Intra-articular Injection of Autologous Protein Solution for Treatment of Canine Osteoarthritis

THESIS

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Abstract

Osteoarthritis (OA) is a common cause of debilitating lameness in dogs. Although osteoarthritis is classified as a non-inflammatory disease, low levels of inflammation are present in the joint and can affect the progression of the disease. Osteoarthritis is characterized by degradation and loss of articular cartilage, osteophyte formation, subchondral bone sclerosis and inflammation of the synovial membrane. As cartilage begins to breakdown, the ratio of naturally occurring inflammatory to anti-inflammatory cytokines becomes imbalanced causing increased breakdown of cartilage. A novel method for up regulation of anti-inflammatory cytokines from whole blood has been reported which produces an autologous protein solution (APS). Injection of APS has been shown to be an effective treatment of OA in horses and has greater anti-inflammatory proteins than platelet-rich plasma, another protein solution used for treatment of OA. The objective of the study was to prospectively evaluate the efficacy of an intra-articular injection of APS for treatment of OA in dogs using the Canine Brief Pain Inventory (CBPI) to score pain, Hudson visual analog scale (HVAS) to score lameness, and peak vertical force (PVF) to evaluate weight-bearing. Twenty client-owned dogs with a unilateral lameness attributable to OA of the elbow or stifle were enrolled and randomly assigned to a joint injection with APS or 0.9% saline solution.
Owners and observers performed assessments blinded prior to injection, and at week 2 and 12 after injection. Radiographs of the affected joint were made prior to injection and at week 12. For dogs that received the APS injection, lameness scores (improved 25.6%; P<0.03), pain scores (improved 15%; P<0.05) and peak vertical force (increased 14.9%; P<0.2) showed significant improvement at week 12 compared with pretreatment values. For control dogs, lameness scores, pain scores and peak vertical force at week 12 were not significantly different from pretreatment values. There was no evidence of radiographic progression of osteoarthritis from week 0 to week 12. A single intra-articular injection of autologous protein solution was an effective means of improving lameness, pain and weight bearing by 12 weeks in dogs with dominant single limb lameness due to OA.
Dedication

This thesis is dedicated to my husband, Adam, for his never-ending love, constant support and encouragement.
I would like to sincerely thank Dr. Alicia Bertone for being a fantastic research mentor, source of support, positivity and dedication to this project. Thanks to my committee members Dr. Bianca Hettlich and Dr. Lisa Zekas for the insight and guidance provided during this process. Thanks to the members of the OSU Clinical Trials department for assistance with patient recruitment and management of the clinical trial. Thanks to Dr. Allison Kilborne, Dr. Claudia Zindl and other members of the Allen and Bertone research laboratory for statistical and technical support. Additionally I would like to thank Dr. Bianca Hettlich and Dr. Lillian Su for performing the joint injections as well as being a constant source of support and encouragement during this project as well as during my residency. Lastly, I thank all of the owners of the dogs in the trial for allowing their beloved pets to be included in this trial as well as the many referring veterinarians who recommended their patients for the trial.
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Table of Contents

Abstract .................................................................................................................................................. ii

Acknowledgments .................................................................................................................................. v

Vita ...................................................................................................................................................... vi

List of Tables ....................................................................................................................................... viii

List of Figures ..................................................................................................................................... ix

Chapter 1: Introduction ...................................................................................................................... 1

Chapter 2: Materials and Methods .................................................................................................... 5

Chapter 3: Results .............................................................................................................................. 14

Chapter 4: Discussion ......................................................................................................................... 22

Appendix A: Forms ............................................................................................................................. 29

Appendix B: Data ............................................................................................................................... 37
List of Tables

Table 1: Breed distribution for dogs treated with APS or control vehicle. .................. 37

Table 2: Weight distribution for dogs treated with APS or control vehicle. ................. 37

Table 3: Sex distribution for Dogs treated with APS or control vehicle. ....................... 38

Table 4: Sex distribution for dogs treated with APS or control vehicle. ....................... 38

Table 5: Affected limb distribution for dogs treated with APS or control vehicle. .......... 38

Table 6: Affected limb distribution for dogs treated with APS or control vehicle. .......... 38
List of Figures

Figure 1: Week 0 and week 12 radiographs from a dog in the control group showing no progression of osteoarthritic disease. ................................................................. 16

Figure 2: Week 0 and Week 12 radiographs from a dog in the treatment group showing no progression of osteoarthritic disease. ................................................................. 16

Figure 3: Graph of the significant decrease in CBPI owner questionnaire indices (median +/- interquartile range) at week 12 compared to baseline in the APS group representing a decrease in pain. ................................................................. 17

Figure 4: Graph of the significant decrease in HVAS owner questionnaire indices (median +/- interquartile range) at week 12 compared to baseline in the APS group representing a decrease in lameness. ................................................................. 18

Figure 5: Graph representing a significant increase in PVF (median +/- interquartile range) at week 12 compared to baseline in the APS group representing increased weight-bearing on the affected limb. ................................................................. 21
Figure 6: Representative Canine Brief Pain Inventory questions.......................... 33

Figure 7: Representative Canine Brief Pain Inventory survey part 2 ...................... 34

Figure 8: Representative Hudson Visual Analogue Scale ...................................... 35

Figure 9: Representative Hudson Visual Analogue Scale part 2 ............................ 36

Figure 10: Box and whisker plots of scored CBPI data ........................................ 39

Figure 11: Box and whisker plots of scored CBPI data continued .......................... 40

Figure 12: Box and whisker plot of scored HVAS data ........................................ 41

Figure 13: Box and whisker plot of HVAS scored data continued .......................... 42
Chapter 1: Introduction

Osteoarthritis is a debilitating joint disease that is the most common disorder of joints in people worldwide\(^1\). Similarly, an estimated 20% of dogs in the U.S over 1 year of age are affected with osteoarthritis\(^2\) with the elbow and stifle joints most commonly affected\(^3\). Osteoarthritis is classified as a non-inflammatory type of arthritis due to low numbers of neutrophils in the joint fluid, however there is some degree of inflammation present in all forms of arthritis. Subsequently the inflammatory process can affect the progression of the disease\(^5,6\). Further characterization of osteoarthritis include primary (idiopathic) or secondary. In dogs, the disorder occurs most commonly as secondary osteoarthritis in response to an abnormality in the joint. Common causes of secondary osteoarthritis in the dog include a developmental disorder such osteochondritis dissecans; joint instability such as with elbow dysplasia, cranial cruciate ligament disease or hip dysplasia; or trauma such as intra-articular fracture\(^4\).

Articular cartilage is a specialized, avascular, aneural connective tissue that provides covering for the osseous structures of joints. The function of articular cartilage includes
absorbing impact and sustaining shear forces. The main components of articular cartilage include chondrocytes and the extracellular matrix. Extracellular matrix is composed of proteoglycans, collagen and water. Proteoglycans consist of a core protein with covalently linked glycosaminoglycan side chains (heparin sulfate, keratin sulfate, chondroitin sulfate, etc). The main proteoglycan in articular cartilage is aggrecan that is composed of a backbone of hyaluronic acid with multiple noncovalently linked proteoglycans. Aggrecans are large branched macromolecules that have a high affinity for water which give the cartilage ability to resist compressive loads. Articular cartilage is primarily made up of type II collagen with smaller amounts of type VI, IX and XI, XII and XIV. Type II collagen provides the tensile strength to articular cartilage. Chondrocytes are the cells of articular cartilage responsible for production of the extracellular matrix. Articular cartilage is divided into 4 zones with increased concentration of collagen towards the surface to provide tensile strength and increased concentrations of proteoglycan in the deeper layers to provide compressive strength.

Osteoarthritis is characterized by degradation and loss of articular cartilage, hypertrophic bone changes with osteophyte formation, subchondral bone remodeling, and chronic inflammation of the synovial membrane. This is a result of a complex system of cartilage destruction from an imbalance of in the chondrocyte homeostasis between

2
extracellular matrix synthesis and degradation. The key component in the process is
degradation of the extracellular matrix of articular cartilage. As cartilage begins to
breakdown, the ratio of naturally occurring inflammatory to anti-inflammatory cytokines
becomes imbalanced causing increased breakdown of the extracellular matrix of articular
cartilage. The primary cytokines involved in this process include upregulation of the
inflammatory cytokines IL-1β and TNFα and down-regulation of anti-inflammatory
cytokines such as IL-1ra, sTNF-RI and sTNF-RII⁹.

Cartilage matrix turnover is constant throughout life. The amplification of the
degenerative process leads to the development of osteoarthritis. There are many
inflammatory factors involved in the progression of the disease including proteases,
proinflammatory cytokines, nitric oxide, eicosanoids, and protease-activated receptors¹⁰.
Treatment options for osteoarthritis are aimed at stopping the inflammatory process,
slowing the progression of the disease and improving joint pain and comfort.

Administration of anti-inflammatory proteins derived from autologous blood is a
treatment option for osteoarthritis in dogs (C-pet, Pall Corporation). Platelet-rich plasma
contains many autologous cytokines and white blood cells that have been shown to
reduce pain and lameness scores and increase weight bearing when injected in an arthritic
canine joint\textsuperscript{11}. A novel method for producing an autologous protein solution (APS) from whole blood has been reported\textsuperscript{12,13}. This method has been shown to up-regulate anti-inflammatory cytokines from human, equine, and canine blood with increased cytokine and anti-inflammatory protein profile compared to platelet-rich plasma. In addition to concentrating white blood cells and platelets, this system mixes the blood product over polyacrylamide beads, which desiccate plasma and concentrate proteins. This therapeutic option is attractive because APS is unlikely to produce adverse immune reaction and can be produced in a short time frame as a point of care treatment. Significantly, in a clinical trial, equine OA patients showed significant reductions in lameness after treatment with APS\textsuperscript{14}. The effectiveness of APS treatment has not been tested in canine OA patients. The following study was designed to determine if an intra-articular injection of APS is a safe and effective treatment for canines with OA. Specifically, we aimed to determine whether there would be significant changes in severity of pain, lameness or weight bearing at 2 or 12 weeks after a single, intra-articular injection of autologous protein solution in dogs with osteoarthritis involving a single joint.
Chapter 2: Materials and Methods

Study design
The study was in compliance with Institutional Animal Care & Use Committee guidelines and was conducted as a prospective, randomized, blinded, placebo-controlled clinical trial. All dogs were client-owned and all owners signed a consent form prior to study enrollment.

Inclusion criteria
20 client-owned dogs examined at The Ohio State University College of Veterinary Medicine for lameness due to osteoarthritis in the elbow or stifle joint were enrolled in the study. Dogs were eligible for inclusion in the study if they were otherwise healthy, >11.4 kg, between 1-12 years of age, had a predominant unilateral load-bearing lameness localized to a single elbow or stifle joint, did not have any palpable laxity of that joint when examined awake, and had radiographic or CT evidence of osteoarthritis of the affected joints (i.e., osteophytes, subchondral sclerosis, narrowing of the joint space without complete loss of the joint space). Additionally, dogs must not have had any
surgical procedure on the affected or contralateral limb in the past 3 months, received systemic steroids or any joint injections in the past 2 months, or received any injections of polysulfated glycosaminoglycans in the past 1 month. Dogs were excluded if there was radiographic evidence of lysis, bone on bone contact, intra-articular fragments or intra-articular fracture. Dogs that were being treated with non-steroidal anti-inflammatory drugs (NSAIDs), pain medications or nutritional supplements (eg, glucosamine, chondroitin sulfate, and omega-3 fatty acids) were eligible for enrollment, provided that administration was discontinued at least 1 week prior to each evaluation for the study.

*Study protocol*

Patient randomization was performed using an online random number generator (www.randomizer.com) and patients were allocated to treatment (n=10) or control (n=10) group in the order they were enrolled into the study. Radiographs or CT of the affected joint were made within 1 month of injection. At the beginning of the study (week 0), owners of participating dogs assigned scores for pain severity and lameness severity with the University of Pennsylvania Canine Brief Pain Inventory (CBPI)\textsuperscript{15-17} and the Hudson visual analogue scale (HVAS)\textsuperscript{18,19}, respectively. Force platform analysis was performed
to measure peak vertical force (PVF). A physical exam was performed by a blinded veterinarian and blood was drawn for complete blood count (CBC) analysis.

After the initial evaluations were completed, dogs in the treatment group were sedated and a blood sample (55 ml) was obtained from a jugular vein. APS was generated from a point-of-use dual-device system (Biomet Biologics, Warsaw, IN) that concentrated plasma and white blood cell proteins and enriched platelet growth factors and was injected intra-articularly. Dogs in the control group were sedated with the same protocol and given an intra-articular injection of saline solution (0.9% NaCl). Physical exam, CBC, force plate analysis, CBPI and HVAS assessments were repeated at 2 and 12 weeks after injection. Radiographs or CT (depending on initial screening method) were made at 12 weeks after injection.

The study was conducted in a blinded manner with the owners and assessing veterinarian unaware of the patients’ allocation to treatment or control groups when they were completing the CBPI or HVAS surveys or when data was collected. Separate veterinarians, skilled at performing joint injections, were utilized for preparation and administration of intra-articular injections. Staff members unaware of group allocation performed force plate analyses. Radiographs were collected and scored by one blinded
radiologist at the conclusion of the study. At 12 weeks after injection, the owners were unmasked to group assignment and owners of dogs in the control group were given the option of having their dogs receive an injection of APS

**Preparation and administration of autologous protein solution**

An autologous protein solution was created from whole blood using a minimally invasive, point-of-use, dual-device concentrating system (Biomet Biologics, Warsaw, IN) in accordance with manufacturer’s directions. Briefly, after patient sedation and preparation, 55 mls of blood was drawn from a jugular vein into a syringe containing 5 ml of ACD-A anticoagulant and gently mixed. Blood was processed through a single use APS separator device, a medical grade plastic container containing a buoy system tuned to the density of red blood cells, and centrifuged for 15 minutes (Drucker Company, Philipsburg, PA). Then, approximately 5-6 ml of intermediate cell solution was transferred to an APS concentrating device consisting of a self-contained medical grade polymer tube containing polyacrylamide (PA) beads that desiccate input platelet-rich plasma and up-regulate anti-inflammatory cytokines with mixing for 30 seconds. The sample was centrifuged for 2 minutes and the APS solution was withdrawn. This process resulted in a final volume of approximately 2.5 ml of APS.
Following sterile preparation of the joint, arthrocentesis was performed to confirm needle presence in the joint space and synovial fluid withdrawn and placed in a sterile container and frozen at -80°C for further investigation. This was followed by intra-articular injection of autologous protein solution.

*Administration of saline solution*

Dogs in the control group were sedated with the same protocol as the treatment group and arthrocentesis was performed as described for the treatment group and synovial fluid obtained for future analysis. Saline solution (0.9% NaCl) was injected intra-articularly.

*Radiographic evaluation*

Radiographs or CT of the affected joint were made within 30 days of enrollment and at week 12. Standard protocols were used to make orthogonal radiographs of either stifle or elbow joint or CT imaging. CT was only used if both time points (pre-injection and week 12) were imaged with this modality. Images were examined at the end of the study period by a board-certified radiologist blinded to treatment group assignment. Joints were assessed for evidence of periarticular osteophyte formation at the osseous-articular chondral junction on the edges of the joint; enthesisophyte formation at points of insertion of tendons, ligaments, joint capsule and fascia; subchondral bone sclerosis; irregularity of
the articular margin; and subchondral bone cyst formation. Scores for severity of osteoarthritic changes were assigned as follows: none (0), minimal (1), mild (2), moderate (3), marked (4). Subchondral bone cyst was noted as absent (0) or present (1).

Physical Exam and CBC

One veterinarian performed a physical exam to document the presence of predominant single limb lameness due to osteoarthritis of either the elbow or stifle joint and assess overall health at weeks 0, 2 and 12. A CBC was performed at weeks 0, 2 and 12.

Assessment of pain and lameness

At weeks 0, 2, and 12, surveys of pain and lameness were completed by the owner using the CBPI and HVAS. The standard CBPI uses lower numbers to represent less severe pain (0 = no pain; 10 = worst pain), whereas the standard HVAS uses higher numbers to represent less severe lameness. The HVAS questionnaire was changed by inverting the scale, so that 0 represented no lameness and 10 represented severe lameness, to make it less confusing for owners to complete both questionnaires at the same time.
Kinetic Gait Analysis

Force-plate analysis was performed on each dog three times during the study (treatment week 0, week 2, and week 12). Kinetic gait analysis was performed by staff, without knowledge of treatment, on the dogs by use of a stationary 2’ x 1’ in ground force plate and computer analysis system. Kinetic gait analysis was performed on the affected limb and contralateral limb at the trot. (Kistler Instrument Corp, Amherst, NY). An examination runway (1 x 5 m) with a central force plate was used for data collection, with the force plate and runway surface covered by a mat to prevent dogs from slipping and avoid recognition of the plate. Five valid repetitions were recorded for the OA and contralateral limb. A valid measurement was defined as a passage by the dog over the force plate during which the paw of the limb of interest fully contacted the surface of the plate and the gait velocity was within the range of 1.3 to 2.1 m/s. Before data collection, all dogs were warmed up by walking and trotting for 5-10 times through the examination runway to acclimatize to the environment and ensure that the dog would trot calmly on the force plate with constant speed that is similar to prior recorded speed. Gait velocity was measured by use of 2 photoelectric switches connected to the computer analysis system. The force-versus-time curves generated by the computer analysis system were used to compute the peak vertical force (PVF) parameters of the affected limb. PVF values were expressed as percentage of body weight (Newton/kg x 100).
**Data Analysis**

Statistical software (GraphPad Inc., ver 6, San Diego, CA) was used to generate descriptive statistics. Data were assessed for normality using graphical methods as well as Shapiro-Wilk normality test. In general, all demographic and analytical data were summarized using traditional methods. Categorical data was summarized using frequency tables. Continuous data was summarized with the mean, standard deviation, median, minimum and maximum. Differences between groups were assessed parametrically using a parametric paired t-test (PVF, AI-PVF, HVAS, CBPI) and non-parametric Wilcoxon matched-pairs (HVAS, CBPI), depending on if the data met the assumptions of normally distributed and equal variance required by the t-test. For subjective clinical outcomes, data was compared between APS and Saline injected OA groups at each time point, and between Week-0 and Week-12 in each treatment group. Data was analyzed both as a per question score and as a sum index score. For objective gait outcomes, a repeated measures ANOVA was used to compare the data between APS and Saline injected OA dogs at each time point, and between Week-0 and Week-12 in each treatment group. For all dogs, 5 valid recorded trials were averaged to create the final force plate measurements used in the statistical analysis. Data was analyzed by using data from all 4 limbs as well as asymmetry indices and coefficient of variance. Gait velocity was held constant for the study and was confirmed by measurement of velocity in force plate trials.
and compared between APS and Saline injected OA groups. Significance level was set at
P<0.05 for all analyses.
Chapter 3: Results

All 20 dogs were client owned. Thirteen breeds were represented (5 mixed breed dogs, 3 Labrador Retrievers, 2 Golden Retrievers, 1 Greater Swiss Mountain dog, 1 Siberian Husky, 1 Newfoundland, 1 German Shepard dog, 1 Doberman Pinscher, 1 Catahoula Leopard Hound, 1 Boxer, 1 Australian Shepard, 1 American Pit Bull terrier, and 1 Akita). The dogs ranged from 2 to 11 years of age (median age 8 years) and weight range was 21.2 to 55kg (median weight, 32.6 kg). There were 12 spayed females, 1 intact female and 7 castrated males. No significant differences were detected in age, body weight or sex between treatment and control groups. There were no significant differences in affected limbs (right v left) or affected joints (elbow v stifle) between treatment and control groups. Volume of APS injected ranged from 2.4 – 2.9 ml. Volume of saline injected for the control group ranged from 2.0 – 2.6 ml. No complications occurred in the preparation or injections of APS or saline. No adverse effects associated with injection of APS or saline solution were reported. All dogs completed the study with one dog (treated) being eliminated due to progressive secondary lameness in the ipsilateral hind limb. This dog not included in subjective clinical outcome analysis or objective gait.
outcomes. Owners of the dogs in the control group were offered to have their dog receive the APS solution after the week 12 evaluation, with post-study treatments performed in 9 dogs.

*Physical Exam and CBC Data*

Physical examination (week 0, week 2, and week 12) and CBC analysis (week 0, week 2, and week 12) were performed. Frequency tables of aberrant values (outside of reference range) were created for physical examinations and blood analysis. Such events were uncommon and showed no significance between groups.

*Radiographic scores*

The radiographic signs of osteoarthritis were not statistically different between APS and control groups at week 0. For all dogs, radiographic scores assigned at week 12 were not significantly different than the scores assigned at week 0 (Figure 1).
**Figure 1**: Week 0 and week 12 radiographs from a dog in the control group showing no progression of osteoarthritic disease.

**Figure 2**: Week 0 and Week 12 radiographs from a dog in the treatment group showing no progression of osteoarthritic disease.

*Owner-assigned pain and lameness scores*

For dogs in the treatment group (n=9), index CBPI scores assigned at week 12 (median, 29; interquartile [25th to 75th percentile] range, 22-31.5) were significantly (p=0.04)
improved compared to scores assigned at week 0 (median, 39; interquartile range, 21-49.5), representing an improvement of 25.6% of baseline. In contrast, for control dogs (n=10), index CBPI scores assigned at week 12 (median, 40; interquartile range, 8.25-51.88) were not significantly (p=0.075) different from scores assigned at week 0 (median, 48.5; interquartile range 25.75-56) (Figure 3).

![Canine Brief Pain Inventory](image)

**Figure 3:** Graph of the significant decrease in CBPI owner questionnaire indices (median +/- interquartile range) at week 12 compared to baseline in the APS group representing a decrease in pain.

Similarly, index HVAS scores assigned at week 12 for the treatment dogs (n=9, median, 39; interquartile range, 27.5-46.5) were significantly (p=0.026) improved, compared with
scores assigned at week 0 (median, 46; interquartile range, 36-56.5), representing an improvement of 15% of baseline. In contrast, for dogs in the control group (n=10), index HVAS scores assigned at week 12 (median, 42; interquartile range, 28.25-66) were not significantly (p=0.156) different from scores assigned at week 0 (median, 54; interquartile range, 43.25-65) (Figure 4).

![Graph of the significant decrease in HVAS owner questionnaire indices](image)

**Figure 4:** Graph of the significant decrease in HVAS owner questionnaire indices (median +/- interquartile range) at week 12 compared to baseline in the APS group representing a decrease in lameness.

For dogs in the control group, index CBPI scores assigned at week 2 (median, 33.5; interquartile range, 4.5 – 51.7) were significantly (p=0.021) improved compared with
scores assigned at week 0 (median, 48.5; interquartile range, 25.75-56). There was no significant difference between index CBPI scores from week 2 (median, 40; interquartile range, 13-48.5) to week 0 (median, 39; interquartile range, 21-49.5) for the treatment group (p=0.73). For dogs in the control group, index HVAS scores assigned at week 2 (median, 44; interquartile range, 36.5 – 57.25) were significantly (p=0.034) improved compared with scores assigned at week 0 (median, 54; interquartile range, 43.25-65). There was no significant difference between index HVAS scores in the treatment group from week 2 (median, 48; interquartile range, 28-59) to week 0 (median, 46; interquartile range, 36-56.5).

When individual scores used to calculate owner-assigned CBPI scores were examined, scores for current pain, and life enjoyment at week 12 for dogs in the treatment group were significantly (p=0.015, p=0.05) improved from scores assigned at week 0. CBPI scores for typical pain for dogs in the control group at week 2 and week 12 were significantly (p=0.019, p=0.046) improved compared to scores assigned at week 0. CBPI scores for worst pain for control dogs at 12 weeks were significantly (p=0.043) improved compared to scores assigned for week 0. For component HVAS scores, bedding stiffness and walking comfort scores at week 12 for dogs in the treatment group were significantly (p=0.015, p=0.058) improved from scores assigned at week 0. HVAS
scores for walking comfort at weeks 2 and 12 for dogs in the control group were significantly improved (p=0.015, p=0.023) compared to scores assigned at week 0.

Kinetic Gait Analysis

For the treatment dogs (n = 9), PVF (expressed as a percentage of body weight) at week 12 (mean ± SE, 87.18 ± 5.4%) was significantly (P=0.019) improved from PVF at week 0 (79.59 ± 5.5%) representing a 15% increase in mean PVF. In contrast, for dogs in the control group (n = 10), PVF at week 12 (84.55 ± 4.93%) was not significantly different, compared with PVF at week 0 (83.26 ± 4.89%) (Figure 5). There was no significant difference between weeks 2 – week 0 for any group.
Figure 5: Graph representing a significant increase in PVF (median +/- interquartile range) at week 12 compared to baseline in the APS group representing increased weight-bearing on the affected limb.

For all dogs, gait velocity ranged from 1.48 to 2.13 m/sec and gait velocity was not significantly different between control dogs and dogs that received the APS or across time. There was no significant difference in coefficient of variance of velocity between groups or across time. Mean absolute difference in gait velocity between week 0 and week 12 was 0.20 m/s (median, 0.19 m/s; range, 0.026 to 0.2 m/s). For both groups, gait velocity at all time points was not significantly different.
Chapter 4: Discussion

The rationale behind the current study was to determine whether an intra-articular injection of autologous protein solution (APS) would result in improvement in pain scores, lameness scores and improved weight bearing in dogs with predominant single limb lameness due to osteoarthritis. This study showed that a single intra-articular injection of APS resulted in significant improvements at 12 weeks in subjective severity of pain and lameness scores (CBPI, HVAS) and objective measurement of weight-bearing (PVF). Additionally no adverse clinical effects or radiographic progression of OA were noted at 12 weeks. This data supports the use of an intra-articular injection of autologous protein solution as a treatment option for canine osteoarthritis.

A summary index score for CBPI and HVAS data was calculated by adding all individual scores. The index represents a summary assessment for all categories of pain and lameness and was chosen to reflect the overall assessment. When component scores were evaluated the control group was noted to have improvement in typical pain and walking
comfort at week 2 and 12 however overall index scores were not significantly different at week 2 or week 12.

Results of the HVAS showed that walking comfort increased in both treatment and control groups at 2 and 12 weeks after injection. The reason for the increase in both groups may be due to the benefit of owner awareness, due to placebo effect, and allowing increased activity (i.e. owners believe their dog may have received the treatment and start walking the dog more to determine if the dog is getting better or worse). One of the mainstay recommendations for treatment of osteoarthritis is daily low impact activity so dogs in this study may have shown benefit from an overall increased activity. In a similar study conducted with APS in equine patients, horses were hospitalized and subjected to daily controlled activity and evaluated by blinded staff rather than owners. In that study, there was no difference in walking comfort noted in either group at all time points.

The mechanism of action of APS is by up-regulation of anti-inflammatory cytokines and growth factors. Previous in-vitro work has shown that APS, produced both from patients who are healthy and patients who have OA, has an increased anti-inflammatory cytokine profile compared to whole blood. Specifically APS has been shown to contain a high
concentration of autologous IL-1 receptor antagonist and sTNF-RI, which inhibit binding of IL-1β and TNF-α leading to reduction in inflammatory mediators such as MMP-13, IL-6, IL-8, nitric oxide species and PGE₂. Previous studies have demonstrated that therapy with a single cytokine agent such as recombinant human IL-1 receptor antagonist protein is effective for treatment of rheumatoid arthritis but has not been shown to be effective in treatment of human knee osteoarthritis. Similarly, recombinant soluble tumor necrosis factor-receptor (sTNF-R) was successful in clinical trials for rheumatoid arthritis but not in osteoarthritis. These findings support the need to target multiple inflammatory mediators as treatment for OA, which is accomplished with APS therapy. Additionally, APS has been shown to exert protective effects on cartilage and proliferative effects on chondrocytes, which suggests that APS therapy is chondroprotective, and has the potential to prevent cartilage loss associated with OA. APS also contains IGF-1, which is important in prevention of chondrocyte apoptosis, and is not found in PRP preparations (Eppley).

The use of a one-time, minimally-invasive, treatment for osteoarthritis by APS is exciting and holds promise in the veterinary field. An autologous product eliminates the possibility of reaction from non-autologous products and the APS therapy was found to be safe in this study with no adverse events or complications reported. The processing of
the product through the device was easy to perform and could be completed in less than 30 minutes making this an attractive same day procedure for pet owners.

Conclusions

This study is the first to assess injection of APS for treatment of canine osteoarthritis. The results of the study indicate that APS injection is safe and effective and should be considered as an option for treatment of canine osteoarthritis for up to 12 weeks duration. Additionally, as evidenced by previous studies\textsuperscript{22}, APS therapy may have regenerative potential. Future studies are necessary to determine duration of effect and a dosing timeline.
References


Appendix A: Forms

OWNER CONSENT FORM

The Ohio State University
Veterinary Medical Center

**Name of Study:** Pilot Clinical Trial on the Use of Intra-Articular Autologous Protein Solution to Improve Lameness in Dogs with Osteoarthritis

**Name of Investigators:** Alicia Bertone, DVM; Bianca Hettlich, DVM; Lillian Su, DVM; Audrey Wanstrath, DVM; Matthew Allen, DVM

**Informed Client Consent for autologous protein solution (APS) therapy in osteoarthritic dogs**

**What is osteoarthritis?** Osteoarthritis is a degenerative disease affecting joints. The bones within a moving joint are cushioned by cartilage, a fibrous tissue, and bounded by a sac of fluid called the synovial sac that keeps the joint lubricated and freely moving. Cells in the cartilage produce inflammatory proteins that comprise cartilage and induce inflammation. The process causes progressively increasing pain and lameness.

**How can blood proteins help?** Proteins from blood, while well known to be responsible for healing, also contain a variety of anti-inflammatory proteins and growth factors. A previous prospective and double-blinded horse clinical trial has shown that APS therapy improved lameness, but studies in the dog are lacking. APS therapy involves taking blood from your dog, then sequestering cells and proteins by centrifugation in a device that results in a smaller volume and higher cell concentrations. This system has an additional device that superconcentrates both the cells and the plasma (non red blood cell part of blood). Plasma contains many anti-inflammatory proteins and growth factors that are known to be supportive to cartilage. This therapeutic option is attractive because the APS comes from your own animals therefore adverse immune reactions are unlikely.
Experience in performance horses has shown APS to be safe. The only risks we are aware of are the risk of anesthesia and infection at the site of injection – neither of which are related to the therapy itself.

**What will happen to my dog?** If your dog appears to be a candidate for this pilot study of APS therapy in osteoarthritis, the study involves testing at no cost to you at the medical facility. If you decide to allow your dog to be enrolled, an x-ray and blood work will be taken. Your dog will be guided through an assessment of lameness and pain using a questionnaire, which you and a veterinarian will be asked to take part in. In addition, your animal will be lead several times across an in-ground flat metal force plate designed to measure the force with which each step is taken. Once the results confirm your animal can be enrolled, your dog will be assigned at random to one of two groups: either the control saline group or the treatment group. Be assured that if your animal is in the control group, you will be offered the option of having the treatment provided at no additional charge after the 3-month evaluation at the end of the study.

**Who pays?** The costs for testing, sedation/anesthesia, and treatment will be paid for by the sponsor of this pilot study. That includes the physical exams, x-rays if necessary, and all biochemical and blood tests as well as the technology used to harvest your dog’s blood APS therapy. While any side effects of injection are unlikely, these could include swelling, inflammation and rarely, infection. The study does not cover costs for treatment of these complications. I understand that upon completion of milestones in the study I will receive a debit card for $150. These milestones include completion of the 12-week evaluation at OSU and 24-week surveys, which you will mail to OSU.

**What are my responsibilities?** Once your dog has enrolled into the study and you have signed the consent form, you will be responsible for maintaining appointments and filling out any required questionnaires. A single owner should fill out questionnaires over the course of the study. These questionnaires help the investigators to assess your dog’s level of discomfort associated with the arthritis. The study timeline is outlined below:

Day 1: Your animal will be physically examined then rated using two questionnaires (one for pain, which you will perform, and the other for lameness which one of the study investigators will score with your help). The force plate test will be performed by one of the study co-investigators. Thereafter, your animal will be sedated and about 2 tablespoons of blood will be drawn from the jugular vein. This volume is known to be
safely withdrawn from animals the size of your dog. Synovial fluid will be withdrawn from the affected joint (the knee, called a stifle in the dog, or the elbow) to make room for the treatment to be administered. The synovial fluid samples will either be discarded, or may be saved for additional tests not planned for at this time. Then, your animal will be injected with either the control (saline) or the APS therapy.

You will be given simple instructions on how to care for your animal for the first day or two after the procedure. You will be asked to fill out two 11-item questionnaires for your return appointments. Finally, you will be asked to comply with the request to withdraw your animal from any pain medications and nutraceuticals 1 week prior to your appointments, if your animal needed them during this time. This is critical in order to determine the effectiveness of the therapy is not masked by pain medication.

Week 2 and Week 12: Your dog will be evaluated as described above using the questionnaires, physical examination, blood analysis and force plate analysis. At week 12, radiographs will be acquired and you will be informed as to whether your dog received the APS or the saline injection. If your dog received saline, then you will have the opportunity to have your dog injected with the APS therapy at this time.

**What’s in it for my dog?** APS therapy is an emerging new technology being used as a biologic therapy. This is an innovative therapy that may substantially improve the osteoarthritis in your dog’s joint and thus enhance your dog’s quality of life.

**Please read the following and sign below:**

- All potential therapeutic options for my dog’s osteoarthritis have been discussed with me.
- I have been informed of the possible benefits and risks associated with this treatment. These include bruising from blood collection, and swelling, discomfort and infection from joint injection.
- I understand that any side effects of this therapy are expected to be minor, but are not fully understood.
- I acknowledge that the study will cover procedures related to, and planned for, in the study, but will not cover any costs of unintended consequences.
- I acknowledge that, in the event of complications associated with blood collection or injection, it will be my responsibility to cover additional costs if I choose to pursue treatment.
• I understand that my dog will receive a blood harvesting procedure and joint injection procedure (taking fluid from the joint and replacing the fluid with one of two possible treatments: a saline control or APS therapy).
• I understand that I will, in the event my animal has been randomly assigned to the control group, be given the opportunity to receive APS therapy at the 3-month follow-up visit.
• I understand that I retain the right to remove my dog from this study at anytime; however, if I do prior to the study’s conclusion, then I forego the $150 incentives for milestones I did not complete.
• I understand that the veterinarians in charge of this study may choose to remove my dog from the study if they believe it is not in my dog’s best interest to continue on the study.
• I understand that case materials, photos and patient information gathered in this study may be used for scientific presentations and publications.
• I have disclosed all medications my dog is on and I will not administer any new (not prescribed) medications during the course of this study (including pain medications, NSAIDs, supplements, etc).
• I agree to maintain appointments, complete the required questionnaires, and withhold pain medications for 1 week prior to visits to the medical facility for study-related appointments.

As a result of discussion with Dr. ________________________, and after reading the above, I voluntarily consent to assignment of my pet to the clinical trial treatment program. I consent to participate in this project and will follow the instruction of the veterinarians-in-charge, as it pertains to therapy and follow-up procedures.

I/We grant permission for photos/images of my pet, along with information about the treatment/study, to be published on The Ohio State University Veterinary Medical Center website, newsletter, Facebook page or other social media outlets and publications.

☐ Yes  ☐ No

Signed ___________________________________________  Date ____________________
Owner or authorized agent of the owner

Witnessed By: _______________________________________  Date ____________________
12 WEEK CANINE BRIEF PAIN INVENTORY- PAGE 1

Canine Brief Pain Inventory (CBPI)

Description of Pain:
Rate your dog's pain.

1. Fill in the oval next to the one number that best describes the pain at its worst in the last 7 days.
   - 0: No Pain
   - 1-10: Extreme Pain

2. Fill in the oval next to the one number that best describes the pain at its least in the last 7 days.
   - 0: No Pain
   - 1-10: Extreme Pain

3. Fill in the oval next to the one number that best describes the pain at its average in the last 7 days.
   - 0: No Pain
   - 1-10: Extreme Pain

4. Fill in the oval next to the one number that best describes the pain as it is right now.
   - 0: No Pain
   - 1-10: Extreme Pain

Description of Function:
Fill in the oval next to the one number that describes how during the past 7 days pain has interfered with your dog’s:

5. General Activity
   - 0: Does not interfere
   - 1-10: Completely interferes

6. Enjoyment of Life
   - 0: Does not interfere
   - 1-10: Completely interferes

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Recorded by:  
(Please keep consistent throughout study)  
Date: (DD/MM/YY)
**Figure 7:** Representative Canine Brief Pain Inventory survey part 2.

```
<table>
<thead>
<tr>
<th>Description of Function (continued):</th>
</tr>
</thead>
</table>

7. **Ability to Rise to Standing From Lying Down**
   - 0: Does not interfere
   - 1 to 10: Completely interferes

8. **Ability to Walk**
   - 0: Does not interfere
   - 1 to 10: Completely interferes

9. **Ability to Run**
   - 0: Does not interfere
   - 1 to 10: Completely interferes

10. **Ability to Climb Up (for example Stairs or Curbs)**
    - 0: Does not interfere
    - 1 to 10: Completely interferes

**Overall Impression:**

11. Fill in the oval next to the one response that best describes your dog’s overall quality of life over the last 7 days.
    - Poor
    - Fair
    - Good
    - Very Good
    - Excellent

---

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**Recorded by:** ____________________________  **Date:** (DDMMYY)

(Please keep consistent throughout study)
12 WEEK HUDSON VISUAL ANALOGUE SCALE - PAGE 1

- Please Read Instructions First
- Please notice the labeling on the left and right sides before marking it.
- Reply to the questions by placing a check mark in the square nearest the most appropriate score.
- Lower scores mean your animal is improving and high scores mean doing poorly

1. How would you describe your overall assessment of your dog in the last month?
   
   □1 □2 □3 □4 □5 □6 □7 □8 □9 □10
   Good □10
   Bad

2. What kind of mood has your dog been in the last month?
   
   □1 □2 □3 □4 □5 □6 □7 □8 □9 □10
   Good □10
   Bad

3. How has your dog's attitude been in the last month?
   
   □1 □2 □3 □4 □5 □6 □7 □8 □9 □10
   Positive □10
   Negative

4. How frequently does your dog display comfort or "happy dog" postures (e.g., lying on back with toy in mouth)? Not applicable:
   
   □1 □2 □3 □4 □5 □6 □7 □8 □9 □10
   Often □10
   Rarely

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?
   
   □1 □2 □3 □4 □5 □6 □7 □8 □9 □10
   A Lot □10
   Not at All

Recorded by: ___________________________ Date: ________________

(Please keep consistent throughout study)

Figure 8: Representative Hudson Visual Analogue Scale

35
Animal ID: ___________________________ Name: ___________________________ Date: ___________________________
(Case #) (DD/MM/YY)

12 WEEK HUDSON VISUAL ANALOGUE SCALE - PAGE 2

6. How willing is your dog to play voluntarily?
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
All the Time
Not at All

7. How often does your dog get exercise?
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
All Day
Never

8. How stiff is your dog when arising for the day?
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
Not at All
Very

9. How stiff is your dog at the end of the day (post-activities)?
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
Not at All
Very

10. Does your dog indicate any lameness at a walk?
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
Never Lame
Always Lame

11. Does your dog indicate any pain when turning suddenly at a walk?
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
Never
Always

Recorded by: ___________________________ Date: ___________________________
(Please keep consistent throughout study) (DD/MM/YY)

Figure 9: Representative Hudson Visual Analogue Scale part 2

36
Appendix B: Data

<table>
<thead>
<tr>
<th>Breed</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akita</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>American Pit Bull Terrier</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Australian Shepard</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Boxer</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Catahoula Leopard Hound</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Doberman Pinscher</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>German Shepard Dog</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Golden Retriever</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Labrador Retriever</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Newfoundland</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Siberian Husky</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Greater Swiss Mountain Dog</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Mix Breed dog</td>
<td>3</td>
<td>2 *</td>
</tr>
</tbody>
</table>

Table 1: Breed distribution for dogs treated with APS or control vehicle.

<table>
<thead>
<tr>
<th>Weight (kg)</th>
<th>Control (n=10)</th>
<th>Treatment (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 Mean ± SD</td>
<td>31.45 ± 9.5</td>
<td>37.11 ± 5.5</td>
</tr>
<tr>
<td>Day 0 Median</td>
<td>30.05</td>
<td>37.6</td>
</tr>
<tr>
<td>Week 2 Mean ± SD</td>
<td>31.35 ± 9.85</td>
<td>37.12 ± 6.17</td>
</tr>
<tr>
<td>Week 2 Median</td>
<td>31</td>
<td>37.7</td>
</tr>
<tr>
<td>Week 12 Mean ± SD</td>
<td>31.07 ± 9.97</td>
<td>35.18 ± 7.07</td>
</tr>
<tr>
<td>Week 12 Median</td>
<td>30.3</td>
<td>37.7</td>
</tr>
</tbody>
</table>

Table 2: Weight distribution for dogs treated with APS or control vehicle.
<table>
<thead>
<tr>
<th>Sex</th>
<th>Control (n=10)</th>
<th>Treatment (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexually intact male</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Castrated male</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Sexually intact female</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Spayed female</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table 3**: Sex distribution for Dogs treated with APS or control vehicle.

<table>
<thead>
<tr>
<th>Age</th>
<th>Control (n=10)</th>
<th>Treatment (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>7.8 ± 3.15</td>
<td>6.8 ± 2.28</td>
</tr>
<tr>
<td>Median</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

**Table 4**: Sex distribution for dogs treated with APS or control vehicle.

<table>
<thead>
<tr>
<th>Affected limb</th>
<th>Control (n=10)</th>
<th>Treatment (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left front</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Left hind</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Right front</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Right hind</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 5**: Affected limb distribution for dogs treated with APS or control vehicle.

<table>
<thead>
<tr>
<th>Left / Right</th>
<th>Control (n=10)</th>
<th>Treatment (n=9)</th>
<th>Front / Hind</th>
<th>Control (n=10)</th>
<th>Treatment (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left limb</td>
<td>5</td>
<td>4</td>
<td>Front limb</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Right limb</td>
<td>5</td>
<td>5</td>
<td>Hind limb</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table 6**: Affected limb distribution for dogs treated with APS or control vehicle.
Figure 10: Box and whisker plots of scored CBPI data.
Figure 11: Box and whisker plots of scored CBPI data continued.
**Figure 12:** Box and whisker plot of scored HVAS data.
Figure 13: Box and whisker plot of HVAS scored data continued.

42