The influence of IL-1 and C-reactive protein levels in synovial fluid of companion dogs with bilateral hip osteoarthritis on various clinical disease parameters

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OBJECTIVE
To evaluate IL-1 and C-reactive protein (CRP) levels in the synovial fluid in dogs with bilateral hip osteoarthritis and their relation to animals’ clinical, radiographic, and thermographic disease signs.

SAMPLE
100 joints from police working dogs.

PROCEDURES
Synovial fluid, IL-1, and CRP levels, weight distribution, joint range of motion, thigh girth, digital thermography, and radiographic signs of the joints were recorded. Data from 4 clinical metrology instruments (CMIs) were collected. Results were compared by age, sex, and Orthopedic Foundation for Animals hip scores with the independent samples t test, ANOVA, and Pearson correlation coefficient (P < .05).

RESULTS
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 body weight of 26.7 ± 5.2 kg. IL-1 levels, particularly above 200 pg/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 a better prognosis. Increasing body weight was related to worse CMI scores.

CLINICAL RELEVANCE
We described the relation of IL-1 and CRP synovial concentration levels with several clinical signs, diagnostic imaging, laboratory findings, and CMI results of animals with osteoarthritis. Further studies are required to determine the interest of each parameter for the prognosis and treatment monitoring.

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 concerns. OA is a relatively low-grade inflammatory disease, where the inflammatory process affects the progression of the disease, frequently without systemic manifestation. IL-1 has been pointed out as the most critical proinflammatory cytokine responsible for the catabolism in OA, in relation to lameness duration. However, some studies have reported low or undetectable IL-1 levels in OA animals. 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. 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.
femoral head osteophyte (CFHO) and caudolateral curvilinear osteophyte (CCO), early radiographic signs related to the development of the clinical signs of hip OA.1,9 Weight distribution, off-loading, or limb favoring at the stance is a standard assessment during the orthopedic examination, as animals tend to bear less weight on a painful limb.20 Stance analysis has been reported as a sensitive evaluation for detecting lameness in dogs.21 Digital thermal imaging relies on heat generated during physiologic functions and its relation with skin temperature control.12 It is a reliable technique to assess inflammatory arthritis pain and differentiate normal from osteoarthritis subjects.13 Pain and functional ability are the most relevant parameters in the evaluation of OA animals and for the assessment of treatment efficacy.14 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).24 The CBPI is divided into 2 sections, a pain severity score (PSS) that assesses the magnitude of the animal pain, and a pain interference score (PIS) that assesses the degree to which pain affects daily activities. It has been demonstrated that the PSS is not being associated with response bias.15 Other CMIs are also used to evaluate different dimensions of OA, like the Canine Orthopaedic Index (COI; divided into 4 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.19,20 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.15 Pedometers are simple and inexpensive devices, capable of measuring ambulatory activity with acceptable accuracy.21 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.22 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 Evora (Orgão Responsável pelo Bem-estar dos Animais da Universidade de Evora; approval No. GD/32055/2018/P1; September 25, 2018). Written, informed consent was obtained from the institution responsible for the animals.

The sample comprised 100 joints of active police working dogs with hip OA. This was a convenience sample, comprised of animals presented for intra-articular treatment for hip OA. For the diagnosis, a complete history, physical, orthopedic, neurologic, and radiographic examinations were obtained and had to be consistent with bilateral hip OA. Additionally, animals should have a body weight ≥ 15 kg, be over 2 years old, and must not have received any medication or nutritional supplements for 6 weeks or more. Animals were excluded if suspected to have any other orthopedic or concomitant disease or if they were not tolerant of data collection. All evaluations were performed at the same moment by the same researcher. All animals remained in active work. The dogs’ handlers were present during the evaluation of their animal.

Stance analysis

Stance analysis was conducted with a weight distribution platform (Companion Stance Analyzer; LiteCure LLC). According to the manufacturer’s guidelines, the equipment was placed in the center of a room, at least 1 m from the walls. It was calibrated at the beginning of each day and zeroed before each data collection. Trainers encouraged aged 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: 

\[
\text{SI} = \left( \frac{\text{WB}_R - \text{WB}_L}{\text{WB}_R + \text{WB}_L} \right) X 0.5
\]

where \( \text{WB}_R \) is the value of weight-bearing for the right pelvic limb and \( \text{WB}_L \) 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%, 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 symmetric 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 distance of 60 cm.26 A lateral view was also obtained, at a distance of 60 cm, with the greater trochanter in the center. All images were produced with a ThermaCAM E25 model (FLIR Systems Inc). The range of temperature was set at 15 to 40°C and emissivity at 0.98. Data from the thermographic images were analyzed with free software tools (FLIR Systems Inc), and the Rainbow HC color 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 stifte.
Pedometers
Pedometers were worn around the patient’s neck and were attached to an adjustable lightweight collar. They were placed 1 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 (0.01 mg/kg) and butorphanol (0.1 mg/kg), given intravenously. VD extended legs and FL views were obtained. An Orthopedic Foundation for Animals hip score was obtained from the VD extended legs view. The presence of seven radiographic OA signs 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; and subchondral bone sclerosis along the cranial acetabular edge and CFHO. In the FL view, the presence of CCO and CFHO was also recorded.

Determination of synovial IL-1 and serum and synovial CRP concentrations
With the patient positioned in lateral recumbency with the affected joint uppermost, a small window of 4 × 4-cm 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 4 X 4-cm 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 needle was introduced just dorsal to the greater trochanter and perpendicular to the table, with the affected joint uppermost, a small window of 4 × 4 cm 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 needle was introduced just dorsal to the greater trochanter and perpendicular to the table.

Statistical analysis
Normality was assessed with a Shapiro-Wilk test, and each measured parameter was compared with an independent sample t test or ANOVA. Different score cutoff points (4, 6, and 8) were analyzed for synovial IL-1 and CRP concentrations, to evaluate the effect of different concentrations on various clinical disease parameters. 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 statistical software (SPSS Statistics version 20; IBM Corp), and a significance level of P < .05 was set.

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 body weight of 26.7 ± 5.2 kg. Four dog breeds were represented: German Shepherd Dogs (n = 17), Belgian Malinois Shepherd Dogs (15), Labrador Retriever (10), and Dutch Shepherd Dog (8). Considering OFA hip grading, 70 joints were classified as mild (70%), 20 as moderate (20%), and 10 as severe (10%). Both sexes were represented (60 limbs from males and 40 from females, all intact), and male dogs showed significantly higher mean temperature on the lateral view than females (P = .05) and also higher flexion values (P = .05). The volume of synovial collected from each joint varied significantly (between 0.5 mL and 1.5 mL) but was enough to perform the analysis. Overall IL-1 concentration in the synovial fluid was 161.8 pg/mL (± 66.5) and in CRP was 2.5 mg/dL (± 1.9). Serum CRP concentration was 0.8 mg/dL (± 0.5). Clinical findings with different cutoff points for IL-1 and CRP concentration in synovial fluid are presented (Table 1). CMI results are presented (Table 2). Variables considered in multiple regression statistically significantly predicted IL-1 synovial concentration F(5,85) = 2.826 (P < .03; R² = .117), with OFA hip score (P < .01) adding statistically significantly to the prediction.

Evaluation of synovial IL-1 and CRP concentration
Considering a 100-ng/mL cutoff point for IL-1 concentration, no significant differences were observed. With a 200-ng/mL cutoff, animals with higher values showed higher frequency in the presence of CCO on the VD view (P < .01), worse OFA hip grades (P < .01), and lower serum CRP concentration (P = .02). Considering a 0.3-ng/dL cutoff point for CRP concentration, animals below this range had worse PSS (P = .04), PIS (P < .01), and function scores (P < .01). With a 1-ng/dL cutoff, animals with higher values had higher pedometer counts (P = .02), lower CFHO frequency (P < .01), and better OFA hip grade (P < .01), PIS (P < .01), and function scores (P < .01). Considering a 2-ng/dL cutoff value, animals with higher values had higher pedometer counts (P = .02), higher mean and maximal thermography values on the lateral view (P = .05 and P < .01, respectively), increased frequency of CCO and CFHO on the VD view (P < .01, for both), better OFA hip grade (P < .01), and higher serum CRP concentration (P = .02). IL-1 synovial levels added statistically
Table 1—Mean values (±standard deviation) of overall 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 cutoff values for synovial IL-1 and CRP concentration.

| Cutoff          | Symmetry index | Deviation | Thermography (ventrodorsal) | Thermography (lateral) | Thigh girth (cm) | Joint flexion (degrees) | Joint extension (degrees) | IL-1 | SF CRP | Serum CRP |
|-----------------|----------------|-----------|----------------------------|------------------------|------------------|------------------------|---------------------------|------|--------|-----------|
| IL-1 with 100-ng/mL cutoff |                |           |                           |                        |                  |                        |                           |      |        |           |
| Above           | 14.5 ± 20.9    | 1.9 ± 2.2 | 25.4 ± 1.3                 | 26.8 ± 1.2             | 28.3 ± 2.1       | 33.8 ± 19.3           | 150.7 ± 11.3              | —    | 0.2 ± 0.9 | 0.6 ± 0.5 |
| Below           | 16.5 ± 20.9    | 3.0 ± 5.3 | 26.0 ± 0.8                 | 27.5 ± 0.8             | 27.9 ± 1.1       | 32.1 ± 2.3            | 153.3 ± 2.1               | —    | 0.5 ± 0.6 | 0.4 ± 0.3 |
| IL-1 with 200-ng/mL cutoff |                |           |                           |                        |                  |                        |                           |      |        |           |
| Above           | 14.4 ± 21.2    | 2.3 ± 2.8 | 25.9 ± 1.1                 | 27.2 ± 1.1             | 28.4 ± 1.6       | 48.3 ± 45.1           | 145.0 ± 27.4              | —    | 0.0 ± 0.0 | 0.4 ± 0.3 |
| Below           | 14.7 ± 20.8    | 2.0 ± 2.6 | 25.4 ± 1.3                 | 26.8 ± 1.2             | 28.2 ± 2.1       | 48.3 ± 45.1           | 151.8 ± 3.3               | —    | 0.2 ± 0.9 | 0.6 ± 0.5 |
| IL-1 with 300-ng/mL cutoff |                |           |                           |                        |                  |                        |                           |      |        |           |
| Above           | 7.1 ± 7.5      | 1.0 ± 1.1 | 25.6 ± 1.0                 | 27.2 ± 1.1             | 29.2 ± 1.6       | 31.0 ± 3.0            | 151.9 ± 3.2               | —    | 0.0 ± 0.0 | 0.5 ± 0.3 |
| Below           | 15.4 ± 21.5    | 2.13 ± 2.7| 25.5 ± 1.3                 | 26.8 ± 1.2             | 28.2 ± 2.1       | 31.0 ± 3.0            | 151.9 ± 3.2               | —    | 0.2 ± 0.9 | 0.6 ± 0.5 |
| CRP with 0.3-ng/mL cutoff |                |           |                           |                        |                  |                        |                           |      |        |           |
| Above           | 3.1 ± 2.9      | 1.0 ± 0.7 | 26.2 ± 1.4                 | 27.6 ± 1.2             | 28.6 ± 0.5       | 32.5 ± 3.1            | 151.8 ± 3.0               | 113.5 ± 25.6 | — | 0.9 ± 0.8 |
| Below           | 12.5 ± 19.7    | 1.7 ± 2.6 | 25.4 ± 1.3                 | 26.8 ± 1.3             | 28.2 ± 2.0       | 36.3 ± 22.7           | 150.7 ± 13.3              | 169.3 ± 69.1 | — | 0.6 ± 0.5 |
| CRP with 1-ng/mL cutoff |                |           |                           |                        |                  |                        |                           |      |        |           |
| Above           | 3.9 ± 2.6      | 1.3 ± 0.5 | 26.2 ± 1.4                 | 27.6 ± 1.2             | 28.7 ± 0.5       | 31.6 ± 2.7            | 150.8 ± 2.2               | 117.3 ± 27.9 | — | 1.1 ± 0.8 |
| Below           | 12.3 ± 19.8    | 1.7 ± 2.6 | 25.4 ± 1.3                 | 26.8 ± 1.3             | 28.2 ± 2.0       | 35.3 ± 22.5           | 150.8 ± 13.2              | 168.2 ± 69.1 | — | 0.6 ± 0.5 |
| CRP with 2-ng/mL cutoff |                |           |                           |                        |                  |                        |                           |      |        |           |
| Above           | 2.7 ± 3.8      | 1.0 ± 0.0 | 26.3 ± 1.9                 | 27.5 ± 1.6             | 29.0 ± 0.3       | 30.8 ± 1.1            | 150.5 ± 3.5               | 132.3 ± 28.3 | — | 1.5 ± 1.1 |
| Below           | 12.1 ± 19.4    | 1.67 ± 3.6| 25.5 ± 1.3                 | 28.8 ± 1.3             | 28.2 ± 1.9       | 35.2 ± 2.2            | 150.8 ± 12.9              | 166.0 ± 69.0 | — | 0.6 ± 0.5 |

— = Not applicable.
Table 2—Mean values (±standard deviation) of clinical metrology instruments scores, by different cutoff values for synovial IL-1 and CRP concentration.

| Cutoff                  | CBPI                  | HVAS (0–10) | PSS (0–10) | PIS (0–10) | LOAD (0–52) | COI                  |
|-------------------------|-----------------------|-------------|------------|------------|-------------|----------------------|
| IL-1 with 100-ng/mL cutoff | Above                | 6.6 ± 1.5  | 3.3 ± 2.3  | 3.3 ± 2.6  | 13.2 ± 11.2 | 3.5 ± 4.1  |
| IL-1 with 200-ng/mL cutoff | Below                | 6.8 ± 1.5  | 2.9 ± 2.3  | 3.2 ± 2.4  | 12.0 ± 11.9 | 3.5 ± 4.2  |
| IL-1 with 300-ng/mL cutoff | Above                | 7.0 ± 1.0  | 2.8 ± 1.8  | 2.6 ± 1.7  | 10.4 ± 7.5  | 2.6 ± 3.1  |
| IL-1 with 300-ng/mL cutoff | Below                | 6.6 ± 1.5  | 3.4 ± 2.4  | 3.5 ± 2.7  | 13.6 ± 11.7 | 3.7 ± 4.3  |
| CRP with 0.3-mg/dL cutoff | Above                | 7.5 ± 0.9  | 1.7 ± 1.1  | 1.7 ± 1.5  | 4.1 ± 3.4  | 0.3 ± 0.5  |
| CRP with 0.3-mg/dL cutoff | Below                | 6.6 ± 1.5  | 3.4 ± 2.4  | 3.5 ± 2.6  | 13.9 ± 11.3 | 3.8 ± 4.2  |
| CRP with 1-mg/dL cutoff | Above                | 7.7 ± 1.1  | 1.6 ± 1.3  | 1.3 ± 0.6  | 5.2 ± 6.1  | 0.8 ± 1.8  |
| CRP with 1-mg/dL cutoff | Below                | 6.8 ± 1.3  | 3.2 ± 2.1  | 3.2 ± 2.3  | 11.9 ± 10.3 | 3.1 ± 3.7  |
| CRP with 2-mg/dL cutoff | Above                | 7.5 ± 1.2  | 1.7 ± 1.4  | 1.4 ± 0.7  | 5.5 ± 7.0  | 1.0 ± 2.0  |
| CRP with 2-mg/dL cutoff | Below                | 6.8 ± 1.3  | 3.1 ± 2.1  | 3.1 ± 2.3  | 11.8 ± 10.2 | 3.1 ± 3.7  |
| 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.

(P < .01) significantly to the prediction of thigh girth $F(3,59) = 11.019 (P < .01; R^2 = .359)$ and joint extension thigh girth $F(3,58) = 3.676 (P = .03; R^2 = .148)$.

Evaluation of radiographic findings

Clinical findings for different OFA hip grades, according to the presence of CCO and CFHO, with different cutoff points for weight, and sex are presented (Table 3). CMI results are presented (Table 4).

Grouping joints by OFA hip grading, animals with a severe grade were significantly older than those with mild (P < .01) and moderate (P < .01) grades and had worse SI and deviation than mild (P = .03 and P = .01, respectively). On the digital thermography DV view, mild hip grades showed higher values than moderate grades (P = .03). Mild grades had higher IL-1 concentration than moderate grades (P < .01) and better HVAS scores than moderate (P < .01) and severe (P < .01) hip grades. They also had better PSS (P < .01), PIS (P < .01), LOAD (P < .01), function (P < .01), gait (P < .01), QOL (P < .01), and COI (P < .01) scores than severe hip grades. IL 1 synovial levels added statistically (P = .01) significantly to the prediction of OFA hip grade $F(1,90) = 6.352 (P = .01)$.

Joints that exhibited CCO on the DV view had worse OFA hip grade (P < .01), lower levels of synovial IL-1 concentration (P = .02), higher serum CRP (P < .01), and worse PSS (P = .05), LOAD (P < .02), function (P = .05), gait (P = .01), and COI (P = .04) scores. IL 1 synovial levels added statistically (P < .01) significantly to the prediction of the presence of CCO on a DV view $F(3,59) = 11.019 (P < .01)$. Joints with CFHO on the DV view showed lower pedometer counts (P = .02), worse OFA hip grade (P < .01), and worse HVAS (P < .01), PSS (P < .01), PIS (P < .01), LOAD (P < .02), stiffness (P < .02), function (P < .01), gait (P = .02), QOL (P = .02), and COI (P < .01) scores. Considering the FL view, joints where CCO was identified had worse SI (P < .01), deviation (P = .03), and OFA hip grade (P < .01). IL-1 synovial levels added statistically (P < .01) significantly to the prediction of the presence of CCO on an FL view $F(3,59) = 3.047 (P = .01)$. No significant variations were observed when CFHO was considered in this view.

Evaluation of increasing body weight

Different cutoff points were considered regarding weight. With a 25-kg cutoff, heavier animals had
Table 3—Mean values (± SD) of overall weight, age, symmetry index, 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 IL-1 and C-reactive protein (CRP), by different OFA hip grades, presence of caudolateral curvilinear osteophyte (CCO) and circumferential femoral head osteophyte (CFHO), different cutoff values for weight, and sex.

| Cutoff                        | Age (years) | Pedometer (mean daily steps) | Symmetry index | Deviation | Thermography (ventrodorsal) Mean °C | Maximal °C | Thermography (lateral) Mean °C | Maximal °C | Thigh girth (cm) | Joint flexion (degrees) | Joint extension (degrees) | IL-1 | Synovial fluid CRP |
|-------------------------------|-------------|-----------------------------|----------------|-----------|-----------------------------------|------------|--------------------------------|------------|-------------------|--------------------------|--------------------------|------|------------------|
| 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              | 150.6 ± 50.5     | 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 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 |
| CCO 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 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 ± 2.7       | 50.8 ± 3.7               | 150.1 ± 16.2              | 163.7 ± 70.9    | 0.4 ± 1.1 |
| CFHO 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 |
| BW with 25-kg cutoff 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   | 0.2 ± 0.9 |
| BW with 25-kg cutoff Below    | 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   | 0.3 ± 0.9 |
| BW with 30-kg cutoff 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 |
| BW with 30-kg cutoff Below    | 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 35-kg cutoff Above    | 7.0 ± 0.0   | 101.0 ± 0.0                 | 23 ± 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 |
| BW with 35-kg cutoff Below    | 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 |
| Male                          | 6.2 ± 2.3   | 457.6 ± 452.6               | 18.3 ± 15.2    | 2.5 ± 2.1 | 24.7 ± 1.3                        | 26.1 ± 1.3 | 24.7 ± 1.3                      | 31.1 ± 1.5 | 30.9 ± 2.3       | 151.1 ± 5.6              | 55.9 ± 4.1               | 168.3 ± 87.6   | 0.3 ± 0.9 |
| Female                        | 6.9 ± 2.8   | 432.0 ± 372.5               | 17.6 ± 12.6    | 2.3 ± 3.2 | 24.6 ± 1.2                        | 26.5 ± 1.1 | 24.5 ± 1.2                      | 30.9 ± 2.1 | 28.5 ± 2.2       | 150.6 ± 4.9              | 55.4 ± 4.2               | 156.6 ± 40.5    | 0.2 ± 0.1 |

BW = Body weight.
lower pedometer counts \( (P = .03) \), higher SI \( (P < .01) \), lower mean and maximal temperature on the lateral view \( (P < .01 \text{ for both}) \), and worse HVAS \( (P < .01) \), PSS \( (P < .01) \), PIS \( (P < .01) \), LOAD \( (P < .01) \), stiffness \( (P < .01) \), function \( (P < .01) \), gait \( (P < .01) \), QOL \( (P < .01) \), and COI scores \( (P < .01) \). Setting a 30-kg cutoff value, heavier animals had lower mean and maximal temperature on the lateral view \( (P < .01 \text{ for both}) \), worse flexion \( (P < .01) \), and lower IL-1 concentration level \( (P = .04) \). For a 35-kg cutoff value, heavier animals had lower SI \( (P < .01) \), lower mean and maximal temperature on the lateral view \( (P < .01 \text{ for both}) \), worse flexion \( (P < .01) \), higher frequency of CCO on an FL view \( (P < .01) \), lower IL-1 concentration level \( (P < .01) \), and CRP serum concentration \( (P < .01) \) and worse HVAS \( (P = .05) \), LOAD \( (P = .05) \), stiffness \( (P = .03) \), gait \( (P < .01) \), QOL \( (P = .03) \), and COI scores \( (P = .03) \).

**Evaluation by sex**

Considering sex, significant differences were observed in thigh girth \( (P < .01) \), with male dogs having higher values. Sex has not contributed to the prediction of synovial IL-1 and CRP concentrations. The correlation between measured parameters and the different Clinical Metrology Instruments are presented (Supplementary Table S1). Correlations between measured parameters are also presented (Supplementary Table S2).

**Discussion**

Osteoarthritis is the most commonly diagnosed joint disease both in human and veterinary medicine.\(^{31}\) To our knowledge, this is the first study to describe the influence of IL-1 and CRP synovial concentration levels in several clinical signs, diagnostic imaging, laboratory findings, and clinical metrology instrument 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.\(^1\) IL-1 drives this process and increases the expression of inflammatory genes and mediators, with median detected concentrations of IL-1β concentrations of 109 pg/mL in knees with mild OA and 122 pg/mL in moderate OA, in an animal model.\(^{32}\) We have recorded mean values of 178.3 pg/mL for joints with mild OA, 130.6 pg/mL for joints with moderate OA, and 144.7 pg/mL for joints with severe OA. Concentration levels were significantly higher in mild cases compared with moderate ones, which may account for the also significantly higher thermographic evaluation. In fact, regression analysis

**Table 4**—Mean values (±standard deviation) of clinical metrology instruments scores, by different OFA hip grades, presence of CCO and CFHO, different cutoff values for weight, and sex.

| Cutoff | CBPI | Stiffness | Function | Gait | QOL | Total |
|--------|------|-----------|----------|------|-----|-------|
|        | HVAS \( (0–10) \) | PSS \( (0–10) \) | PIS \( (0–10) \) | LOAD \( (0–52) \) |       |       |
| 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 |

**See** Tables 2 and 3 for remainder of key.
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 may be an indicator of higher inflammatory activity in the early stages of the disease. This, however, did not translate into greater disease-related impairment, since animals with mild OA exhibited better scores in several CMIs. This is in 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.33

In some cases, even if in cases without overt lameness, subtle shifts in body weight distribution at a stance may be present.26,28 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 VA 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 when 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 cutoff point of 200 pg/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 or glucocorticoids.6,35–37 It has been measured in dogs with stiff OA, with concentrations being higher in cases of OA secondary to naturally occurring cranial cruciate ligament rupture when compared to normal stifles. However, in cases of OA from other anatomical locations, synovial CRP levels were lower.30 In this study, we have observed CRP synovial levels, similar to what has previously been recorded in dogs with knee OA (2.5 mg/dL in this study vs 2.22 mg/dL).30 Registered serum levels were higher than what has been recorded in a cohort of dogs with OA (0.8 mg/dL in this study vs 0.05 mg/dL).38 It was interesting to observe that higher values of synovial CRP corresponded to better OFA hip grades and higher activity levels as measured with the pedometer and PIS and function scores. However, they also had higher thermography values, which are usually related to increased inflammatory activity. We also reported higher serum CRP concentration levels than what has been previously reported.38 In the same study, CRP levels increased after treatment. It is unclear if higher CRP levels are a consequence of high activity levels, microlesions 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.39 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.14,40 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 35-kg cutoff value, the highest considered, animals additionally showed worse clinical and imaging findings, higher thermography values, worse flexion, and higher frequency of CCO.

In dogs, males are frequently present as being at higher risk to develop OA than females, possibly due to sex hormone or activity differences. An additional reason is related to differences in body weight. Additionally, neutered dogs are more likely to develop OA.31 We only found a significant difference in thigh girth. This finding may be related to the fact that all animals included in the study were intact and were active police working dogs, with similar activity levels, regardless of sex. This finding should be evaluated in future studies.

This study presents some limitations, namely the absence of a control group. This is related to the nature of the sample, composed of animals presented for treatment for hip OA. Further studies, including a control group, with nonlame dogs, would be of interest to validate this comparison. It would also be of interest to evaluate other proinflammatory cytokines or inflammatory serum markers. 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
This study described the influence and direct relation of IL-1 and CRP synovial concentration levels in dogs with OA. It provides important information for the characterization of the multiple dimensions of this complex and prevalent disease. Further studies are required in order to determine the interest of each of the evaluated parameters for the prognosis and treatment monitoring of the disease.

Acknowledgments
The Pall Corporation provided the V-PET kits used in this study, and Companion, LiteCure LLC provided the Stance Analyzer used in this study.

The authors thank Manuel Pereira for the assistance in the analysis of the data, FujiFilm Europe GmbH for providing the CRP tests, Concessus and Companion, LiteCure LLC for providing the stance analyzer used in this study, and Specman Lda for providing the digital thermography camera.

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**Supplementary Materials**

Supplementary materials are posted online at the journal website: avmajournals.avma.org