[53–55]. Previous reports on the effect of a single administration of a platelet concentrate showed improvements in pain, kinetics and joint range of motion, up to the last evaluation point considered, which ranged from 12 weeks to 6 months[56,57]. Different reports on the use of stanozolol in animals described it as being able to resolve signs of lameness, while reducing osteophyte formation, subchondral bone reaction, and promoting articular cartilage regeneration[42,43]. A previous study on a canine model has provided information on the efficacy of IA hyaluronan in animals with OA of pain, function, lameness and kinetics when compared to pre-treatment and saline control, with maximum benefits noted at 4–8 weeks and gradually tampered down by a 6-month evaluation time point[58]. OA is characterized by variable degrees of clinical and functional impairments, with the severity of pain correlating with the functional status rather than radiographic grading of osteoarthritis, which does not correlate with functional status. Treatment, therefore, could be planned according to the clinical features and functional status instead of radiological findings[59,60]. For that reason, we compared well established to novel therapeutic approaches, while evaluating the impact of documented predisposing and clinical factors of OA. All treatments were able to improve clinical signs of OA compared with the treatment groups, in all of the evaluated dimensions, from pain, function and quality of life. As a whole, patients in PCG and HG took longer to return to baseline values, which may indicate that these approaches are better therapeutic approaches for the management of OA. It was interesting to see that patients in the CG did not remained or returned to initial values and scores at the first follow-up evaluations, in some cases taking 60 days to do so. It has been documented that placebo saline injections may have an effect in functional improvements that can last up to a 6-month follow-up[61], and a similar phenomenon may have occurred in this study.

We also investigated variables which could influence patient's response to treatment, regarding different OA dimensions. Age showed an impact in functional scores as HVAS, stiffness, gait and COI, as did the degree

Variable	Wheight distribution				HVAS (p = 0.000)		CBPI				LOAD (p = 0.000)	
	Symmetry Index (p = 0.008)		Deviation (p = 0.024)				PSS (p = 0.412)		PIS (p = 0.000)			
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Age	0.91 (0.83–10.01)	0.074	0.95 (0.86–1.05)	0.314	1.13 (1.02–1.25)	0.024*	1.08 (0.97–1.21)	0.163	0.95 (0.86–1.06)	0.374	0.93 (0.83–1.04)	0.190
Body weight			1.02 (0.97–1.07)	0.509	0.98 (0.93–1.03)	0.378	0.99 (0.93–1.05)	0.770	1.02 (0.97–1.08)	0.479	0.99 (0.94–1.06)	0.941
Sex												
Male	1.00				1.00		1.00		1.00		1.00	
Female	1.09 (0.59–2.02)	0.763	0.88 (0.49–1.57)	0.657	0.31 (0.15–0.62)	0.001*	1.24 (0.64–2.38)	0.524	1.12 (0.61–2.08)	0.715	1.18 (0.63–2.21)	0.616
Treatment		0.002*		0.005*		0.003*		0.154		0.000*		0.000*
Control	1.00				1.00		1.00		1.00		1.00	
HG	0.53 (0.27–1.04)	0.067	0.44 (0.22–0.88)	0.020*	0.27 (0.12–0.63)	0.002*	0.31 (0.12–0.78)	0.013*	0.07 (0.03–0.21)	0.000*	0.11 (0.04–0.28)	0.000*
PCG	0.20 (0.93–0.45)	0.000*	0.31 (0.15–0.62)	0.001*	0.26 (0.12–0.56)	0.001*	0.53 (0.24–1.16)	0.114	0.09 (0.03–0.24)	0.000*	0.31 (0.15–0.64)	0.001*
SG	0.39 (0.19–0.82)	0.012*	0.85 (0.44–1.66)	0.642	0.33 (0.14–0.77)	0.010*	0.57 (0.25–1.33)	0.195	0.08 (0.03–0.23)	0.000*	0.17 (0.07–0.37)	0.000*
THG	0.46 (0.22–0.96)	0.039*	0.43 (0.21–0.89)	0.023*	0.98 (0.93–1.03)	0.151	0.70 (0.31–1.59)	0.397	0.05 (0.02–0.14)	0.000*	0.09 (0.94–1.06)	0.000*
OFA score		0.831		0.179		0.026*				0.175		0.007*
Mild	1.00				1.00		1.00		1.00		1.00	
Moderate	0.85 (0.45–1.58)	0.605	1.01 (0.57–1.80)	0.973	1.29 (0.68–2.47)	0.435	1.09 (0.58–2.03)	0.79	1.74 (0.95–3.19)	0.074	2.93 (1.50–5.70)	0.002*
Severe	1.11 (0.47–2.62)	0.811	2.21 (0.95–5.2)	0.67	3.46 (1.39–8.55)	0.007*	0.79 (0.25–2.5)	0.70	0.86 (0.29–2.5)	0.784	1.56 (0.49–4.92)	0.451

Variable	COI									
	Stiffness (p = 0.001)		Function (p = 0.000)		Gait (p = 0.000)		QOL (p = 0.033)		Total (p = 0.000)	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	P	HR (95% CI)	p	HR (95% CI)	p
Age	1.19 (1.05–1.35)	0.007*	1.09 (0.99–1.21)	0.093	1.27 (1.13–1.43)	0.000*	0.98 (0.88–0.11)	0.835	1.15 (1.04–1.26)	0.005*
Body weight	1.03 (0.97–1.09)	0.324	1.03 (0.97–1.09)	0.346	1.01 (0.96–1.07)	0.717	1.04 (0.99–1.10)	0.106	0.98 (0.93–1.05)	0.603
Sex										
Male	1.00		1.00		1.00		1.00		1.00	
Female	2.02 (0.99–4.09)	0.051	1.40 (0.74–2.67)	0.304	1.28 (0.67–2.45)	0.453	2.58 (1.33–5.01)	0.005*	1.79 (0.95–3.37)	0.071
Treatment		0.032*		0.000*		0.002*		0.303		0.012*
Control	1.00		1.00		1.00		1.00		1.00	
HG	0.25 (0.09–0.64)	0.004*	0.09 (0.03–0.27)	0.000*	0.16 (0.06–0.42)	0.000*	0.45 (0.19–1.05)	0.064	0.41 (0.19–0.90)	0.026*
PCG	0.34 (0.15–0.79)	0.012*	0.40 (0.19–0.81)	0.011*	0.28 (0.13–0.61)	0.001*	0.55 (0.26–1.15)	0.113	0.28 (0.13–0.59)	0.001*
SG	0.41 (0.16–1.02)	0.054	0.27 (0.11–0.66)	0.004*	0.45 (0.19–1.03)	0.058	0.92 (0.45–1.85)	0.808	0.53 (0.24–1.14)	0.103
THG	0.34 (0.14–0.80)	0.014*	0.33 (0.15–0.73)	0.006*	0.32 (0.15–0.69)	0.004*	0.69 (0.32–1.47)	0.333	0.39 (0.18–0.85)	0.018*
OFA score		0.0159		0.014*		0.081		0.338		0.048*
Mild	1.00		1.00		1.00		1.00		1.00	
Moderate	0.97 (0.49–1.89)	0.936	1.07 (0.54–2.14)	0.841	0.66 (0.34–1.28)	0.222	0.81 (0.44–1.47)	0.477	0.61 (0.33–1.15)	0.127
Severe	2.48 (0.94–6.59)	0.068	4.59 (1.65–12.82)	0.004*	2.11 (0.79–5.58)	0.134	1.75 (0.67–4.55)	0.252	2.17 (0.86–5.45)	0.101

Table 3. Results Cox proportional hazard regression with the different outcome evaluations. See Table 1 for legend. *Indicates significance.

of OA, with patients with severe OA showing an impact on HVAS and function score. These findings could be explained with the fact that OA is a progressive, degenerative disease, which ultimately has a toll on joint function, without, however, a corresponding increase in pain levels. It is also well established that individuals with higher body mass index experience greater pain than individuals with lower index[62]. We evaluated the effect of

Procedure	Day								
	0	8	15	30	60	90	120	150	180
Treatment	X								
Stance analysis	X	X	X	X		X			X
Digital radiography	X			X		X			X
HVAS	X	X	X	X	X	X	X	X	X
CBPI	X	X	X	X	X	X	X	X	X
COI	X	X	X	X	X	X	X	X	X
LOAD	X	X	X	X	X	X	X	X	X

Table 4. Procedures conducted in each evaluation moment. Days are counted from treatment day. *CBPI* Canine Brief Pain Inventory, *COI* Canine Orthopedic Index, *HVAS* Hudson Visual Analogue Scale, *LOAD* Liverpool Osteoarthritis in Dogs.

body weight in the evolution of OA, which is not the same as body mass index, but since patients in this sample were working dogs, with an ideal body condition score, we chose to evaluate body weight instead which did not influenced any of the evaluation made.

Comparing the effect of different treatments, as a whole they had an effect on all dimensions evaluated, except PSS. Reasons for that may be related with the nature of these animals are working dogs and, for that reason, tend to show few signs of overt pain. In fact, pain is more easily and commonly detected through its impact on measurable parameters, such as weigh bearing, or on active exercises[57]. This may be reflected on an effect of different treatments on the PIS score, but not on the PSS score. PCG and HG registered effects for longer periods, and better improvements according to the Cox hazard regression with the different evaluations made. Considering measurable parameters, patients in PCH showed an 81% and 69% improvement in SI and deviation, respectively, while HG showed 61% and 57% improvements. These seem to be the preferred treatments for functional impairments due to OA. In addition to these evaluations, PCG and HG also registered greater improvements in several scores as HVAS, stiffness, function, gait and COI. Better impact on pain interference was observed in THG, which could be attributed to the high anti-inflammatory effect of corticosteroids, and the relation between pain and inflammation.

Side effects related after IA treatment are documented, and usually include injection pain and local inflamma- tion, that take 2–10 days to resolve[43,58,63,64]. We observed increased lameness in all groups, which spontaneously resolved within 48–72 h.

This study presents some limitations, namely the inclusion of two joints from each dogs, as an association may occur between limbs. This effect has been described in humans[65,66]. Still, the inclusion of contralateral limbs from the same patient is common in animal models, even for the calculation of a symmetry index[27,42,58]. A reason for this is that quadrupeds show more complex compensation mechanism than just side-to-side. Results from the weight bearing evaluation of the patients of this study show that the major compensation occurs in the contralateral thoracic limb, rather than side-to-side. In addition, a majority of joints considered in this study had mild OA. Further studies should include a larger number of the remaining hip grades to determine if similar results are obtained. The safety and efficacy of repeated IA injections should also be investigated.

Conclusions

To our knowledge, this is the first prospective, negative controlled, double-blinded study to compare the effect of commonly used and novel IA treatments for the management of OA, in a naturally occurring canine model, with a long follow up period. It provides important information for the characterization of the effects of these treatment modalities, duration of observed improvements function and pain, in addition to information regarding candidates for each one.

Methods

The study protocol was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval nº GD/32055/2018/P1, September 25th, 2018), and complies with ARRIVE guidelines. All experiments were performed in accordance with relevant guidelines and regulations. Written, informed consent was obtained from the Institution responsible for the animals. In a prospective, longitudinal, double-blinded, negative controlled study, patients were selected after screening of the Portuguese Gendarmerie Canine Unit, based on history, physical, orthopedic, neurological and radiographic examinations compatible with bilateral hip OA. The sample comprised one hundred (N = 100) hip joints of fifty active Police working dogs. It constituted a convenience sample, similar in size to previously published reports on this topic[58,67,68]. Inclusion criteria comprised age over two years, bodyweight over 20 kg, symptomatic in both limbs, with the same OA grade on both hips, and patients should not have received any medication or nutritional supplement for over six weeks. Cases with any other documented or suspected orthopaedic or neurological disease, or any other concomitant disease, were ruled out through physical and radiographic, examination, complete blood count and serum chemistry profile.

After selection, patients were randomly assigned to one of five groups, 10 dogs per group, and treated bilaterally: control group (CG, n = 20 joints), triamcinolone hexacetonide group (THG, n = 20 joints), platelet concentrate group (PCG, n = 20 joints), stanozolol group (SG, n = 20 joints) and hylan G-F 20 group (HG, n = 20

joints). Evaluations were conducted on days 0 (treatment day), 8, 15, 30, 60, 90, 120, 150 and 180 days post treatment. An outline of all procedures on each moment is presented in Table 4. All evaluations and procedures were performed by the same researcher.

On treatment day, patients in CG received an IA administration of 2 ml of 0.9%NaCl, given IA. On the same day, patients in THG received an IA administration of 20 mg/1 ml of triamcinolone hexacetonide (Bluxam, Riemser Pharma, Portugal). In SG, IA administration of stanozolol (Estrombol, Laboratório Fundacion), at a 0.3 mg/kg dose was performed[69,70]. Patients in HG received 2 ml of hylan G-F 20 (Synvisc, Sanofi, Portugal). For patients in PCG, 3 ml of platelet concentrate, prepared with the commercially available V-PET kit, according to the manufacturer's instructions, was applied. For the preparation of the platelet concentrate, fifty-five milliliters of whole blood were collected from the jugular vein of the patient and then introduced into the provided closed system. There, the blood flowed by action of gravity through a filter, where the platelets where concentrated. The final platelet concentrate was then collected and was used in the following 5 min of its preparation.

IA administrations and radiographic examination were conducted under light sedation, induced with a combination of medetomidine (0.01 mg/kg) and buthorphanol (0.1 mg/kg), given intravenously. A VD extended legs projection was used, and joints classified according to the Orthopedic Foundation for Animals hip grading scheme[14]. A full description of the OFA hip grading scheme is available online. For the IA administration, patients were placed in lateral recumbency, with the affected limb uppermost. A window of 4 × 4 cm in the area surrounding the greater trochanter was clipped and aseptically prepared. The limb was then placed in a neutral position, parallel to the table. A 21-gauge with 2.5″ length needle was then introduced just dorsal to the greater trochanter, perpendicular to the long axis of the limb, until the joint was reached[71]. Confirmation of correct needle placement was obtained through the collection of synovial fluid, and the treatment or saline were administered. Stance analysis was conducted with a weight distribution platform (Companion Stance Analyzer; LiteCure LLC, Newark, Delaware, United States). The equipment was placed in the centre of an observation room, at least 1-m from the walls. Complying with manufacturer's guidelines, the platform was calibrated at the beginning of each testing day and zeroed before each data collection. After an acclimatization period, animals were encouraged to stand on the weight distribution platform. To secure a correct position, the patient's trainer helped to ensure it placed one foot on each quadrant of the platform, while maintaining a natural stance with its their centre of gravity and stability (measured by the platform) near the middle of the platform. When required, gentle restraint was used to maintain the patient's head in a natural, forward-facing position. For all animals, at least 20 measure- ments were performed, and the mean value was determined. The left–right symmetry index (SI) was calculated according to the following formula: $SI = [(WB_R - WB_L)/((WB_R + WB_L) \times 0.5)] \times 100$[27,72], where WB_R is the value of weight-bearing for the right pelvic limb and WB_L is the value of weight-bearing for the left pelvic limb. Negative values were made positive. We also considered deviation from the normal 20% weight-bearing for a pelvic limb[18], calculated by subtracting WB to 20. Before completion of an online copy of the HVAS, CBPI, COI and LOAD, handlers received the published instructions for each of them. The CMIs were completed sequentially by the same handler in each of the follow-up assessments, without knowledge of their previous answers, in a calm room with as much time as needed to answer all items. As CBPI has two section (PSS and PIS), and COI has four dimensions (stiffness, function, gait and QOL), were considered all sections and dimensions in the analysis. After treatment, animals were rested for three consecutive days and resumed their normal activity over a period of 5 days. On days 1 and 3 after the procedure, the veterinarian examined all patients in order to determine possible signs of exacerbated pain, persistent stiffness of gait and changes in posture. If no complaints were registered, the animal could resume its normal activity[73,74]. If a deterioration of the animal's condition was detected, rescue analgesia would be provided and based on the administration of a combination of opioids (tramadol, 2–5 mg/kg BID or TID) and gabapentin (10–20 mg/kg TID), as needed.

The outcome considered was as return to or drop below baseline values of SI or deviation and scores of the considered CMIs at the 180-day post treatment. Demographic data consisting of age, sex and body weight, was noted. Results are expressed as mean ± SD. Kaplan–Meier estimators were conducted to generate survival curves, survival probability and compared with the Breslow test. Cox proportional hazard regression analysis was carried out to investigate the influence of the variables of interest (age, sex, body weight and OFA score) on treatment survival. Treatments were compared to control at initial evaluation with a Wilcoxon signed-rank test. Patients with values or scores above baseline values at 180 days post treatment were censored. All results were analyzed with IBM SPSS Statistics for Windows, Version 20.0. (Armonk, NY: IBM Corp., released 2011) and a significance level of $p < 0.05$ was set.

Data availability

All data generated or analysed during this study are included in this published article.

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Author contributions

J.C.A. designed the protocol, conducted treatments and prepared the manuscript. P.J. and A.S. selected patients and conducted treatments. C.L. and L.M.C. revised the protocol and prepared the manuscript. All authors have read and approved the manuscript.

Competing interests

The V-PET kits used in this study were provided by the Pall Corporation and the Stance Analyser used in this study was provided by Companion, LiteCure LLC.

Additional information

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frontiers in Veterinary Science

ORIGINAL RESEARCH
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Intra-articular Injections With Either Triamcinolone Hexacetonide, Stanozolol, Hylan G-F 20, or a Platelet Concentrate Improve Clinical Signs in Police Working Dogs With Bilateral Hip Osteoarthritis

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Objectives: To compare the effect of intra-articular treatment with triamcinolone hexacetonide (TH), stanozolol, hyaluronan, and a platelet concentrate in police working dogs with bilateral hip osteoarthritis (OA).

Study Design: Prospective, longitudinal, double-blinded, negative controlled study.

Sample Population: Fifty police working dogs with naturally occurring hip OA.

Methods: Animals were randomly assigned to a control group (CG, $n = 10$), TH group (THG, $n = 10$), platelet concentrate group (PCG, $n = 10$), stanozolol group (SG, $n = 10$), and Hylan G-F 20 group (HG). On days 0 (T0), 8, 15, 30, 90, and 180 days post-treatment, weight-bearing distribution was evaluated. In those days, and on days 60, 120, and 150, four clinical metrology instruments were completed. Kaplan–Meier estimators were conducted and compared with the log-rank test. Cox proportional hazard regression analysis was performed to determine treatment survival. Significance was set at $p < 0.05$.

Results: Patients had a mean age of 6.5 ± 2.4 years and body weight of 26.7 ± 5.2 kg. At T0, hips were classified as mild ($n = 35$), moderate ($n = 10$), and severe ($n = 5$), according to the Orthopedic Foundation for Animals grading scheme. No differences were found between groups at that moment considering age, body weight, OFA hip score, and all assessments performed. All treatments improved clinical signs in various OA dimensions in some groups, with a broad effect interval. PCG showed a lower range of variation while maintaining a positive result for more extended periods ($p < 0.01$ for symmetry index and $0.01 < p < 0.04$ in the majority of scores). Breed, age, sex, and OFA grade did not significantly influence response to treatment.

Conclusions and Clinical Relevance: This is the first prospective, negative controlled, double-blinded study to compare the effect of a single administration of these IA treatments in dogs with hip OA. HG and PCG recorded more significant improvements throughout the 180-day follow-up. In particular, PCG also registered a lower variation in results, seemingly the best therapeutic option. Nevertheless, improvements were still observed in THG and SG, and these treatment options can be considered, mainly when the first two treatments are not available.

Keywords: animal model, osteoarthritis, pain, intra-articular, platelet, triamcinolone, hylan G-F 20

INTRODUCTION

Osteoarthritis (OA) is the most commonly diagnosed joint disease in veterinary medicine, with at least 80% of the cases of lameness and joint diseases in companion animals broadly classified as OA (1–3). Risk factors for developing OA are well documented and include breed, neutering, higher body weight, and age > 8 years (4). For the evaluation of hip OA, pelvic radiographs are frequently performed (5–7).

Weight distribution, off-loading, or limb favoring at stance is a commonly used subjective assessment during orthopedic examination (8). Animals with OA may not be overtly lame but exhibit subtle shifts in body weight distribution at a stance due to pain or instability, which are detectable with force plate gait analysis and weight distribution platforms (9, 10). Body weight distribution at a stance may even be an equivalent or superior measurement of pain associated with hip OA than vertical impulse or peak vertical force (10, 11). Pain is a hallmark of OA, affecting more than just the functional aspect of the disease, and the evaluation of treatment success should encompass the assessment of these multiple dimensions of OA (12, 13). Clinical metrology instruments (CMIs) aim to evaluate multiple dimensions of OA, and the commonly used instruments in dogs are the Canine Brief Pain Inventory (CBPI, divided into a pain severity score—PSS, and a pain interference score—PIS) and the Liverpool Osteoarthritis in Dogs (LOAD) (12, 14–20). Additional validated CMIs include the Hudson Visual Analog Scale (HVAS), a valid tool to assess the degree of lameness in dogs, with force plate analysis as a criterion- referenced standard, and the Canine Orthopedic Index (COI, divided into four scores: stiffness, gait, function, and quality of life—QOL) (21–23).

The medical approach to OA aims at slowing disease progression, relieving pain, and improving overall function (14, 24), and it is well suited to be addressed through the use of local therapy by intra-articular (IA) injection (25, 26). IA corticosteroids have been used for several decades. Currently, different guidelines for the management of human OA provide varying strength of recommendation for the use of IA corticosteroids, from weak to strong recommendation (27–31). Some reports present deleterious effects of IA corticosteroids, namely, the induction of a low- quantity and high-viscosity synovial fluid. These results are often based on multiple injections, particularly of methylprednisolone, while a single dose does not seem to cause long-term detrimental effects (32, 33). Triamcinolone hexacetonide (TH), in particular, can provide pain relief, improve mobility for prolonged periods, and reduce the severity of structural changes (28, 34–36). Hyaluronan is also a commonly used treatment modality in OA management, although its action mechanism is not entirely known (37, 38). It has been proposed to have anti-inflammatory, anti-nociceptive, and chondroprotective properties (39–42). High-molecular-weight products seem to produce better results (43–46). Autologous platelets are a regenerative treatment modality for OA, acting through a supraphysiologic release of growth factors directly at the treatment site, promoting tissue regeneration and attraction of mesenchymal stem cells (47–50). In dogs, a single IA PRP (platelet-rich plasma) injection has resulted in clinical improvements for 12 weeks in some reports, and up to 6 months according to others. In some cases, these improvements occur without the progression of radiographic signs (51–54).

Multiple injection protocols have also been described, producing a positive effect on joint range of motion, pain, lameness, and kinetics (55). More recently, the use of stanozolol, a synthetic derivative of testosterone, has been described in animal models. When administered IA, it induced fibroblasts to increase collagen production, decrease nitric oxide production, and induce osteoblast proliferation and collagen synthesis. It also has a chondroprotective and cartilage regeneration effect while reducing osteophyte formation and subchondral bone reaction (56–61).

To compare long-term outcomes and to identify factors associated with response to treatment, we compared the effect of the IA administration of TH, Hylan G-F 20, stanozolol, and a platelet concentrate in the treatment of police working dogs with bilateral hip OA. We hypothesize that the different treatments will be able to improve CMIs scores and weight- bearing distribution in dogs with OA, compared to a control group (CG).

METHODS

The study protocol was approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem- estar dos Animais da Universidade de Évora, approval no GD/32055/2018/P1, September 25, 2018). Written informed consent was obtained from the institution responsible for the

animals. Fifty active police working dogs with bilateral hip OA were selected to participate in this prospective, longitudinal, double-blinded, negative controlled study. They were included based on history, physical, orthopedic, neurological, and radiographic examinations compatible with bilateral hip OA. Hips were classified according to the Orthopedic Foundation for Animals hip grading scheme at the initial evaluation, on day 0 (62, 63). Animals suspected or with any other orthopedic, or concomitant disease (ruled out through physical examination, complete blood count, and serum chemistry profile) were excluded. Additionally, animals were >2 years old, weighed >20 kg and had no other medications or nutritional supplements administered for the previous 6 weeks and during the study period. Patients were randomly assigned to five different groups, using the statistical analysis software, according to the treatment being administered: a CG (n = 10), receiving an IA administration of 2 ml of NaCl 0.9% per hip joint; a triamcinolone hexacetonide group (THG, n = 10), receiving 20 mg/ml of TH (Bluxam, Riemser Pharma, Portugal) per hip joint; a platelet concentrate group (PCG, n = 10), which received 3 ml of platelet concentrate per hip joint; a stanozolol group (SG, n = 10), to which 0.3 mg/kg of stanozolol (Estrombol, Laboratório Fundacion) per hip joint (64, 65) was administered; and a hyaluronan group (HG, n = 10), which received 2 ml of Hylan G-F 20 (Synvisc®, Sanofi, Portugal) per hip joint. All treatments were administered only on day 0 (treatment day) through IA administration. According to the manufacturer's instructions, this specific platelet concentrate was prepared with the commercially available kit (V-PET®, PALL Corporation). Briefly, 55 ml of whole blood was collected from the jugular vein and introduced into the provided closed system for its preparation. The blood was then allowed to flow by gravity through a filter, where the platelets were concentrated. The platelet concentrate was then recovered and administered within 5 min of preparation.

All IA administrations and radiographic examinations were conducted under light sedation, obtained with the simultaneous intravenous administration of medetomidine (0.01 mg/kg) and butorphanol (0.1 mg/kg). For IA administrations, patients were placed in lateral recumbency with the treatment joint dorsal. The anatomical reference for access was the greater trochanter, around which a 4 × 4 cm window was clipped and aseptically prepared. After preparation, an assistant placed the limb in a neutral position, parallel to the table. A 21-gauge with 2.5 length needle was then introduced just dorsal to the greater trochanter, perpendicular to the limb's long axis until the joint was reached (66). Confirmation of correct needle placement was obtained by collecting synovial fluid, withdrawing as much synovial fluid as possible, and the respective substance was administered. Ultrasound guidance was available if required to confirm the correct needle placement. After treatment, animals were rested for three consecutive days and examined by a veterinarian on days 1 and 3 post procedure to determine signs of exacerbated pain, persistent stiffness of gait, and posture changes. If no complaints were registered, the animal was allowed to resume its normal activity (54, 67). On days 0, 8, 15, 30, 90, and 180 post-treatment, weight distribution was conducted with a stance

TABLE 1 | Mean values (± standard deviation) at initial evaluation of evaluation conducted for control and treatment groups.

Treatment group	Age	Weight (kg. mean ± SD)	Symmetry Index (mean	Deviation (mean ± SD)	HVAS (0–10)	PSS (0–10)	PIS (0–10)	LOAD (0–52)	Stiffness (0–16)	Function (0–16)	Gait (0–20)	QOL (0–12)	COI (0–64)
CG	6.5 ± 2.5	28.3 ± 5.4	33.6 ± 24.3	2.1 ± 1.8	7.2 ± 0.9	2.4 ± 1.5	2.5 ± 1.7	12.3 ± 8.3	3.1 ± 2.9	3.1 ± 3.5	4.1 ± 4.0	3.6 ± 2.5	13.9 ± 12.6
THG	6.0 ± 2.6	27.4 ± 6.0	41.7 ± 41.7	4.4 ± 3.3	6.2 ± 1.7	3.2 ± 2.4	3.8 ± 2.9	18.2 ± 12.6	5.1 ± 3.9	4.3 ± 5.0	7.6 ± 5.8	4.6 ± 3.6	21.6 ± 17.7
HG													
SG	7.2 ± 2.6	26.5 ± 3.2	20.3 ± 21.8	5.6 ± 4.5	6.5 ± 1.4	3.3 ± 2.4	3.3 ± 2.2	16.6 ± 10.4	3.5 ± 3.1	4.5 ± 3.5	7.1 ± 4.7	4.7 ± 2.8	19.8 ± 12.7

CG, Control group; COI, Canine Orthopedic Index; HG, Hylan G-F 20 group; HVAS, Hudson Visual Analog Scale; LOAD, Liverpool Osteoarthritis in Dogs; OFA, Orthopedic Foundation for Animals; PCG, Platelet Concentrate group; PIS,

TABLE 2 | Survival probability calculated with Kaplan-Meier estimators (in days) and compared with the log rank test.

Variable	Log rank test	Treatment									
		CG		HG		PCG		SG		THG	
		Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI
Symmetry Index	0.000*	28.6 ± 7.5	13.9–43.2	111.8 ± 20.5	71.5–152.1	171.0 ± 9.5	152.3–189.7	80.3 ± 19.6	43.7–116.9	94.5 ± 16.8	78.8–115.7
Deviation	0.031*	48.8 ± 16.6	16.3–81.3	130.5 ± 22.3	86.8–174.2	147.0 ± 18.6	110.5–183.5	90.8 ± 23.9	43.8–137.8	99.0 ± 23.7	52.5–145.5
HVAS	0.012*	49.7 ± 17.5	15.4–84.0	117.0 ± 18.7	80.3–153.7	144.0 ± 17.3	110.2–177.8	129.8 ± 18.9	92.7–166.9	66.1 ± 20.1	26.7–105.5
PSS	0.485	63.2 ± 24.3	15.6–110.8	142.5 ± 16.9	109.4–175.7	150.0 ± 14.3	122.1–177.9	94.6 ± 23.2	49.2–140.0	90.2 ± 24.9	41.4–139.0
PIS	0.000*	8.7 ± 0.7	7.3–10.1	114.0 ± 22.7	69.6–158.4	138.0 ± 15.8	106.9–169.1	109.6 ± 25.6	59.4–159.8	118.6 ± 23.0	76.5–119.0
LOAD	0.000*	40.7 ± 16.3	10.6–70.8	141.8 ± 16.3	109.8–173.8	120.0 ± 18.7	83.3–156.6	123.8 ± 20.1	84.5–163.1	124.3 ± 22.5	80.2–129.4
Stiffness	0.592	64.7 ± 24.0	17.7–111.7	129.8 ± 20.5	89.7–169.9	141.0 ± 15.7	110.1–171.9	111.8 ± 22.5	67.7–155.9	130.8 ± 16.4	98.7–162.9
Function	0.013*		28.4-102.4		148.3–187.7		107.9–162.0		81.7–167.3		69.3–155.9
Gait											

*CG, Control group; COI, CanineOrthopedic Index; HG, Hylan G-F 20 group; HVAS, Hudson Visual Analog Scale; LOAD, Liverpool Osteoarthritis in Dogs; OFA, Orthopedic Foundation for Animals; PCG, PlateletConcentrategroup; PIS, Pain Interference Score; PSS, Pain Severity Score; QOL, Quality of Life; SG, Stanozolol group; THG, Triamcinobone hexacetonide group. * indicates significance.*

analysis platform (Companion Stance Analyzer; LiteCure LLC, Newark, Delaware, United States), placed in the center of a room, at least 1 m from the walls. It was calibrated at the beginning of each testing day and zeroed before each data collection. Animals stood on the platform, with one foot on each quadrant of the platform, while maintaining a natural stance with their center of gravity near the platform's middle. When required, gentle restraint was used to maintain the patient's head in a natural forward-facing position. The left–right symmetry index (SI) was calculated according to the following formula: $SI = [(WB_R - WB_L)/((WB_R + WB_L) \times 0.5)] \times 100$, where WB_R is the value of weight-bearing for the right pelvic limb and WB_L is the value of weight-bearing for the left pelvic limb. Negative values were made positive (19, 68). Since all animals included in the study had bilateral disease, we also considered a deviation from the normal 40% weight-bearing for the combined pelvic limbs (10), calculated by subtracting WB to the normal 40%. On days 0, 8, 15, 30, 60, 90, 120, 150, and 180 post-treatment after treatment, an online copy of the HVAS, CBPI, COI, and LOAD was completed by the dogs' trainers after receiving the published instructions for each of them. Dogs' trainers were unaware of which treatment the animal received. The CMIs were completed in sequence by the same handler in all follow-up moments, without knowing their previous answer. The two sections of the CBPI (PSS and PIS) and COI's four dimensions (stiffness, function, gait, and QOL) were considered separately in the analysis. All evaluations were performed at the same moment by the same researcher blinded to the group and identity of the patient.

For the considered IA treatments, some side effects are documented and include local pain and local inflammation. These are usually self-limiting and take 2–10 days to resolve (69). The occurrence of these side effects was monitored during treatment follow-up assessments and recorded.

Demographic data as age, sex, body weight, and breed were recorded. Kaplan–Meier estimators were conducted to generate survival curves and survival probability and compared with the log-rank test. Cox proportional hazard regression analysis was carried out to investigate interest variables' influence (age, sex, body weight, breed, and OFA score) on survival. All results were analyzed with IBM SPSS Statistics version 20, and a significance level of $p < 0.05$ was set. With the CBPI, a specific measure of success was defined and set as a reduction of ≥1 in PSS and ≥2 in PIS (70). The time for PIS and PSS scores to drop below the defined level of reduction was evaluated. For the remaining CMIs scores and weight-bearing evaluation, the outcome considered was a return to or drop below values recorded at the initial evaluation. Patients with values or scores above baseline values at the evaluation moment the event was recorded were censored.

RESULTS

The sample included 50 police working dogs, of both genders (30 males and 20 females), with a mean age of 6.5 ± 2.4 years and body weight of 26.7 ± 5.2 kg. Four dog breeds were represented: German Shepherd Dogs (GSD, $n = 17$), Belgian Malinois Shepherd Dogs (BM, $n = 15$), Labrador Retriever (LR,

TABLE 3 | Results Cox proportional hazard regression with the different outcome evaluations.

Weight distribution					CBPI							
Variable	Symmetry Index ($p = 0.014$)		Deviation ($p = 0.251$)		HVAS ($p = 0.036$)		PSS ($p = 0.881$)		PIS ($p = 0.025$)		LOAD ($p = 0.006$)	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Treatment		0.001*		0.159		0.005*		0.529		0.001*		0.000*
Control	1.00		1.00		1.00		1.00		1.00		1.00	
HG	0.23 (0.08–0.65)	0.006*	0.25 (0.07–0.84)	0.026*	0.19 (0.06–0.64)	0.007*	0.29 (0.08–1.16)	0.081	0.06 (0.15–0.26)	0.000*	0.06 (0.02–0.25)	0.000*
PCG	0.24 (0.21–0.26)	0.000*	0.29 (0.09–0.96)	0.042*	0.26 (0.09–0.82)	0.021*	0.57 (0.19–1.73)	0.322	0.12 (0.03–0.43)	0.001*	0.19 (0.06–0.62)	0.005*
SG	0.21 (0.07–0.60)	0.004*	0.42 (0.12–1.47)	0.176	0.31 (0.09–1.08)	0.066	0.65 (0.19–2.18)	0.487	0.09 (0.02–0.36)	0.001*	0.08 (0.02–0.28)	0.000*
THG	0.29 (0.09-0.83)	0.021*	0.53 (0.18–1.54)	0.245	0.58 (0.20–1.66)	0.311	0.72 (0.23–2.25)	0.573	0.08 (0.02–0.32)	0.000*	0.09 (0.03–0.36)	0.001*
OFA score		0.582		0.608		0.195		0.998		0.621		0.211
Mild	1.00		1.00		1.00		1.00		1.00		1.00	
Moderate	0.70 (0.29–1.69)	0.430	0.75 (0.30–1.85)	0.528	1.49 (0.63–3.52)	0.358	1.024 (0.41-2.57)	0.960	1.53 (0.65–3.61)	0.330	2.18 (0.89–5.34)	0.088
Severe	0.58 (0.17–1.94)	0.378	1.44 (0.37–5.58)	0.597	3.00 (0.88–10.29)	0.079	0.99 (0.22–4.41)	0.990	1.25 (0.31–5.00)	0.749	1.03 (0.23–4.61)	0.972
Breed		0.861		0.293		0.752		0.856		0.631		0.073
LR	1.00		1.00		1.00		1.00		1.00		1.00	
GSD	0.74 (0.24–2.28)	0.589	0.44 (0.13–1.54)	0.202	0.52 (0.13–2.14)	0.368	1.42 (0.36–5.61)	0.619	0.52 (0.14–1.92)	0.327	0.76 (0.21–2.76)	0.672
BM	0.63 (0.21–1.91)	0.411	0.31 (0.09–1.13)	0.076	0.47 (0.12–1.85)	0.283	0.92 (0.27–3.15)	0.888	0.46 (0.14–1.58)	0.217	0.35 (0.09–1.33)	0.121
DSD	0.79 (0.22–2.86)	0.724	0.58 (0.12–2.74)	0.493	0.58 (0.13–2.72)	0.493	1.39 (0.54–2.92)	0.684	0.43 (0.10–1.82)	0.249	0.17 (0.03–0.83)	0.028*
Sex												
Male	1.00		1.00		1.00		1.00		1.00		1.00	
Female	1.51 (0.76–2.99)	0.238	0.77 (0.34–1.75)	0.536	0.33 (0.12–0.87)	0.025*	1.25 (0.54–2.92)	0.607	0.85 (0.37–1.97)	0.704	1.17 (0.49–2.79)	0.721
Age	0.90 (0.77–1.06)	0.216	0.9 (0.75–1.09)	0.274	1.09 (0.91–1.29)	0.363	1.13 (0.93–1.37)	0.222	0.912 (0.77–1.08)	0.299	0.9 (0.76–1.07)	0.229

	COI									
Variable	Stiffness ($p = 0.034$)		Function ($p = 0.023$)		Gait ($p = 0.069$)		QOL ($p = 0.325$)		Total ($p = 0.053$)	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Treatment		0.099		0.022*		0.056		0.840		0.163
Control	1.00		1.00		1.00		1.00		1.00	
HG	0.19 (0.05–0.74)	0.016*	0.09 (0.02–0.43)	0.002*	0.20 (0.06–0.71)	0.012*	0.64 (0.19–2.09)	0.463	0.29 (0.09–0.92)	0.036*
PCG	0.23 (0.07–0.77)	0.018*	0.33 (0.11–1.02)	0.054	0.21 (0.07–0.67)	0.009*	0.57 (0.21–1.58)	0.282	0.29 (0.09–0.87)	0.030*
SG	0.31 (0.09–1.09)	0.069	0.28 (0.08–1.01)	0.051	0.36 (0.11–1.15)	0.083	0.87 (0.28–0.67)	0.807	0.54 (0.17–1.66)	0.279
THG	0.31 (0.09–1.09)	0.069	0.33 (0.11–1.02)	0.054	0.29 (0.09–0.92)	0.036*	0.65 (0.22–1.96)	0.444	0.49 (0.17–1.41)	0.188
OFA score		0.223		0.068		0.439		0.303		0.041*
Mild	1.00		1.00		1.00		1.00		1.00	
Moderate	1.05 (0.39–2.84)	0.918	0.89 (0.34–2.36)	0.823	0.63 (0.24–1.64)	0.344	0.48 (0.19–1.22)	0.123	0.69 (0.28–1.73)	0.431
Severe	3.11 (0.79–12.13)	0.102	5.09 (1.18–21.98)	0.029*	1.34 (0.39–4.629	0.641	0.78 (0.22–2.77)	0.696	4.19 (1.08–16.24)	0.038*
Breed		0.069		0.559		0.255		0.320		0.997
LR	1.00		1.00		1.00		1.00		1.00	
GSD	0.46 (0.10–2.00)	0.298	2.54 (0.56–11.47)	0.226	0.71 (0.18–2.78)	0.622	2.31 (0.65–8.25)	0.199	0.92 (0.26–3.28)	0.898
BM	1.39 (0.40–4.81)	0.606	2.04 (0.52–7.99)	0.309	1.15 (0.33–3.96)	0.824	0.87 (0.33–7.49)	0.814	0.92 (0.29–2.93)	0.893
DSD	0.22 (0.03–1.51)	0.124	1.18 (0.22–6.22)	0.845	0.29 (0.05–1.58)	0.153	1.57 (0.33–7.49)	0.572	1.00 (0.24–4.15)	0.995
Sex										
Male	1.00		1.00		1.00		1.00		1.00	
Female	1.49 (0.60–3.69)	0.386	1.58 (0.62–4.01)	0.337	1.19 (0.52–2.74)	0.685	3.16 (1.35–7.39)	0.008*	2.02 (0.89–4.55)	0.089
Age	1.08 (0.91–1.29)	0.378	1.16 (0.96–1.41)	0.119	1.19 (0.99–1.43)	0.071	1.06 (0.87–1.29)	0.572	1.14 (0.94–1.37)	0.180

*BM, Belgian Malinois Shepherd Dog; CBPI, Canine Brief Pain Inventory; COI, Canine Orthopedic Index; DSD, Dutch Shepherd Dog; GSD, German Shepherd Dog; HG, Hylan G-F 20 group; HVAS, Hudson Visual Analog Scale; LOAD, Liverpool Osteoarthritis in Dogs; LR, Labrador Retriever; OFA, Orthopedic Foundation for Animals; PCG, Platelet Concentrate group; PIS, Pain Interference Score; PSS, Pain Severity Score; QOL, Quality of Life, SG, Stanozolol group; THG, Triamcinolone hexacetonide group. * indicates significance.*

n = 10), and Dutch Shepherd Dogs (DSD, n = 8). Considering OFA hip grading, 35 animals were classified as mild (70%), 10 were classified as moderate (20%), and 5 were classified as severe (10%). The platelet concentrate obtained had a four-fold platelet concentration, a two-fold leukocyte concentration, and a 50% reduction in platelet concentrate hematocrit than whole blood values. These values are in line with those previously described for V-PET® (71). The results of the evaluation performed at day 0, by group, are presented in **Table 1**, where no significant differences were found between groups. Results of the Kaplan–Meier estimators are presented in **Table 2**. All treatments were able to produce better results than CG, with variable periods of duration. Better results were observed in the PCG and HG in all considered outcome measures, with a lower range with a 95% confidence interval. Results of the Cox proportional hazard regression are presented in **Table 3**. Treatment was the covariable that contributed more frequently to the outcomes observed. In fact, in some cases (as SI, HVAS, PIS, and others), it was the only one. Only overall COI also influenced the OFA score, with dogs with a severe hip grade having a 4.1-fold probability of returning to baseline values, compared with dogs with a mild grade. LOAD was the only outcome measure influenced by breed, with DSD showing a lower risk baseline values. All patients were followed up to the 180-day evaluation moment. Post-injection increased lameness was observed in eight patients in PCG, four in SG, three in HG, and two in THG, which spontaneously resolved within 48–72 h. No additional treatment or medications was administered to the animals during this period.

DISCUSSION

OA is a chronic disease with no cure. Therefore, the main focus of OA management is to control clinical signs, mainly pain levels (72, 73). Hip OA, in particular, is very common in large breed dogs such as German Shepherd Dogs and Labradors. It has a toll on the quality of life, particularly in working dogs, to whom it also affects performance (74, 75). To our knowledge, this is the first prospective, negative controlled, double-blinded study to compare the long-term effects of these different IA approaches for the management of dogs with bilateral hip OA.

Clinical presentation of patients with OA is characterized by variable degrees of clinical and functional impairments. It is well established that clinical signs and the severity of pain, in particular, correlate with the functional status rather than radiographic grading of OA. For that reason, treatment should be planned according to clinical features and functional status instead of radiological findings (62, 76–78). With that in mind, we evaluated the impact of predisposing and clinical factors of OA as demographic characteristics of interest. The IA TH administration has been described as having long-term safety while improving the joint range of motion and pain compared with saline injection (79–82).

Similarly, IA hyaluronan improves pain, function, lameness, and kinetics compared to pre-treatment and saline control in patients with OA (69). Reports of canine OA treatment with this same platelet concentrate present improvements in pain, kinetics, and joint range of motion, lasting from 12 weeks to 6 months (52, 54). The use of IA stanozolol has been published in horses and an ovine model and presented as able to resolve signs of lameness, reduce osteophyte formation and subchondral bone reaction, and promote articular cartilage regeneration (58, 59). In the study presented here, all treatments improved clinical signs in various dimensions of OA in police working dogs with bilateral disease. While being able to do so, the 95% confidence interval was wide for those treatments in some groups. Values and scores in PCG showed a lower range of variation while maintaining a positive result for more extended periods. Except for pain scores, mean values in CG did not return to baseline values immediately at the first follow-up periods, as would possibly be expected. A functional improvement following NaCl IA injections has been described, and, in some instances, effects were noted up until 6-month post-administration (83). This fact can be associated with the removal of inflammatory mediators presented in the synovial fluid, and an effect similar to a joint lavage produced by the administration of saline (83), and may be the reason for the recorded evaluation in CG. Also noteworthy, while any treatment did not significantly influence PSS scores, PIS scores were. It is not uncommon that police working dogs do not show overt signs of pain, which is easily detected through its effect on daily activities and performance (84). Probably for that reason, all treatments were able to produce an 88–94% improvement compared to CG, as evaluated with the PSS. The weight-bearing evaluation platform has been deemed a repeatable and accessible device to measure static weight distribution, compared to a pressure-sensitive walkway (10, 85, 86). A significant improvement was observed only with SI considering the two weight-bearing evaluations evaluated. Dogs presenting with pelvic limb lameness tend to distribute weight more by side-to-side compensation than pelvic-to-thoracic (87, 88). This compensation mechanism may be the reason for this result, and the same compensation mechanism may be present in animals with bilateral disease, such as hip OA. This may be the reason for the wide ranges observed in standard deviations of the SI at the initial evaluation. Despite being a bilateral disease, it is not to say that both joints are affected equally, causing the animal to off-load one limb while supporting more weight on the contralateral limb. The degree of this compensation mechanism can vary between individual dogs. The same can be considered for the wide ranges in COI scores, since this CMI focuses on the ability of the dog to perform daily activities, and the clinical signs of OA patients can vary quite significantly (21).

Also, dogs included were active police working dogs, known to be stoic and not to show overt pain signs (75, 84). The fact that they were signaled to undergo treatment for hip may indicate that these animals were, at the time, in pain (86). With HVAS, HG and PCG registered more significant improvements throughout the 180-day follow-up, also with a lower variation with the 95% confidence interval. When using LOAD, all treatments produced improvements that ranged from 81 to 94%. When using the various dimensions of COI, PCG and HG were the treatments consistently leading to improvements. With this information in mind, and considering the variety of evaluations performed, the platelet concentrate and Hylan G-F 20 seem to be the best IA

therapeutic choices for treating bilateral hip OA. Nevertheless, TH and stanozolol were also able to improve patients' condition and are valid therapeutic options that should be considered, mainly when the first two treatments are not available.

Heavier dogs are more prone to develop OA earlier in life (89, 90), and being overweight is a risk factor for OA. While being related, these two concepts are not the same. Since the animals that comprised the sample were active working dogs, with a body condition score of 4 or 5/9, none was overweight. Also, since represented dog breeds were all large, we chose not to include body weight as a possible influencing factor in our models. Age did not have a significant role in any of the evaluations performed, but increasing age, particularly over 8 years, is a predisposing factor for OA (4). This lack of effect may be attributed to the fact that the sample animals' mean age was below 8 years. It is also possible that age is not a factor by itself, and instead reflects the progression of the disease, which, in turn, may affect response to treatment. OFA grading only influenced function evaluation, with animals with a severe classification showing a significantly worse evolution than those graded as mild. Hylan G-F 20 seems to be the better therapeutic option for these patients since HG was the only group to show significant improvements compared to control. The reason for this may be related to the mechanism of action of hyaluronan, which supplements the viscosity and elasticity of synovial fluid (37). The remaining treatments act by interacting with joint cells and tissues, which may not be as responsive or even present in enough number to show a better response. Certain dog breeds are also at increased risk of developing hip OA since it is a common consequence of hip dysplasia and influenced by a wide range of breed-specific genes (polygenetic trait) (4, 62). Dog breeds included in this sample are known breeds at risk to develop OA and similar size and conformation. With the considered evaluation, no significant differences were observed regarding response to treatment.

There are recommendations for different administration frequency in human, canine, and horse reports. For corticosteroids, a period of at least 6–12 weeks should be respected between administrations, without exceeding two to four injections of the same joint within a year (91, 92). In horses, a study considering triamcinolone acetonide showed no difference between single or multiple administrations (93). For hyaluronan, some reports indicate that three injections weekly are more effective in reducing pain in humans when compared to a single administration, although both protocols improved joint function (94). For canine platelet products, two administrations 2–3 weeks apart have been recommended (52). We chose to administer a single IA inject to compare all treatments before evaluating multiple-administration protocols. Also, available canine recommendations are usually based on recommendations for other species or on data from canine surgical models, raising the need for information from dogs with naturally occurring OA. The fact that the animals enrolled in this study are working dogs means that their musculoskeletal structures are under greater demand than in a companion animal (95). While results may remain significant for a more extended time in companion animals, due to lower physical demand, most of the animals included in this study were being treated at an early age and with less radiographic changes than what is described in companion animals (4).

With all used IA treatments, some side effects are documented and include local pain and local inflammation. These are usually self-limiting and take 2–10 days to resolve, being attributed to a joint capsule expansion following the IA administration (59, 69, 96, 97). Similarly, we observed increased lameness in eight patients in PCG, four in SG, three in HG, and two in THG, which spontaneously resolved within 48–72 h. PCG was the group where the higher treatment volume was administer, which may account for higher number of increased lameness observed.

This study presents some limitations, namely, the inclusion of a majority of dogs with mild OA. For that reason, further studies should include a larger number of dogs with moderate and severe OA to determine if similar results are obtained. Still, a significant difference between mild and severe OA was observable in the COI score. Different volumes were administered in different groups, ranging from 1 ml (in THG) to 3 ml (in PCG). This difference in volumes may impact clinical signs following the administration, as a higher volume can produce joint capsule dilation and, consequently, pain. In our study, this did not significantly impacted the overall results, as this increased lameness resolved within 72 h in all groups, but is a variation to consider in future studies. Different numbers of administration should also be tested.

CONCLUSIONS AND CLINICAL RELEVANCE

To our knowledge, this is the first prospective, negative controlled, double-blinded study to compare the effect of these different IA treatment modalities in police working dogs with bilateral hip OA. It describes each treatment modality's effect on pain level and functional evaluation, their duration, and relevant information regarding patient selection for each treatment. HG and PCG recorded greater improvements throughout the 180-day follow-up. In particular, PCG also registered a lower variation in results, seemingly the best therapeutic option. Improvements were still observed in THG and SG, and these treatment options can be considered, mainly when the first two treatments are not available.

DATA AVAILABILITY STATEMENT

The datasets generated for this article are not readily available because the data used in this study is a property of the Guarda Nacional Republicana, a governmental police force from Portugal and, by law, confidential. The authors obtained specific approval in order to use the data. Requests to access the datasets should be directed to the Divisão de Medicina Veterinária (ari.dsad.dmv@gnr.pt).

ETHICS STATEMENT

This study is a part of a project approved by the ethical review committee of the University of Évora (Órgão Responsável pelo Bem-estar dos Animais da Universidade de Évora, approval no. GD/32055/2018/P1, September 25, 2018).

AUTHOR CONTRIBUTIONS

JA designed the protocol, conducted treatments, and prepared the manuscript. PJ and AS selected patients and conducted treatments. CL and LC revised the protocol and prepared the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The V-PET kits used in this study were provided by the Pall Corporation and the Stance Analyser used in this study was provided by Companion, LiteCure LLC®.
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

IV. CONCLUSIONS AND RESEARCH PERSPECTIVES

Osteoarthritis is a disease as old as joints themselves, and its presence and prevalence continued to accompany all species, particularly with an increase in the population's life expectancy. With a significant impact on the quality of life and function of individuals, OA is difficult to manage, with treatment often offering an unsatisfactory level of control. The complexity of treatment is reflected by the vast number of treatment modalities and different approaches that are used to manage it. In most cases, satisfactory control of symptoms is only achieved through a multi-modal approach. As a chronic progressive disease, in tissues with limited regeneration ability, early diagnosis, associated with precise monitoring, are crucial for long-term success. The use of intra-articular therapies is an attractive option for the management of the disease. This is particularly interesting for regenerative therapies, which aim to have a direct action on the tissues most directly affected by the disease. Being a common approach in humans and horses, it is not yet a frequent option in canine medicine. This was one of the starting points for this study. The lack of data to guide the action of clinicians in monitoring their patients, associated with the particular characteristics of the dog, which make it an optimal model for the study of human OA, were the major determinants for the project developed.

One of the most significant difficulties felt in designing the experimental protocol of the study was the selection of treatments and evaluation modalities to include and those to exclude. In the last decades, several new treatments have appeared or become more accessible, as well as objective measures of patient assessment and response to treatment. As a principle for that choice, we selected what, in our view, would be the options of interest to the clinician who daily treats these patients, and is not necessarily an investigator. For the evaluation of results, objective quantification methods were selected, which can be developed in laboratory facilities but also in the daily in-house Veterinary Medical Centers.

The evaluation of animals over an extended follow-up period, and the use of several evaluation modalities, allowed us to obtain an accurate characterization of each treatment effects at different levels, namely, molecular, imagiological, and clinical. A large amount of data gathered made it possible to exceed significantly the scientific production initial proposed. This production is reflected in the several manuscripts produced, from the preliminary evaluations to the characterization of patients, to the evaluation and validation of the different monitoring modalities, the characterization of each of the treatments used, and, in the end, the comparison between them. This whole process required the acquisition of many skills, from the execution of the procedures themselves to working

with the stance analysis platform and the thermographic camera, the realization of the laboratory component for the analysis of the synovial fluid, to the realization of various statistical analysis methods.

In practical terms, with the results obtained it was possible to present important data to work as a guideline for clinicians in the early diagnosis of the OA disease. Also, to reassess and validate different clinical metrology instruments, to compare projections and to validate digital thermography as a method of monitoring osteoarthritis, and to list the strengths of each treatment depending on the patient's clinical presentation. The study results also point out new lines for future studies. For instance, it would be very interesting to evaluate different platelet products, considering that different compositions will lead to different results. Or the use of accelerometers, centering the study on the patient's functional capability and quality of life, and not only on the results of exams. Also, the interaction and relation with several companies allowed an approach of the academic and business worlds.

This dissertation presents a contribution to several aspects of veterinary and human medicine, contributing to better medical knowledge, deeply immersed in the world concept of One Health / One Medicine.

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APPENDIX I – The Canine Brief Pain Inventory

Description of Pain:

Rate your dog's pain. (0-10, 0 = no pain, 10 = extreme pain)

1. Which one number best describes the pain at its **worst** in the last 7 days.
2. Which one number best describes the pain at its **least** in the last 7 days.
3. Which one number best describes the pain at its **average** in the last 7 days.
4. Which one number best describes the pain as it is **right now**.

Description of Function:

Which one number best describes how during the past 7 days **pain has interfered** with your dog's (0-10, 0 = does not interfere, 10 = completely interferes):

5. General Activity
6. Enjoyment of Life
7. Ability to Rise to Standing From Lying Down
8. Ability to Walk
9. Ability to Run
10. Ability to Climb Up (for example Stairs or Curbs)

Overall Impression:

11. Which response best describes your dog's overall quality of life over **the last 7 days**? (Bad, Fair, Good, Very Good Excelent)

APPENDIX II – The Canine Orthopedic Index

Description of Stiffness:

The following questions concern the amount of joint stiffness your dog has experienced in the **past 7 days**.

Stiffness is the restriction or slowness in the ease with which your dog moves his/her joints.

Please select **one** answer for each question below.

1. How severe is your dog's stiffness after first wakening **in the morning**?
   - None;
   - Mild;
   - Moderate;
   - Severe;
   - Extreme.
2. **Later in the day**, how severe is your dog's stiffness after lying down for at least 15 minutes?
   - None;
   - Mild;
   - Moderate;
   - Severe;
   - Extreme.
3. How much of a problem does your dog have **rising to standing** after lying down for at least 15 minutes?
   - No problems;
   - Mild problems;
   - Moderate problems;

- Severe problems;
- Extreme problems.

4. In general, over the past 7 days, how much difficulty has your dog had with his or her joints?
   - None;
   - Mild;
   - Moderate;
   - Severe;
   - Extreme.

Description of Function:

Please indicate how much of a problem each of the following activities has been for your dog over the **past 7 days**.

Please select **one** answer for each question below.

5. **Jumping up** (as in getting into the car or onto the bed)?
   - No problems;
   - Mild problems;
   - Moderate problems;
   - Severe problems;
   - Extreme problems.

6. **Jumping down** (as in getting out of the car or off of the bed)?
   - No problems;
   - Mild problems;
   - Moderate problems;

- Severe problems;
- Extreme problems.

7. **Climbing up** (as in stairs, ramps or curbs)?
   - No problems;
   - Mild problems;
   - Moderate problems;
   - Severe problems;
   - Extreme problems.
8. **Climbing down** (as in stairs, ramps or curbs)?
   - No problems;
   - Mild problems;
   - Moderate problems;
   - Severe problems;
   - Extreme problems.

Description of Gait:

The following questions concern your dog's gait over **past 7 days**.

Gait refrs to the manner in which your dog uses its legs as it moves

Please select **one** answer for each question below.

9. On average, how severe was your dog's limp **during** mild activities (such as short walks)?
   - None;
   - Mild;
   - Moderate;
   - Severe;
   - Extreme.

10. On average, how severe was your dog's limp **during** mild activities (such as long walks, playing or running)?
    - None;
    - Mild;
    - Moderate;
    - Severe;
    - Extreme.
11. How often did your dog limp **the day after** moderate activities (such as long walks, playing or running)?
    - Never;
    - Rarely;
    - Occasionally;
    - Frequently;
    - Constantly.
12. How often have you been aware of your dog's joint problems?
    - Never;
    - Rarely;
    - Occasionally;
    - Frequently;
    - Constantly.
13. How often did your dog dog 'pay' for over-activity, with increased pain or stiffness the following day?
    - Never;
    - Rarely;
    - Occasionally;

- Frequently;
- Constantly.

Description of Quality of Life:

Please select **one** answer for each question below.

14. In the past 7 days, what has been your level of concern that your dog's joint problems will shorten his or her life?
    - None;
    - Mild;
    - Moderate;
    - Severe;
    - Extreme.
15. In the past 7 days, what has been your level of concern that your dog is generally slowing down?
    - None;
    - Mild;
    - Moderate;
    - Severe;
    - Extreme.
16. Overall, how would you rate your dog's quality of life over the past 7 day?
    - Excellent;
    - Very Good;
    - Good;
    - Fair;
    - Poor.

APPENDIX III – Liverpool Osteoarthritis in Dogs

Mobility:

Generally

1. How is your dog's mobility in general?

   - Very good;

   - Good;

   - Fair;

   - Poor;

   - Very poor.

2. How disabled is your dog by his/her lameness?

   - Not at all disabled;
   - Slightly disabled;
   - Moderately disabled;
   - Severely disabled;
   - Extremely disabled;

3. How active is your dog?

   - Extremely active;
   - Very active;
   - Moderately active;
   - Slightly active;
   - Not at all active;

4. What is the effect of cold, damp weather on your dog's lameness?
    - No effect;
    - Mild effect;
    - Moderate effect;
    - Severe effect;
    - Extreme effect;

5. To what degree does your dog show stiffness in the affected leg after a 'lie down'?
    - No stiffness;
    - Mild stiffness;
    - Moderate stiffness;
    - Severe stiffness;
    - Extreme stiffness;

At exercise

6. At exercise, how active is your dog?
    - Extremely active;
    - Very active;
    - Fairly active;
    - Not very active;
    - Not at all active;

7. How keen to exercise is your dog?
    - Extremely keen;
    - Very keen;

- Fairly keen;
- Not very keen;
- Not at all keen.

8. How would you rate your dog's ability to exercise?
   - Very good;
   - Good;
   - Fair;
   - Poor;
   - Very poor;

9. What overall effect does exercise have on your dog's lameness?
   - No effect;
   - Mild effect;
   - Moderate effect;
   - Severe effect;
   - Extreme effect.

10. How often does your dog rest (stop/sit down) during exercise?
    - Never;
    - Hardly ever;
    - Occasionally;
    - Frequently;
    - Very frequently.

11. What is the effect of cold, damp weather on your pet's ability to exercise?

- No effect;
- Mild effect;
- Moderate effect;
- Severe effect;
- Extreme effect.

12. To what degree does your dog show stiffness in the affected leg after a 'lie down' following exercise?

- No stiffness;
- Mild stiffness;
- Moderate stiffness;
- Severe stiffness;
- Extreme stiffness;

13. What is the effect of your dog's lameness on his/her ability to exercise?

- No effect;
- Mild effect;
- Moderate effect;
- Severe effect;
- Extreme effect.

APPENDIX IV – Hudson visual analogue scale

Hudson Visual Analogue Scale

1. How would you describe your **overall assessment** of your dog in the **last month**? (0-10, 0 = bad, 10 = good)
2. What kind of **mood** has your dog been in the last month? (0-10, 0 = bad, 10 = good)
3. How has your dog's attitude been in the last month? (0-10, 0 = bad, 10 = good)
4. How frequently does your dog display comfort or "happy dog" postures (e.g., lying on back with toy in mouth? (0-10, 0 = bad, 10 = good)

Tell us what type of daily activities your dog engages in (e.g., fetching newspapers, playing frisbee) and then answer question 5.

5. Has your dog **changed the amount** of these activities? (0-10, 0 = not at all, 10 = a lot)
6. How willing is your dog to **play voluntarily**? (0-10, 0 = not at all, 10 = a lot)
7. How **often** does your dog get **exercise**? (0-10, 0 = not at all, 10 = a lot)
8. How **stiff** is your dog when **arising for the day** (0-10, 0 = not at all, 10 = a lot)
9. How **stiff** is your dog **at the end of the day** (post-activities)? (0-10, 0 = not at all, 10 = a lot)
10. Does your dog indicate any **lameness at a walk**? (0-10, 0 = not at all, 10 = a lot)
11. Does your dog indicate any **pain when turning suddenly at a walk**? (0-10, 0 = not at all, 10 = a lot)

APPENDIX VI – Efficay of a single intra-articular administration of methylprednisolone Acetate and triamcinolone acetonide in a natural occurring oestoarthritis canine model

EFFICACY OF A SINGLE INTRA-ARTICULAR ADMINISTRATION OF METHYLPREDNISOLONE ACETATE AND TRIAMCINOLONE ACETONIDE IN A NATURAL OCCURRING OSTEOARTHRITIS CANINE MODEL

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RESUMEN CORTO/RESUME

The goal of this study was to describe the use and effectiveness of intra-articular MPA and TA in the management of naturally occurring hip osteoarthritis (OA) in an animal model. Twenty police working dogs were divided in two groups according to the drug administered through intra-articular (IA) injection: GT (20mg of triamcinolone acetonide – TA, per hip joint) and GMPA (40mg of methylprednisolone acetate – MPA, per hip joint). Seven different time points were considered during the study for data collection purposes: T0 (before treatment), T1 (15 days after treatment), T2, T3, T4, T5, T6, and T7 (1, 2, 3, 4, 5 and 6 months after treatment respectively). Response to treatment was measured using the Canine Brief Pain Inventory (CBPI) and Hudson Visual Analogue Scale (HVAS). Significant results were considered when $p<0.05$.

Treatment successfully reduced pain interference scores (PSS) in two animals of GT at T1 (20%), three at T2-T3 (37.5%) and two at T4-T7 (28.6%). For GMPA, treatment was successful in two animals at T1 (20%), four at T2 (40%), three at T3 (30%) and two at T4-T5 (20%). Considering pain interference score (PIS), treatment was a success in two animals in both GTA and GMPA from T1-T7. However, no significant differences were registered when comparing each time point with T0 at T1 nor between groups. No significant differences were registered with HVAS.

IA TA and MPA may be a treatment option for dogs with naturally occurring OA. Still, further studies are required.

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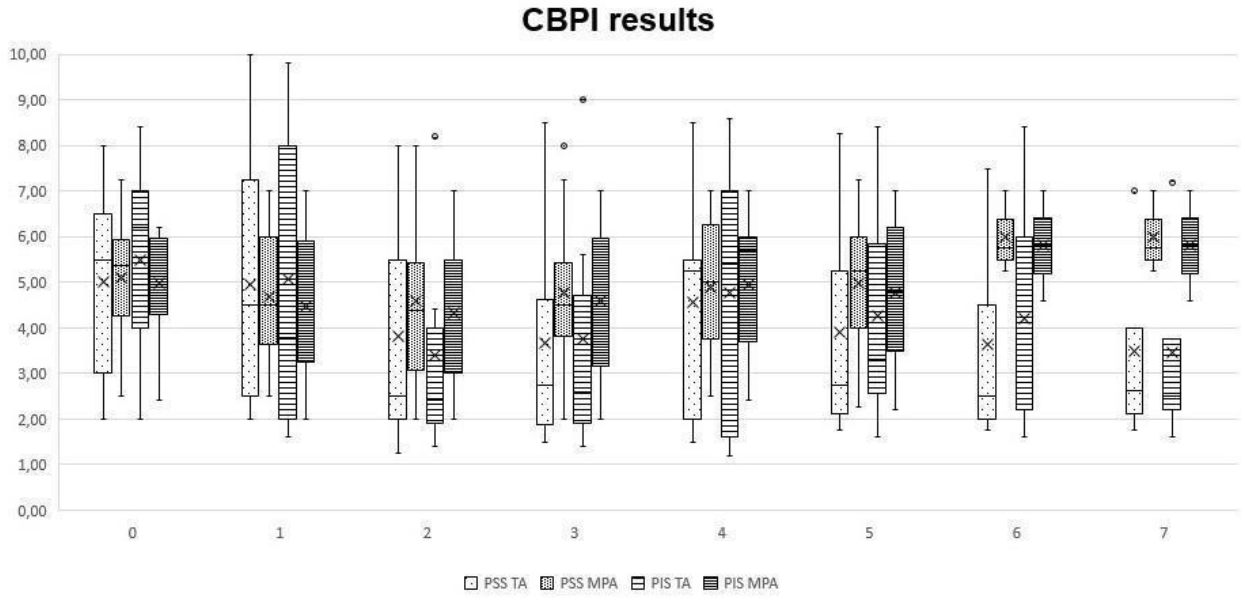

APPENDIX VII - Preliminary study on efficacy of a single intra-articular administration of triamcinolone acetonide, hyaluronan and a combination of both for management of hip osteoarthritis in dogs

EFFICACY OF A SINGLE INTRA-ARTICULAR ADMINISTRATION OF METHYLPREDNISOLONE ACETATE AND TRIAMCINOLONE ACETONIDE IN A NATURAL OCCURRING OSTEOARTHRITIS CANINE MODEL

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RESUMEN CORTO/RESUME

To describe the use and effectiveness of intra-articular triamcinolone acetonide (TA), hyaluronan (HA) and a combination of both (TA+HA), thirty police working dogs with naturally occurring hip osteoarthritis (OA) were selected. Sample comprised 6 females and 24 males, with a mean age of 6 years old and weight of 33.3 kg. Animals were randomly divided in three groups and treated with either a single administration of 20mg of TA (GT), 20mg of HA (GH) or a combination of 20mg of TA and 20mg of HA (GTH) per hip joint..

Response to treatment, measured by the Canine Brief Pain Inventory (CBPI) and the Hudson Visual Analogue Scale (HVAS), was evaluated at T0 (before treatment), T1 (after 15 days), T2, T3, T4, T5 and T6 (after 1, 2, 3, 4 and 5 months respectively). Results were compared using a Kruskal-Wallis test or a Wilcoxon signed ranks test.

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Comparing results of the different time points considered with T0, significant differences were observed in GH at T1 for HVAS ($p=0.03$) and PIS ($p=0.04$); and in GTH at T1 ($p=0.05$ for HVAS and $p<0.05$ for PIS), T2 ($p<0.04$ for PIS), T3 ($p<0.03$ for HVAS and $p=0.05$ for PIS), T4 ($p<0.03$ for HVAS and $p<0.05$) and T5 ($p<0.05$ for HVAS). No significant differences were found between groups when comparing results by time point. Intra-articular TA and HA may be a treatment option for dogs with naturally occurring OA, but their simultaneously use seems to improve the results. Further studies are required.

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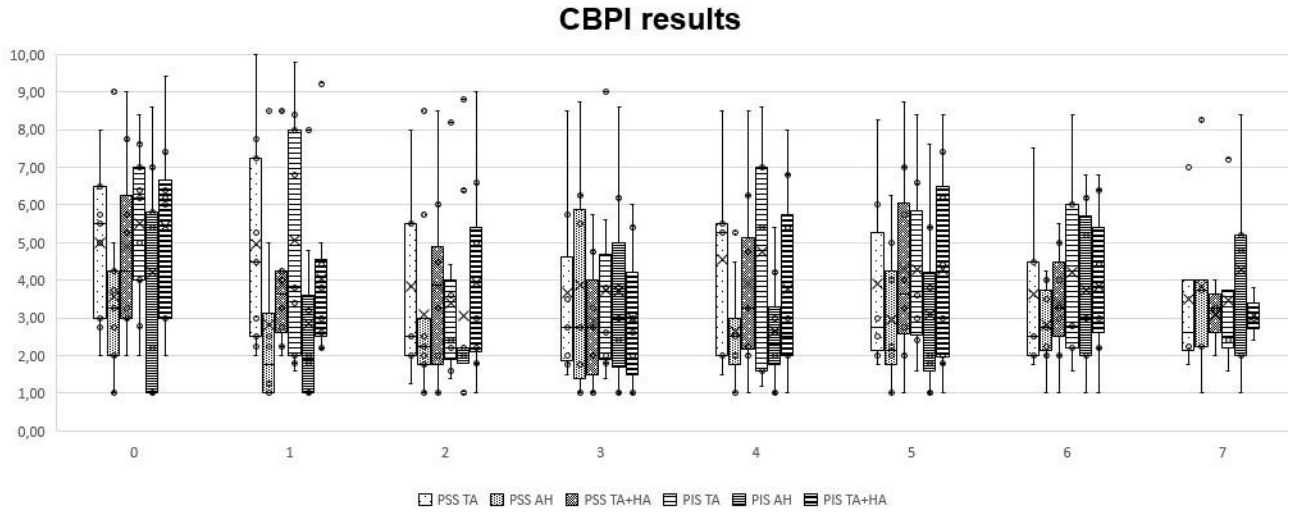

APPENDIX VIII - A preliminary report on the efficacy of a single administration of a platelet concentrate (V-PET) for the management of naturally occurring osteoarthritis

EFFICACY OF A SINGLE INTRA-ARTICULAR ADMINISTRATION OF METHYLPREDNISOLONE ACETATE AND TRIAMCINOLONE ACETONIDE IN A NATURAL OCCURRING OSTEOARTHRITIS CANINE MODEL

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RESUMEN CORTO/RESUME

The goal of this study was to describe the use and effectiveness of intra-articular a platelet concentrate (V-PET) in the management of naturally occurring hip osteoarthritis (OA) in an animal model. Twelve working dogs were treated with 3ml of platelet concentrate per hip, prepared with V-PET. Evaluations were conducted at T0 (before treatment), T1 (15 days after treatment), T2, T3, T4 and T5 (1, 2, 3, and 4 months after treatment). Response to treatment was evaluated with the Canine Brief Pain Inventory (divided in Pain Severity Score (PSS) and Interference Score (PIS)), Liverpool Osteoarthritis in Dogs (LOAD), Canine Orthopedic Index (divided in stiffness (Stiff), function, gait and quality of life (QOL)) and Hudson Visual Analogue Scale (HVAS).

When comparing each moment with T0, significant differences where found at T1 (p=0.03 for HVAS and PIS, p=0.00 for PSS and Gait, p=0.05 for Function, p=0.01 for QOL), T2 (p=0.00 for PSS, PIS and Gait, p=0.03 for Function and LOAD), T3 (p=0.01 for HVAS, PSS and Gait, p=0.05 for PIS and QOL, p=0.03 for Function), T4 (p=0.02 for PSS) and T5 (p=0.01 for PSS and Function, p=0.03 for Gait).

Intra-articular V-PET is a good treatment option for OA. More studies are required.

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APPENDIX IX - Comparison of a ventro-dorsal and lateral digital thermographic imaging in dogs with hip bilateral osteoarthritis

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Digital thermal imaging is a non-invasive, nonradiating, contact-free, physiologic diagnostic tool, that depends on heat resulting from physiological functions related to skin temperature control (Hildebrandt, Zeilberger, John Ring, & Raschner, 2012). It can be used to assess soft tissue injuries including muscle stains, sprains and tendinopathies but also OA and has been described as being useful in several species, from humans to horses and cats, but it's clinical utility has rarely been studied in small animals (Hildebrandt et al., 2012; M. H. Vainionpää et al., 2013). This study aimed to compare the ventro-dorsal view and lateral view thermographic images in dogs with bilateral hip osteoarthritis (OA).

Two hundred and eighty-two (n=282) sets of images were considered. Each comprised a dorso-ventral (DV), left lateral (LL) and right lateral (RL) images. Before imaging, dogs were maintained in a room at 21°C for 30 min. For the DV, they were positioned standing in a symmetrical upright position, as symmetrically as possible, without the trainer or veterinarian touching the dog's torso, and each image included the area from the last lumbar to the first coccygeal vertebra (M. Vainionpää et al., 2012). Each lateral view was set with the greater trochanter in the centre. All mages were taken

with a FLIR ThermaCAM E25 from a distance of 60 cm. Mean and maximal temperatures were recorded, and analyzed with a Wilcoxon Signed Ranks Test and Spearman correlation.

For the right hip, mean temperature recorded on a DV was 25.7ºC (±0.84) and 29.1ºC (±0.18) on the RL. Maximal values were 25.7ºC (±0.13) on the DV and 32.2ºC (±0.19) on the RL. In both cases, values were significantly different ($p<0.01$) and a showed week correlation (0.33 and 0.13, respectively). For the left hip, mean temperature on a DV was 24.97ºC (±0.12) and 28.7ºC (±0.18) on the LL view. Maximal values were 25.9ºC (±0.13) on the DV and 32.0ºC (±0.20) on the LL. Values varied significantly in both cases ($p<0.01$) and a showed low correlation (0.38 and 0.12, respectively).

Results obtained with a dorso-ventral and a lateral image are significantly different. Further studies are required, to determine which approach best reflects evaluation and treatment results.

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APPENDIX X - A comparison of weight bearing, thigh girth and joint range of motion in Police working dogs with bilateral hip osteoarthritis

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In dogs, hip OA is commonly a consequence of HD. This is the most common orthopaedic condition in dogs, often bilateral, with a prevalence of up to 71% in affected breeds, and causes joint inflammation with variable degrees of clinical discomfort (King, 2017). Physical examination is an essential part of the diagnosis process, and several parameters should be determined. Coxofemoral range of motion (ROM) can be diminished, particularly during extension (Smith, Karbe, Agnello, & McDonald-Lynch, 2011). Thigh girth is a useful measurement, not only in the initial assessment but also as an outcome measure, since the quadriceps muscle group is particularly prone to atrophy secondary to decreased limb function (McCarthy, Millis, Levine, & Weigel, 2018). Stance analyser has been reported as a sensitive for detecting lameness in dogs, being more sensitive in large breed dogs) (Clough, Canapp, Taboada, Dycus, & Leasure, 2018). . It has been proposed that body weight distribution at a stance may, in fact, be an equivalent or superior measurement of pain associated with hip OA than both VI and PVF. This study aimed to describe individual limb disease severity in dogs with bilateral hip osteoarthritis (OA).

Fifty police working dogs (N=50) with bilateral hip osteoarthritis were evaluated. Four breeds were represented: German Shepherd dog (n=17), Belgian Malinois (n=15), Labrador Retriever (n=10) and

Dutch Shepherd Dog (n=8). Weight bearing (WB) of each limb was collected with a Stance Analyzer. Thigh girth (TG) was measured with a Gullick II measuring tape at a distance of 70% thigh length, measured from the tip of the greater trochanter, with the leg in an extended position while in lateral recumbency. Hip joints range of motion were obtained at extension (Ext) and flexion (Flex) with a flexed stifle. Results were compared with an independent samples T-Test, with $p<0.05$.

Animals included had a mean age of 6.4 (±2.4) years old and body-weight of 26.7kg (±5.3). For the left pelvic limb, mean WB was 19.2% (±4.8), TG was 30.6cm (±2.9), Flex was 55.6° (±4.1) and Ext was 149.2° (±9.5). For the right pelvic limb, mean WB was 18.7% (±4.2), TG was 30.4cm (±2.6), Flex was 55.2° (±4.5) and Ext was 150.6° (±7.1). No significant differences were observed when comparing measurements from left and right limbs.

No significant variations between limbs were observed in WB, TG, Ext and Flex. Further studies are required, to determine if the same is true in animals of different sizes, breeds and ages.

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APPENDIX XI - Comparison of different Clinical Metrology Instruments in dogs with osteoarthritis

Poster presentations

Comparison of different Clinical Metrology Instruments in dogs with osteoarthritis

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OBJECTIVES

To compare the scores obtained with four Clinical Metrology Instruments (CMI) in dogs with bilateral hip osteoarthritis (OA).

METHODS

Three hundred responses (n = 300) to the Canine Brief Pain Inventory (CBPI, divided in pain severity score – PSS and pain interference score – PIS, the Liverpool Osteoarthritis in Dogs (LOAD), the Canine Orthopedic Index (COI, with four domains: stiffness, gait, function and quality of life – QOL) and the Hudson Visual Analogue Scale, were compared. For each animal, simultaneous responses to all CMI were obtained. In the cases of the CBPI and COI, respective sections were considered, as the overall score for COI. Results were compared with Spearman correlation and a Huynh-Feldt correction tests.

RESULTS

All other scores showed a negative correlation with HVAS, particularly PSS (−0.81), PIS (−0.83) and LOAD (−0.71). PSS and PIS showed a good correlation with all other scores, but particularly LOAD (0.80 and 0.82) and COI (0.80 and 0.82). LOAD scores had high correlation with all scores, especially COI (0.91) and its dimensions (>0.88). COI dimensions also showed good correlation comparing one to another, particularly stiffness and gait.

STATEMENT (CONCLUSIONS)

Scores of different CMI showed good correlation amongst themselves. Still, some variations were observed which might traduce that different CMI capture different aspects of OA.

Simple spatio-temporal gait analysis is not better than the human eye

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OBJECTIVES

Visual gait analysis suffers from subjectivity, and it is difficult to compare lameness grades between clinic visits and between veterinarians, but objective spatio-temporal analysis is generally restricted to the laboratory setting. Could simple video analysis offer a more objective and repeatable method of evaluation for mild grades of lameness in clinics?

METHODS

Video recordings obtained prospectively from 58 dogs using a standardised protocol were analysed using freely available software to determine ratios of stance and swing times. Captured still images were used to calculate symmetry ratios of head, shoulder and pelvic height between left and right limbs. Analysis time was recorded to assess practicability.

RESULTS

On clinical evaluation, 37 dogs were sound and 21 dogs were lame (9 forelimb, 12 hindlimb). The majority of lame dogs were graded as 1/5 or 2/5: none exceeded 3/5. On average, video recording took 7 minutes, stance/swing time analysis took 10 minutes, and height-ratio calculation took 19 minutes per dog. Measurement repeatability was good. Only the symmetry ratio for head height was significantly different between sound and forelimb lame dogs ($p = 0.001$), but graphical assessment indicated this was skewed by the most lame dogs.

STATEMENT (CONCLUSIONS)

While the described methods are simple and cheap, the simple spatio-temporal measures tested here could not discriminate between sound and mildly lame dogs (for which the need for such measures may be considered greatest), and busy clinicians may find the time requirements impractical. The veterinarian's eye, aided by video playback, remains the most sensitive lameness detection tool in clinical practice.

APPENDIX XII - Efficacy of a single intra-articular administration of autologous platelet therapy in police working dogs with hip osteoarthritis

Oral presentations

Efficacy of a single intra-articular administration of autologous platelet therapy in police working dogs with hip osteoarthritis

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OBJECTIVES

To describe the use and effectiveness of autologous platelet therapy in dogs with hip osteoarthritis (OA).

METHODS

In a prospective study, fifteen dogs with bilateral hip OA were treated with 3ml of platelet concentrate, prepared with the V-PET set, per hip joint. Response to treatment, measured by the Canine Brief Pain Inventory (CBPI, divided in pain interference score – PIS and Pain Severity Score – PSS), Liverpool Osteoarthritis in Dogs (LOAD), Canine Orthopedic Index (COI, divided in four dimensions: function, gait, stiffness and quality of life – QOL) and the Hudson Visual Analogue Scale (HVAS), was evaluated at T0 (before treatment), T1 (after 15 days), T2, T3, T4, T5, T6 and T7 (after 1, 2, 3, 4, 5 and 6 months respectively). Results were analyzed with a Paired Samples T-Test.

RESULTS

Significant differences were observed at T1 ($p < 0.01$ for HVAS, PSS, COI, Gait and QOL; $p = 0.01$ for PIS; $p = 0.02$ for Function; $p < 0.05$ for Stiffness), T2 ($p < 0.01$ for PSS, PIS and Gait; $p = 0.01$ for COI; $p = 0.02$ for HVAS, Function and QOL; $p = 0.04$ for Stiffness), T3 ($p < 0.01$ for HVAS, PSS, PIS, Function and Gait; $p = 0.01$ for COI; $p = 0.02$ for QOL), T4 ($p < 0.01$ for PSS; $p = 0.03$ for PIS and Gait), T5 ($p < 0.01$ for COI, Function and Gait; $p = 0.03$ for PSS, PIS and Stiffness), T6 ($p < 0.01$ for PSS, Function and Gait; $p = 0.04$ for PIS; $p < 0.05$ for COI) and T7 ($p < 0.01$ for PSS, Function and Gait; $p = 0.01$ for COI; $p < 0.05$ for PIS).

STATEMENT (CONCLUSIONS)

A single administration of autologous platelet therapy produced significant improvements for several months.

Radiographic measurement of mechanical tibial joint angles in Dachshunds: what is normal?

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OBJECTIVES

To establish mean mechanical tibial joint angles in normal Dachshunds. To facilitate surgical planning when managing angular limb deformities in a breed predisposed to Pes Varus.

METHODS

Caudocranial and mediolateral radiographs of normal tibiae from dachshunds were retrospectively evaluated. The mechanical medial proximal (mMPTA), mechanical medial distal (mMDTA), mechanical caudal proximal (mCaPTA) and mechanical cranial distal (mCrDTA) tibial angles were measured on three occasions, by two separate observers using previously established methodology. Data were analysed to assess normality with Shapiro-Wilk test, reliability via Interclass correlation coefficient (ICC) and the mean and standard deviation were calculated.

RESULTS

Thirty-three craniocaudal and thirty mediolateral radiographs met the inclusion criterion. Data were normally distributed, intra-observer and inter-observer reliability was good (>0.8) for all measures. The mean and standard deviation for mMPTA, mMDTA, mCaPTA and mCrDTA were $93.201° \pm 4.287°$, $97.63° \pm 4.022°$, $74.768° \pm 3.866°$, $85.183° \pm 5.677°$ respectively.

STATEMENT (CONCLUSIONS)

Previous studies have established normal tibia joint reference angles (JRAs) in small, medium and large breeds.

APPENDIX XIII – A comparison of four intra-articular treatment modalities in a natrually occurring canine osteoarthritis model

A COMPARISON OF FOUR INTRA-ARTICULAR TREATMENT MODALITIES IN A NATURAL OCCURRING CANINE OSTEOARTHRITIS MODEL

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RESUMEN CORTO/RESUME

To compare the effect of a single intra-articular administration of different treatments in a natural occurring canine osteoarthritis (OA) model, one hundred (N=100) hip joints with naturally occurring osteoarthritis (OA) and randomly assigned to five groups: control group (CG, n=20), triamcinolone hexacetonide group (THG, n=20), platelet concentrate group (PCG, n=20), stanozolol group (SG, n=20) and hylan G-F 20 group (HG). Evaluations were conducted on days 0 (treatment day), 8, 15, 30, 60, 90, 120, 150 and 180 days post treatment, and consisted of weight distribution analysis and data from four Clinical Metrology Instruments. Cox proportional hazard regression analysis was carried out to investigate the influence of the variables of interest on treatment survival. All results were analyzed with IBM SPSS Statistics version 20 and a significance level of $p< 0.05$ was set.

The sample included joints of 100 pelvic limbs (n=50 left and n=50 right), with a mean age of 6.5±2.4 years and body weight of 26.7±5.2kg, classified as mild (n=70), moderate (20) and severe (10). Patients in HG and PCG took longer to return to baseline values and scores. PCH and HG experienced 57%-81% improvements in functional evaluation and impairments due to OA, and may be a better options for these cases. Better impact on pain interference was observed in THG, with a 95% improvement over CG.

This study provides important information for the characterization of the effects of these treatment modalities, duration of observed improvements function and pain, in addition to information regarding candidates for each one.

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APPENDIX XIV – Characterization of the effect of the intra-articular administration of stanozolol in a natural occurring canine osteoarthritis model

CHARACTERIZATION OF THE EFFECT OF THE INTRA-ARTICULAR ADMINISTRATION OF STANOZOLOL IN A NATURAL OCCURRING CANINE OSTEOARTHRITIS MODEL

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RESUMEN CORTO/RESUME

In this study, we aimed describe the effect of the intra-articular administration of stanozolol in a natural occurring canine osteoarthritis model. Forty (N=40) hip joints of active police working dogs were randomly assigned to a control (CG) and treatment (SG) group. Weight distribution, joint range of motion, thigh girth, digital thermography, radiographic signs, synovial fluid interleukin-1 levels were recorded at treatment day (day 0), and at 8, 15, 30, 90 and 180 days post-treatment. The Hudson Visual Analogue Scale, the Liverpool Osteoarthritis in Dogs, the Canine Brief Pain Inventory and the Canine Orthopedic Index. Results were compared using Repeated Measures ANOVA, with a Huynh-Feldt correction, Wilcoxon Signed Ranks Test, with $p<0.05$.

Sample included joints from 40 pelvic limbs, graded as mild (n=36, 90%), moderate (n=2, 5%) and severe (n=2, 5%), with a mean age of 6.5±2.4 years and body weight of 26.7±5.2kg. No differences were found between groups at day 0. Comparing CG to SG, symmetry index showed significant improvements in SG from 15 days ($p=0.05$) up to 180 days ($p=0.01$). SG also showed lower values during thermographic evaluation in both views taken, and improved joint extension at 90 ($p=0.02$) and 180 days ($p<0.01$). Pain and function scores, improved up to 90.180 days. In SG, improvements in some radiographic findings was observed.

To our knowledge, this is the first study to describe the effect of a single injection of stanozolol in a naturally occurring canine model, with a long follow up period.

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APPENDIX XV - The influence of IL-1 and C-reactive protein concentrations levels in the synovial fluid of patients with osteoarthritis

THE INFLUENCE OF IL-1 AND C-REACTIVE PROTEIN CONCENTRATIONS LEVELS IN THE SYNOVIAL FLUID OF PATIENTS WITH OSTEOARTHRITIS

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RESUMEN CORTO/RESUME

Osteoarthritis (OA) affects all mammals, being an important and costly disease. We aimed to evaluate IL-1 and C-reactive protein (CRP) levels in the synovial fluid in dogs with naturally occurring hip osteoarthritis (OA), and its relation with patients' clinical, radiographic and thermographic disease signs.

One hundred (N=100) joints of active police working dogs with hip OA were evaluated. SF IL-1 and CRP levels, weight distribution, joint range of motion at flexion and extension, thigh girth, digital thermography and hip grade were recorded. Data from four Clinical Metrology Instruments, the Hudson Visual Analogue Scale, Liverpool Osteoarthritis in Dogs, Canine Brief Pain Inventory and Canine Orthopedic Index, were collected. Results were compared by different IL-1 and CRP concentration levels and OFA scores with the Independent Samples T-Test, ANOVA and Pearson correlation coefficient, with $p<0.05$.

Sample included 100 pelvic limbs, equally distributed between left and right pelvic limbs 30 males and 20 females, with a mean age of 6.5±2.4 years and body weight of 26.7±5.2kg. IL-1 levels, particularly above 200pg/mL, may be related with the development of radiographic signs, which then reflects on worse CMI scores. It was unclear if CRP level was a consequence of inflammatory activity within the joint or a reflection of better prognosis. Increasing body weight was related with worse CMI scores.

This is the first study to describe the relation of IL-1 and CRP synovial concentrations levels with several clinical signs, diagnostic imaging, laboratorial findings and clinical metrology instruments results of patients with OA.

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APPENDIX XVI - Description of limb weight bearing redistribution in police working dogs with hip osteoarthritis

DESCRIPTION OF LIMB WEIGHT BEARING REDISTRIBUTION IN POLICE WORKING DOGS WITH HIP OSTEOARTHRITIS

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RESUMEN CORTO/RESUME

This study aimed to describe weight bearing redistribution present in police working diagnosed with bilateral hip osteoarthritis (OA), and in response to treatment. Fifty Police working dogs with bilateral hip OA was performed at the time of initial evaluation and after treatment, on days 0 (treatment day) and on 8, 15, 30, 90 and 180 post-treatment. Results were compared by breed, age, sex, weight and OFA scores with the Independent Samples T-Test, one-way ANOVA and Pearson correlation coefficient, with $p< 0.05$.

Patients had a mean age of 6.5±2.4 years and body weight of 26.7±5.2kg, of both sexes (30 males and 20 females). Four breeds were represented: German Shepherd Dogs (n=17), Belgian Malinois Shepherd Dogs (n=15), Labrador Retriever (n=10), and Dutch Shepherd Dog (n=8). Thirty-five animals were classified as mild (70%), 10 as moderate (20%) and 5 as severe (10%). No significant differences were observed when comparing weight bearing for different breeds, sex, hip grades or different cut-offs for weight at the initial evaluation. A weight shift from pelvic to thoracic limbs was observed, with a weak significant correlation found between a pelvic limb and the opposing contralateral thoracic limb. Labrador Retrievers showed higher symmetry index and deviation from normal values than other breeds. During the follow-up period, after treatment, weight bearing of all limbs correlated with the remaining limbs, reflecting a more balanced weight distribution.

This study first describes weight-bearing redistribution in dogs with bilateral hip OA and also in response to treatment, using a novel evaluation modality.

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APPENDIX XVII - Use of an autologous platelet therapy in police working dogs with hip osteoarthritis

Objectives: To describe the effect of the platelet concentrate V-PET in dogs with hip osteoarthritis (OA).

Methods: In a prospective, randomly controlled, double-blind study, forty (N=40) joints of active police working dogs with hip OA were randomly assigned to control and V-PET groups. Weight distribution, joint range of motion, thigh girth, radiographic signs, and four clinical metrology instruments (CMI) were recorded at treatment day, 8, 15, 30, 90, and 180-days post-treatment. Results were compared with repeated measures ANOVA, with a Huynh-Feldt correction, or Wilcoxon Signed Ranks Test, with $p<0.05$. Kaplan-Meier estimators were conducted and compared with the log-rank test.

Results: Patients had a mean age of 6.5±2.4 years and bodyweight of 26.7±5.2kg. At T0, 32 (80%) joints were graded as mild, 6 (15%) as moderate, and 2 (5%) as severe OA. No differences were found between groups on treatment day. Comparing control to V-PET groups, weight-bearing showed significant improvements with V-PET from 8 days ($p<0.01$) up to 180 days ($p=0.01$), alongside joint flexion up to 90 days ($p=0.05$), and extension up to 180 days ($p=0.01$). Several CMI scores also improved up to 90 to 180 days post-treatment. In radiographic signs in the control group progressed, while in the V-PET group showed some improved signs. Kaplan-Meier estimators showed significantly better CMI and weight-bearing results in the V-PET group.

Impact: A single injection of V-PET had a positive effect on several clinical and imaging signs, lasting up to 180 days.

APPENDIX XVIII - Comparison of the intra-articular treatment with a platelet concentrate, high-density hyaluronan, triamcinolone hexacetonide, and stanozolol in dogs with hip osteoarthritis

Objectives: To compare the effect of the intra-articular treatment with triamcinolone hexacetonide (TH), stanozolol, hyaluronan, and platelet concentrate (PC) in Police working dogs with bilateral hip osteoarthritis (OA).

Methods: In a prospective, longitudinal, double-blinded, negative controlled study, fifty (N=50) police working dogs with hip OA were randomly assigned to a control group (CG, n=10), TH group (THG, n=10), PC group (PCG, n=10), stanozolol group (SG, n=10) and Hylan G-F 20 group (HG). On days 0, 8, 15, 30, 90, and 180 days post-treatment, weight-bearing distribution was evaluated. Three clinical metrology instruments (CMI - Canine Brief Pain Inventory, Liverpool Osteoarthritis in Dogs, and Canine Orthopedic Index) were also completed. Kaplan-Meier estimators were conducted and compared with the log-rank test. Cox proportional hazard regression analysis was performed to determine treatment survival. Significance was set at $p<0.05$.

Results: Patients had a mean age of 6.5±2.4 years and bodyweight of 26.7±5.2kg. At initial evaluation, hips were graded as mild (n=35), moderate (n=10), and severe (N=5). No differences were found between groups at that moment. All treatments were able to improve CMI scores and weight-bearing, but in some groups with a wide interval of efficacy. PCG showed a lower range of variation while maintaining a positive result for more extended periods. Breed, age, sex, and OFA grade did not significantly influence response to treatment.

Impact: This is the first study to compare the effect of IA treatments in dogs with hip osteoarthritis. PC and hyaluronan showed better results in the management of OA.