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The intravenous administration of blood cell secretome can improve clinical signs in dogs with osteoarthritis

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ABSTRACT

We aimed to evaluate the effect of the intravenous (IV) administration of blood cell secretome (BCS) compared to its intra-articular (IA) administration.

In this cross-over study, ten dogs with bilateral hip osteoarthritis were initially assigned to an IA group (IAG). After a 180-day follow-up, the same 10 animals were assigned to an IV group (IVG). IAG received a single IA administration of 3 ml of BCS per hip joint, and IVG received a single 6 ml IV administration of BCS. Following each treatment, follow-up was conducted on days 0, 8, 15, 30, 60, 90, 120, 150, and 180. In each follow-up, copies of the Canine Brief Pain Inventory (divided into pain interference score - PIS and Pain Severity Score - PSS), Liverpool Osteoarthritis in Dogs (LOAD), and Canine Orthopedic Index (COI, divided into function, gait, stiffness, and quality of life) were obtained. Results were analyzed with the Mann-Whitney test, and Kaplan-Meier estimators were calculated and compared, $p < 0.05$.

The animals had a mean age of 7.6 ± 2.9 years and bodyweight of 27.8 ± 3.6 kg. Seven hips were classified as moderate osteoarthritis, and three as severe. No differences were found between groups from days 0 to +60d, with both groups showing clinically significant improvements. Improvements in the IAG lasted up to +120d. Kaplan-Meier estimators showed that dogs in IAG took longer to record the considered events. IVG also showed positive clinically significant results with different instruments.

IV administration of BCS can improve the overall condition of dogs with hip OA, but the IA administration produces longer-lasting results.

1. Introduction

Osteoarthritis (OA) remains a commonly diagnosed disease in dogs (Anderson et al., 2018), having a significant toll on the animal's overall quality of life (Alves et al., 2020a; Alves et al., 2022a). Over time, multiple therapeutic approaches to the management of OA have been introduced to the market, targeting different aspects of the disease, from non-steroidal anti-inflammatory drugs (NSAIDs) (Pye et al., 2022), oral joint supplements (Alves et al., 2017; Moreau et al., 2003), intra-articular (IA) injections (Alves et al., 2022b; Alves et al., 2022c; Alves et al., 2021a; Alves et al., 2020b; Alves et al., 2021b; Alves et al., 2021c), biological products (Alves et al., 2020c; Alves et al., 2021d; Alves et al., 2021e), photobiomodulation (Alves et al., 2022d), or anti-nerve growth factor monoclonal antibodies (Enomoto et al., 2019). NSAIDs, such as meloxicam, often remain the first line of approach for OA's medical management (Walton et al., 2014). They have a longstanding clinical history to support their use, associated with relative ease of administration, which helps to increase adherence to treatment (KuKanich et al., 2012). Treatment adherence is paramount for the management of chronic conditions such as OA. Having products that require less

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frequent administrations, in contrast to daily administration, may improve adherence to treatment (Enomoto et al., 2019).

Blood Cell Secretome (BCS) is an autologous biologic treatment containing high concentrations of anti-inflammatory mediators, growth factors, and lipid mediators, prepared through extended coagulation of venous blood in a defined environment (Sawyere et al., 2016; Shirokova et al., 2020; Bogers, 2018). It differs from other biological products, such as platelet-rich plasma (PRP). While PRP also relies on the supra-physiological administration of growth factors and mediators, obtained from autologous platelets (Alves et al., 2023). With PRP, high concentrations of platelets are administered, while BCS is acellular. In humans, BCS reduced pain scores and improved clinical outcomes up to 1 or 2 years (Baselga García-Escudero and Miguel Hernández Trillos, 2015; Evans, 2005; Baltzer et al., 2009). In dogs, its intra-articular administration has produced clinical improvements for several months, measured with different clinical metrology instruments (Alves et al., 2022e; Alves et al., 2022f). Since OA is a localized disease, intra-articular administrations are a good approach for managing the disease (Alves et al., 2021b). However, some clinicians may not choose this approach due to a lack of awareness of the possibility, technical difficulties in the execution of the procedure, and the need to sedate the patient in most cases. As BCS is an acellular treatment, the intravenous (IV) of the high concentrations of anti-inflammatory mediators and growth factors may still add clinical benefits.

For that reason, we aimed to evaluate the effect of the IV administration of BCS compared to its IA administration. We hypothesized that the IV administration of BCS can improve OA-related clinical signs compared to the IA administration.

2. Materials and methods

This study's protocol was reviewed by the Ethical Review Committee of the University of Évora (Órgão Responsável pelo Bem-Estar dos Animais da Universidade de Évora, process n° GD/21660/2022) and complies with the NIH guidelines for Humane Care and Use of Animals. All methods were carried out in accordance with relevant guidelines and regulations, and the manuscript adheres to ARRIVE guidelines. Written, informed consent was obtained from the Institution responsible for the animals (Guarda Nacional Republicana, Portuguese Gendarmerie).

In this double-blinded, crossover pilot study, 10 animals were enrolled. The sample was a convenience sample, constituted by active police working dogs presented for treatment of bilateral hip OA at the Clinica Veterinária de Cães of the Guarda Nacional Republicana (Canine Veterinary Clinic, National Republican Guard). The diagnosis of hip osteoarthritis was made following a consistent history, physical, orthopedic, neurological, and radiographic examinations. Animals with signs or suspicion of any other disease or OA in any other joint and that received any treatment for over six weeks at the time of the initial enrolment were excluded. Only animals ≥2 years, with a body weight ≥ 15 kg, were included (Alves et al., 2020a; Alves et al., 2021b). Hip x-rays were graded following the Orthopedic Foundation for Animals hip grading scheme (Puckler et al., 2016). All animals were in active service and remained in active service after treatment.

After selection, the 10 patients were initially treated with an IA administration of BCS (IAG). After a 180-day follow-up, all animals received an IV BCS (IVG) administration. A 15-day washout period was followed between the two. For IAG, 3 ml of BCS per hip joint was administered. For IVG, 6 ml of BCS was administered. BCS was prepared as described before (Alves et al., 2022e; Alves et al., 2022f). Briefly, BCS was prepared with a commercially available kit (Orthogen® Device, Orthogen AG, Düsseldorf, Germany), following the manufacturer's guidelines. Access to the jugular vein was prepared with the animal in lateral recumbency. For each device, 15 ml of whole blood was collected directly into the device from the jugular vein. Two devices were prepared per dog. All blood collections were performed early in the morning, with the patient fasted. The blood was then submitted to extended coagulation for 4.5 h at 37 °C (MF-6 W incubator, HCP-Technology, Nortrup, Germany). After this period, the device was centrifuged for 3 min at 1500*g* (M-Universal, MPW, Warsaw, Poland), and a vial containing sterile BCS was collected. The BCS used in the IVG was produced at the time of the IAG treatment. Vials were kept frozen until the moment of use and thawed in the refrigerator.

The intra-articular administration technique has been described before (Alves et al., 2021a). The dogs were placed under light sedation, induced with an IV combined administration of medetomidine (0.01 mg/kg) and butorphanol (0.1 mg/kg) (Alves et al., 2021e). The dog was placed in lateral recumbency, the joint to be accessed uppermost. Then, a window of 4x4cm is aseptically prepared, with the greater trochanter in the center. With all the material prepared, an assistant places the limb in a neutral position. A 2,5″ 21-gauge needle is then introduced just dorsal to the greater trochanter, perpendicular to the long axis of the limb until the joint is reached, confirmed through the collection and removal of as much synovial fluid as possible. The BCS was then administered.

An IV catheter was placed in the cephalic vein for the IV administration. The 6 ml of BCS was administered in small boluses of 1 ml over 5 min. The animals remained under medical surveillance for 3 h following the administration. For blinding purposes, the hair around the hip joint was clipped for dogs in IVG. For dogs in IAG, the hair in the thoracic limb was also clipped, as if an IV catheter was placed in the cephalic vein. The administrations were conducted in a secluded room by a single veterinarian who was not responsible for communications with the handler and follow-ups. If required, rescue analgesia would be instituted (meloxicam, 0.2 mg/kg PO SID).

Response to treatment was evaluated on treatment day, 8, 15, 30, 60, 90, 120, 150, and 180 days post-treatment with the Canine Brief Pain Inventory (CBPI, divided into pain severity score - PSS, and pain interference score – PIS), the Canine Orthopedic Index (COI, divided into stiffness, gait, function, and quality of life – QOL), and the Liverpool Osteoarthritis in Dogs (LOAD). Their Portuguese versions have been previously validated (Alves et al., 2022g; Alves et al., 2022h; Alves et al., 2022i).The dogs' handlers completed a digital copy of these instruments. The same handler completed all questionnaires for each dog throughout the entire study.

At each evaluation moment, groups were compared using a Mann-Whitney test with a Bonferroni correction. The Kaplan-Meier test was used to generate time-to-event curves, and event probability was compared with the Log Rank test. For each instrument considered, different events were set based on what has been determined as a clinically important change in score. With the CBPI, a reduction of ≥1 in PSS and ≥ 2 in PIS was considered (Brown et al., 2013), while with the LOAD, the set event was a reduction of ≥4 (Innes et al., 2022; Alves and Innes, 2023). For the COI, a reduction of ≥3.5 of the overall score, reduction≥3.5, has been determined as being a clinically important change (Innes et al., 2022). For the different dimensions of the COI, stiffness, gait, function, and QOL), we considered as a reference the baseline values since it was the moment when medical assistance was sought for the animal, meaning it has some level of clinical significance (Alves et al., 2021d; Alves et al., 2021f). The Kaplan-Meier test was used to evaluate the time for the improvements to reduce below the set levels. Patients showing a clinically significant improvement at the +180d evaluation were censored. Results were analyzed with IBM SPSS Statistics version 20, $p < 0.05$.

3. Results

The sample included 10 dogs (6 males and 4 females) with a mean age of 7.6 ± 2.9 years and a body weight of 27.8 ± 3.6 kg. The breeds represented were German Shepherd Dog ($n = 6$), Labrador Retriever ($n = 3$), and Belgian Malinois Shepherd Dog ($n = 1$). Seven hips were classified as moderate OA and 3 as severe. All dogs were followed until the end of the study. At least 6 ml of BCS was obtained with one device,

enough to treat both hips or for IV administration. No additional treatment or medications were administered throughout the study, and no side effects were observed.

Table 1 presents the results of the CBPI, the LOAD, and the COI. No differences were observed between groups up to the +60d evaluation, with both groups presenting clinically significant improvements compared to their respective baseline values. Differences were observed from that moment on, with improvements lasting up to +120d in the IAG. The improvements in PSS and LOAD for each group are presented in Figs. 1 and 2, respectively.

Table 2 presents the results of the Kaplan-Meier time-to-event estimators with each clinical metrology instrument. Fig. 3 shows the evolution of the Pain Interference Score. Dogs in IAG took longer to record the considered events.

4. Discussion

Managing OA patients is a lifelong commitment, and it is important to develop and introduce treatment modalities that can improve treatment adherence. This study's results show that the IV administration of BCS may improve the clinical signs of OA at a similar level to its IA administration, although results with an IA administration last longer.

The improvements observed in IAG were consistent with previous reports on the effect of BCS (Alves et al., 2022e; Alves et al., 2022f) and other autologous blood products that share a similar action mode to BCS (Fahie et al., 2013; Franklin and Cook, 2013; Damiá et al., 2018). The

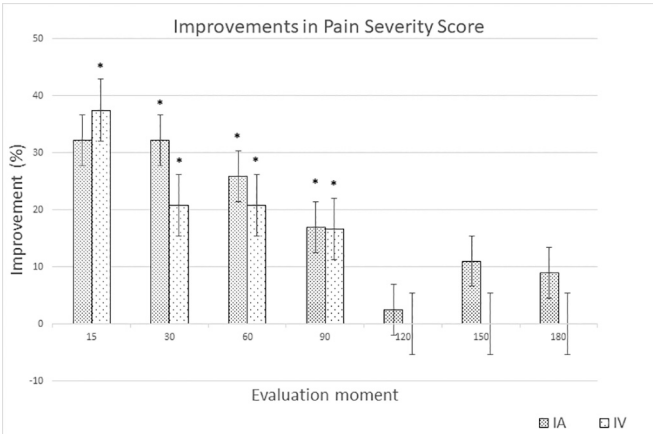

Fig. 1. Improvements (%) in Pain Severity Score for the intra-articular group (IAG) and the intra-venous group (IVG), compared to baseline values. * indicates a clinically significant improvement (reduction ≥1).

Table 1
Evolution of Clinical Metrology instruments (median score, interquartile range, and percentual variation compared to T0), by group. CBPI – Canine Brief Pain Inventory; COI – Canine Orthopedic Index; LOAD – Liverpool Osteoarthritis in Dogs; PIS – Pain Interference Score; PSS – Pain Severity Score; QOL – Quality of Life. * indicates significance when comparing groups at each follow-up moment.

Clinical Metrology Instrument		Group	T0		p	+15d			p	+30d			p	+60d			p
			Med	IQR		Med	IQR	%		Med	IQR	%		Med	IQR	%	
		IA	5,9	1,7		4,0	1,9	32,2		4,0	2,4	32,2		4,4	2,4	25,8	
	PSS (0–10)	IV	6,0	2,3	0,61	3,8	4,5	37,5	0,96	4,8	3,0	20,8	0,96	4,8	3,3	20,8	0,61
		IA	6,5	2,8		4,8	2,5	26,2		4,3	1,4	33,8		4,4	1,5	32,3	
CBPI	PIS (0–10)	IV	6,0	2,4	0,74	4,8	4,0	20,0	0,89	4,0	3,4	33,3	0,54	4,4	4,6	26,7	0,96
		IA	25,5	6,8		21,5	3,8	15,7		20,5	3,5	19,6		21,0	2,5	17,6	
LOAD (0–52)		IV	26,0	8,0	0,54	22,0	14,0	15,4	0,61	21,0	14,0	19,2	0,74	21,5	12,0	17,3	0,89
		IA	7,0	6,8		5,0	1,8	28,6		5,0	2,5	28,6		6,0	2,8	14,3	
	Stiffness (0–16)	IV	8,0	3,0	0,61	6,0	4,0	25,0	0,74	6,0	4,0	25,0	0,74	6,0	4,0	25,0	0,67
		IA	8,0	1,8		6,0	3,0	25,0		6,0	4,3	25,0		6,0	3,0	25,0	
	Function (0–16)	IV	8,0	7,0	0,96	10,0	5,5	25,0	1,00	8,0	5,0	0,0	0,28	5,0	4,0	37,5	0,61
		IA	12,0	2,5		10,5	5,0	12,5		10,0	4,5	16,7		9,0	5,3	25,0	
	Gait (0–20)	IV	12,0	4,0	0,48	10,0	7,0	16,7	0,96	11,0	8,0	8,3	0,82	11,0	8,0	8,3	0,89
		IA	7,0	1,3		7,0	2,5	0,0		7,0	2,8	0,0		7,0	1,3	0,0	
	QOL (0–12)	IV	7,0	3,0	0,82	6,0	6,0	14,3	0,67	7,0	5,0	0,0	0,54	7,0	5,0	0,0	0,82
		IA	34,0	5,5		28,5	12,5	16,2		28,0	6,8	17,6		28,0	11,5	17,6	
COI	Overall (0–64)	IV	35,0	18,0	0,82	32,0	19,0	8,6	0,96	32,0	19,0	8,6	0,89	29,0	19,0	17,1	0,82

Evolution of Clinical Metrology instruments (median score, interquartile range, and percentual variation compared to T0), by group. CBPI – Canine Brief Pain Inventory; COI – Canine Orthopedic Index; LOAD – Liverpool Osteoarthritis in Dogs; PIS – Pain Interference Score; PSS – Pain Severity Score; QOL – Quality of Life. * indicates significance when comparing groups at each follow-up moment

Clinical Metrology Instrument		Group	+90d			p	+120d			p	+150d			p	+180d			p
			Med	IQR	%		Med	IQR	%		Med	IQR	%		Med	IQR	%	
		IA	4,9	1,6	16,9		5,8	1,6	2,5		5,3	2,3	11,0		5,4	2,7	8,9	
	PSS (0–10)	IV	5,0	2,8	16,7	0,14	6,0	2,0	0,0	0,48	6,0	2,0	0,0	0,82	6,0	2,0	0,0	0,48
		IA	4,5	2,1	30,8		5,4	2,4	16,9		5,6	2,1	13,8		6,0	2,6	7,7	
CBPI	PIS (0–10)	IV	5,4	1,8	10,0	0.04*	6,2	2,0	-3,3	0,07	6,2	2,0	-3,3	0,11	6,2	2,0	-3,3	0,28
LOAD (0–52)		IA	21,0	2,0	17,6	0,01*	21,5	2,0	15,7	0,01*	24,5	4,0	3,9	0,02*	26,0	3,8	-2,0	0,04*
		IV	27,0	1,0	-3,8		29,0	5,0	11,5		29,0	5,0	11,5		29,0	5,0	11,5	
	Stiffness (0–16)	IA	6,5	3,3	7,1	0,54	8,0	2,8	-14,3	0,11	7,0	3,3	0,0	0,04*	7,5	2,8	−7,1	0,07
		IV	8,0	2,0	0,0		8,0	3,0	0,0		8,0	3,0	0,0		8,0	3,0	0,0	
	Function (0–16)	IA	6,0	3,3	25,0	0,61	8,0	2,5	0,0	<0,01*	7,5	2,8	6,3	0,01*	6,5	4,8	18,8	0,04*
		IV	8,0	4,0	0,0		10,0	1,0	25,0		10,0	1,0	25,0		10,0	1,0	25,0	
COI	Gait (0–20)	IA	10,5	5,0	12,5	0,42	11,5	3,3	4,2	0,82	11,0	4,3	8,3	0,82	11,5	3,0	4,2	0,89
		IV	10,0	2,0	16,7		12,0	0,0	0,0		12,0	0,0	0,0		12,0	0,0	0,0	
	QOL (0–12)	IA	6,5	1,5	7,1	0,48	7,5	2,3	−7,1	0,02*	7,0	1,5	0,0	0,04*	7,0	2,3	0,0	0,04*
		IV	6,0	2,0	14,3		9,0	2,0	28,6		9,0	2,0	28,6		9,0	2,0	28,6	
	Overall (0–64)	IA	29,5	13,8	13,2	0.04*	35,0	8,0	−2,9	<0,01*	32,5	12,3	4,4	0,07	32,5	13,8	4,4	0,07
		IV	32,0	10,0	8,6		39,0	4,0	11,4		39,0	4,0	11,4		39,0	4,0	11,4	

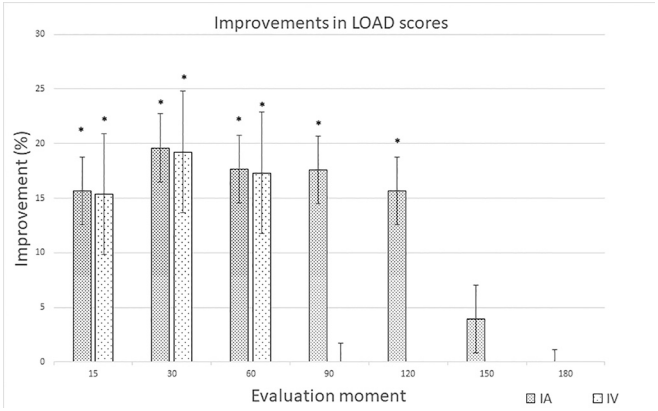

Fig. 2. Improvements (%) in Liverpool Osteoarthritis in Dogs (LOAD) scores for the intra-articular group (IAG) and the intra-venous group (IVG), compared to baseline values. * indicates a clinically significant improvement (reduction ≥4).

use of different IA modalities has gained increased interest recently (Alves et al., 2021b). This approach makes clinical sense, as OA is a local disease and, compared to systemic medications, it can increase bioavailability and safety, especially when comorbidities are present (Wehling et al., 2017). However, the need for sedation and the technical execution of the technique can pose a barrier to some clinicians despite the advantages. We should also consider that, despite being a local disease, OA can, particularly in more advanced cases, have an impact on multiple dimensions, including movement, affection, and activity (Lascelles et al., 2019).

Our results show a clinically significant improvement with the IV administration of BCS from the first evaluation point at +15d. Although it was not objectively recorded, many handlers reported an "increase in energy" and "vitality" following the IV administration compared to the initial evaluation. While performing the IV administration of BCS, its high content of anti-inflammatory mediators and growth factors is certain to reach several tissues rather than the joint tissues directly, as with an IA administration. The reports we received from the handlers may reflect that effect on different tissues but cannot be determined in the present study. The improvements observed lasted until the +60d evaluation moment, as measured with the considered clinical metrology instruments, particularly the CBPI and the LOAD. The Kaplan-Meier test results also show significant differences between the two groups in these two instruments. The mean duration of the clinically significant effects was slightly higher than 60 days, with some dogs showing improvements for longer periods, as shown in Table 2 and the time-to-event curves.

It is important to remember that the dogs in IVG had previously been treated with an IA administration of BCS. A previous IA administration may have primed the joint for a future IV treatment, leading to a positive result. With that in mind, future studies are needed to determine if the same effect would be observed in a patient being treated with IV BCS as a first treatment or by doing both simultaneously. For that reason, future studies should address this possibility, perhaps in a cross-over study. On the other hand, an IV administration can bring other advantages, namely, it does not raise technical difficulties in the execution of the IA procedure, does not require sedation, and has a possible positive systemic effect. We selected to administer the same volume of IVG based on the recommended volume for an IA administration, as used in IAG (Alves et al., 2022e; Alves et al., 2022f)However, a lower dose could also have been effective, and follow-up administrations could contribute to maintaining the improvements observed. This possibility needs to be evaluated. Additional limitations of the present study include the selection of a convenience sample without a sample size calculation and the lack of a placebo group. We chose not to include a control group because all animals had clinical signs of OA, and it would not be easy to justify not treating these dogs.

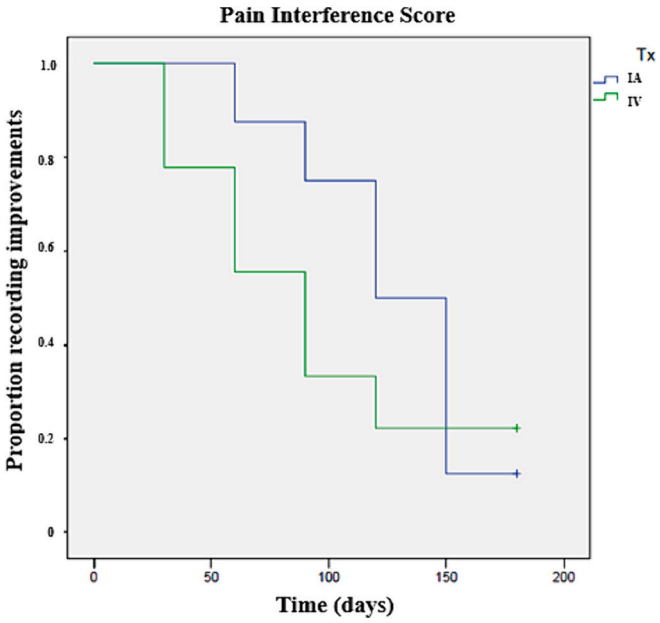

Fig. 3. - Kaplan-Meier curves demonstrating a significant difference between the intra-articular group (IAG) and the intra-venous group (IVG), in time (days) for the improvement in Pain Interference Score of the Canine Brief Inventory score to reduce below ≥1 improvement.

Table 2
Time-to-event (in days) for the clinical metrology instruments considered, in the intra-articular group (IAG) and the intra-venous group (IVG), calculated with Kaplan-Meier estimators and compared with the Log Rank test. "Events" considered were a score change below the improvement level set as clinically significant for each score: ≥1 in PSS, ≥2 in PIS[32], ≥4 in LOAD (Brown et al., 2013), ≥3.5 in COI[33], or when an improved score, compared to baseline values, was no longer observed for stiffness, function gait, and QOL. Legend: CBPI – Canine Brief Pain Inventory; COI – Canine Orthopedic Index; LOAD – Liverpool Osteoarthritis in Dogs; PIS – Pain Interference Score; PSS – Pain Severity Score; QOL – Quality of Life. * indicates significance.

Clinical Metrology Instrument		Log Rank test	Group							
			IAG				IVG			
			mean	SD	95 % CI		mean	SD	95 % CI	
CBPI	PSS	0,03*	116,3	13,5	89,8	142,6	83,3	20,6	62,9	143,7
	PIS	0,04*	127,5	12,7	102 6	152,4	93,3	17,9	58,2	128,4
LOAD		0,04*	123,7	9,8	104,5	143,2	93,3	17,1	58,2	128,4
COI	Stiffness	0,34	12,8	10,5	103,1	144,4	93,3	13,7	66,5	120,2
	Function	0,61	105,0	14,0	77,5	132,5	83,3	19,9	44,4	122,3
	Gait	0,61	105,0	14,0	77,5	132,5	83,3	19,9	44,4	122,3
	QOL	0,83	116,3	14,5	87,9	144,6	110,0	19,4	71,9	148,1
	Overall	0,35	123,8	15,5	93,4	154,0	86,7	12,4	54,1	119,3

As with different evaluation modalities, and as a proxy completes the clinical metrology instruments, there is a risk for a caregiver placebo effect (Alves and Innes, 2023; Brown, 2014; Brown et al., 2008). The LOAD and the COI have not shown a major placebo effect in previous reports due to an emphasis on the questions on activity (Walton et al., 2013). Also, a placebo effect has not been found at the animal level (Conzemius and Evans, 2012; Piel et al., 2014). While it is possible that a certain level of caregiver placebo effect could be present in the present results, and since some treatments were more invasive (Hróbjartsson et al., 2014), it should not have influenced the results significantly. Still, future studies should also include an objective evaluation measure to complement the information obtained from the clinical metrology instruments.

5. Conclusions

The results of the present study show that the IV administration of BCS can improve the overall condition of dogs with hip OA. While providing a similar improvement to the IA administration of BCS, the effect of IV BCS doesn't last as long but is easier to perform. Future studies should examine the effect of a previous IV administration and different administration volumes.

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CRediT authorship contribution statement

J.C. Alves: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A. Santos:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Ana Filipe:** Writing – original draft, Investigation, Conceptualization. **L. Miguel Carreira:** Writing – review & editing, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing interests.

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