Comparison of Radiographic Guidance to Magnetic Resonance Imaging Guidance for Injection of the Collateral Ligaments of the Distal Interphalangeal Joint in an Equine Cadaver Model

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ABSTRACT

Desmopathy of the collateral ligament of the distal interphalangeal joint is a common cause of lameness in the horse and carries a poor prognosis for soundness. Intrallesional treatment has been suggested as a way to improve outcome; however, limited reports describe methods for injecting this ligament. This study compares the accuracy of injecting the collateral ligament of the distal interphalangeal joint using magnetic resonance imaging (MRI) versus radiographic guidance. Equine cadaver digit pairs (n=10) were divided by random assignment to injection of the ligament by either technique and assessed using post-injection MRI or gross sections. Images from the proximal, middle, and distal portions of the ligament were blindly evaluated for successful injection. McNemar’s test was performed to determine statistical difference between injection techniques. Fisher’s exact test was used to evaluate number of injection attempts and injection of the medial or lateral collateral ligament. MRI-guided injection was successful more frequently than radiographic-guided injection on post-injection MRI (24 of 30 versus 9 of 30; p=0.0006) and gross sections (26 of 30 versus 13 of 30; p=0.0008). At each level of the ligament (proximal, middle, and distal), MRI-guided injection resulted in more successful injections than radiographic guidance. Statistical significance occurred at the proximal aspect of the collateral ligament based on post-injection MRI (p=0.0143) and the middle portion of the ligament based on gross sections (p=0.0253). Based on these results, injection guided by standing, low-field MRI should be considered an option for delivering intrallesional regenerative therapy to horses with desmopathy of these collateral ligaments.
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Attributions

Several authors were involved in the design and implementation of the research or production of this thesis.

Jennifer G. Barrett- DVM, PhD, Diplomate ACVS (Marion duPont Scott Equine Medical Center, Virginia-Maryland Regional College of Veterinary Medicine) is the committee chair and principal advisor. Her research interests include regenerative therapies and tendon, ligament, and cartilage healing. Dr. Barrett’s PhD is in molecular biology. She was vital in the development of the study design, manuscript production, and review of the thesis.

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Thesis Organization

This thesis is formatted with a journal publication as the central portion of the document. The manuscript submitted for publication is entitled “Comparison of radiographic guidance to magnetic resonance imaging guidance for injection of the collateral ligaments of the distal interphalangeal joint in an equine cadaver model” and it includes introduction, methods, results, discussion, and reference sections. The following introduction provides a literature review of distal interphalangeal joint desmopathy. The conclusion of the thesis provides final comments that outline directions for future research.
Chapter 1

Introduction

Pathology of the collateral ligaments of the distal interphalangeal joint is the second most common diagnosis for equine lameness based on magnetic resonance imaging (MRI) of the hoof. Injury of this ligament affects equine athletes in a wide variety of disciplines including jumping, racing, and dressage. Previous reports have shown variable response to treatment, with 28-60% of horses returning to their previous level of exercise. The poor response to treatment has led to interest in delivering regenerative cell therapies to the collateral ligament by intralesional injection to stimulate healing. Intralesional injection has been recommended for improved healing of tendon and ligament injuries in other portions of the body; however, limited studies have evaluated delivery to structures within the hoof. A recent report describes injection into the collateral ligament of the distal interphalangeal joint using radiographic guidance after MRI or ultrasonographic diagnosis of desmopathy. During radiographic guidance the needle is directed based on identification of bony landmarks, but the ligament itself is not visible. Alternatively, this study describes a technique for guiding injection with MRI, which allows visualization of the entire course of the ligament when MRI-compatible needles are utilized. The goal of this study was to compare the accuracy of this novel technique, MRI-guided injection into the collateral ligament of the distal interphalangeal joint, to a previously described technique, radiographic guidance, in an equine cadaver model.

Collateral Ligament Anatomy

Ligaments are dense, white, connective tissue cords that connect bones. The collateral ligaments of the distal interphalangeal joint are symmetrically located on the dorsomedial and dorsolateral side of the limb, spanning from the collateral fossa of the middle phalanx to an insertion fossa on the distal phalanx, dorsal to the ungular cartilage (Figure 1). The fibers of the collateral ligament project obliquely from the middle phalanx then course vertically toward the insertion. Separated from bone by the collateral synovial recess, the ligament is embedded in the joint capsule with the majority lying deep to the hoof capsule. The ligament is beige-yellow with white striations, and in cross section it is oblong with individual fibers grossly visible.
In general, ligaments receive blood supply from the surface and vessels course through the superficial layers along the length of the ligament with minimal blood supply originating from the bony interfaces; however, the vascular supply of the collateral ligament has not been well defined. Other ligaments contain C-type pain fibers and proprioceptive nerve endings, but no published reports have characterized the innervation of this specific ligament.

Collateral ligaments consist of highly organized, linear, collagen fibers with slender cells (Figure 2). The majority of cells are fibroblasts or fibrocytes, but cell morphology can differ within these cell populations depending on the location within the ligament. Approximately 75%-80% of ligament’s organic solid content is collagen. At the molecular level, collagen is a right-handed superhelix composed of three intertwined polypeptide chains. The fibrils appear crimped or kinked when viewed using scanning electron microscopy, and when viewed using polarizing filters these kinks result in alternating light and dark striations. It is thought that this crimping lends ligament some of its elastic biomechanical properties. Ligament tissue contains approximately 95% type 1 collagen with lesser amounts of collagen types III and V present in the basement membranes and vasculature, respectively.

Proteoglycan accounts for 23% of the solid structure of ligament tissue. Water distribution within the ligament is controlled by these molecules, the most common being dermatan sulfate. Elastin is the third solid component, making up 1-2% of the ligament’s structure. Other, less prevalent components include actin, laminin, integrins and many other molecules that have yet to be characterized.
The overall composition of the ligament is determined by fibrocytes, which synthesize and degrade macromolecular components. There is evidence that the mechanical environment of the ligament may activate ion channels or influence cell metabolism, thereby controlling the matrix composition of the structure. Matrix also provides a negative feedback on the cells to modulate further matrix synthesis.

Figure 2: Photomicrograph demonstrating the ordered collagen arrangement of a normal collateral ligament of the distal interphalangeal joint. (Dyson, Blunden, and Murray 2008) Used under Fair Use guidelines.

Collagen type I organizes into progressively larger units bound by connective tissue cross-links: molecules, fibrils, fibers, and bundles. The connective tissue divisions of ligament are less prominent grossly than those of tendon; however, the subdivisions are vital to normal ligament function. For example, periarticular ligaments contain multiple fascicle bundles that are taut during different positions of the joint, in either flexion or extension, to provide stability throughout the range of motion.

The collateral ligament of the distal interphalangeal joint relies heavily on calcified fibrocartilage for strength at the origin and insertion. The distal phalanx does not contain periosteum at the insertional zone, so Sharpey’s fibers are not present compared to the enthesis of other ligaments. Instead, these direct insertion sites contain four distinct zones as the ligament merges from soft tissue to bone (Figure 3). The first zone is the structure of the ligament body, consisting mostly of type 1 collagen and dermatan sulfate. The second zone is composed of fibrocartilage with rounder fibroblasts and increased numbers of chondrocytes. A “tide mark” separates zone 2 from the calcified cartilage of zone 3. Contrary to the degeneration of cartilage cells in mineralized portions of other tissues, most of the fibrocartilaginous cells in this portion of the ligament insertion remain metabolically active in their lacunae. The final zone is composed of bone, where the collagen of the ligament merges with the collagen of the bony matrix. The collagen is still type 1 in this area, but the proteoglycan becomes chondroitin sulfate instead of dermatan sulfate.
Fibrocartilaginous metaplasia is a common, non-pathologic finding at the entheses of this ligament. These areas look like bands of pallor from proteoglycan deposition and mild disruption of the collagen fiber pattern.16,17 These may represent early pathologic change, but were documented in normal horses in both referenced studies.

**Collateral Ligament Biomechanics**

Collateral ligaments have two primary functions: passive control of bone position during joint function and stabilization of a joint when an extrinsic load is applied.13 Kinematic analysis has shown that large changes in position occur in the bones of the distal interphalangeal joint during the weight bearing phase of the inner limb as the horse moves on a circular track.24 The inside hoof is immobile on the ground while the horse’s body mass moves over the limb, resulting in adduction with the maximum angle when the heels leave the ground. That study documented medial to lateral motion of the distal phalanx in the direction of travel (2.0 ± 1.8°) and axial rotation in the opposite direction (10.2 ± 3.9°). This results in stretching of the joint space on the side away from the direction of travel (right side of the hoof as the horse travels to the left). Similar findings were described in a separate report, which notes that these movements place particular stress on the collateral ligaments of the

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Figure 3: Photomicrograph of the collateral ligament of the distal interphalangeal joint at its proximal enthesis. Note the transected fascicles of the ligaments (1). Fibers in the non-mineralized fibrocartilage zone (2) are separated from the calcified fibrocartilage (3) by a tidemark. The bone (4) in this zone is the middle phalanx. (Masson’s trichrome, original magnification x 4). Figure courtesy of N. A. White.
distal interphalangeal joint. These changes are exacerbated by uneven footing or improper hoof balance.

**Ligament Injury and Healing**

Ligament injury occurs due to biomechanical trauma, although the characteristics of injury vary by the specific location of the damaged structures. Periarticular ligaments tend to sustain damage due displacement of the bones when an extrinsic load is applied. This leads to tearing of the fibers at the yield point or complete disruption at the failure point of the tissue (Figure 4). Chronic exposure to conditions that place the ligament near its yield point may lead to structural changes in ligament composition. Exercise within a certain range will increase the strength and stiffness of the structure, but loading outside this range may lead to deterioration without complete failure. Histologic evaluation of damaged distal interphalangeal joint collateral ligaments has demonstrated the presence of degenerative changes without signs of inflammation, thus “desmopathy” has been proposed as the most accurate term for pathology of this ligament, since “desmitis” connotes inflammatory injury.

![Figure 4: Load versus deformation curve for ligament fibers showing progressive stretching as load increases (A, B) to the yield point where individual fibers begin to fail (C), and rupture of the entire ligament structure at the failure point (D). (Zachazewski, Magee, and Quillen 1996). Used under Fair Use guidelines.](image_url)
The initial changes seen with mild injury to the collateral ligament include clustering of fibroblasts and bands of pallor between normal collagen fibers. Extensive fibrocartilaginous change, tortuous fissuring degeneration, and complete disruption of the ligament may be seen in more severe cases of desmopathy (Figure 5).17

Healing of ligament pathology depends on multiple local factors that are specific to the ligament, species, and possibly breed.26 These factors include the degree of injury, blood supply, amount of mechanical stress, periligamentous environment, and presence of inflammatory mediators.23 In general, healing occurs as a continuum theoretically divided into the following phases: 1) inflammation, 2) proliferation, 3) remodeling, 4) maturation (Figure 6).

Edema and leukocytes are present in the ligament during the inflammatory phase.23 A fibrin clot forms and platelets release growth factors that initiate the inflammatory cascade.13 Proliferation of cells, extracellular matrix production, and the peak of collagen turnover define phase 2, or the proliferative phase.23 There is no evidence that any damaged ligament can
naturally produce normal matrix during this phase of healing, and it produces a higher ratio of type III collagen compared to normal ligament. There is decreased proliferative activity during phase 3 as the scar stabilizes and remolds, and the amount of fluid and edema in the tissue normalizes during this final remodeling period. The matrix becomes denser and more organized as it contracts over time. This scar tissue tends to be stiffer, resulting in decreased performance and higher risk for reinjury. The exact sequence of healing events as they occur specifically in the collateral ligament of the distal interphalangeal joint has not been elucidated.

**Diagnosis of Lameness**

Horses with desmopathy of the collateral ligaments of the distal interphalangeal joint typically display the greatest degree of lameness when the affected limb is on the inside of a circular track. This is regardless of the medial versus lateral orientation of the lesion, likely due to increased strain on the ligaments as the horse moves its weight over the inside limb. The severity of the lameness grade is highly variable, as is the response to flexion tests. Commonly, there are no specific localizing signs; however, coronary swelling at the site of injury has been reported. Anesthesia of the palmar digital nerves at the proximal sesamoid bones should abolish lameness caused by desmopathy of these collateral ligaments. The palmar digital nerve block at the proximal edge of the heel bulb improves lameness in 72% and resolves lameness in 65% of cases. Intra-articular anesthesia of the distal interphalangeal joint is inconsistent with a reported efficacy ranging from improvement in 24% of cases to resolution of lameness in 62% of cases. This difference might be due to a longer time between injection and jogging (10 minutes versus 5 minutes) in the latter study, allowing for increased diffusion of the anesthetic from the synovial fluid into the periarticular tissues.

**Diagnostic Imaging**

Desmopathy of the collateral ligament of the distal interphalangeal joint has been diagnosed with multiple imaging modalities. Magnetic resonance imaging (MRI) is considered the gold standard for diagnosing this condition since it has the highest sensitivity and specificity. The tightly bound nature of water particles within the collagen cross links of normal ligament tissue make for a short T2 or spin-spin relaxation time, thus normal ligament typically has a low signal intensity on both T1 and T2 weighted sequences.
signal intensity may vary by level within the ligament and between individuals. Typically, the signal intensity is higher at the origin of the collateral ligament at the middle phalanx compared to the middle portion and insertion due to relationship of the fibers to the primary magnetic field. T2-weighted fast-spin echo sequences are considered the best to delineate anatomic detail of the collateral ligaments on low-field MRI, and proton density turbo-spin echo sequences were the best on high-field MRI. A complete examination includes sequences in multiple planes with different timing parameters in order to best characterize pathology.  

Acute injuries of the collateral ligaments may appear as increased signal intensity on T1- and T2-weighted images as well as in fat-suppressed images. The increased signal intensity on T1-weighted images may be due to disorganization of the collagen alignment, while increased intensity on T2-weighted and fat-suppressed images may be due to increased fluid in the tissue, namely edema or hemorrhage. Chronic injuries may only show abnormally increased signal intensity on T1-weighted images as the fluid content of the ligament normalizes. 

Poor positioning of the digit within the magnet can confound evaluation of signal intensity within the collateral ligament of the distal interphalangeal joint. Magic angle artifact appears as increased signal intensity within the ligament when the ligament is angled with respect to the magnetic field, reaching a maximum intensity at an angle of 55 degrees. At this inclination, water proton motion is less restricted by the dipolar interaction of the water molecules and collagen helix, leading to longer T2- (spin-spin) relaxation times. T1-weighted sequences are more susceptible to this artifact due to shorter echo times for this pulse sequence. The anatomy of the collateral ligament of the distal interphalangeal joint decreases the limb angle required to generate this artifact. The angle of the collateral ligament fibers at the middle phalanx ranges from 20-82°, allowing magic angle artifact to be observed with as little as a 4° angle of the limb. The effect of this artifact could be minimized using a T2-weighted fast-spin echo sequence with a 120 ms echo time while still maintaining image quality. 

Digital radiography is more universally available to equine practitioners than MRI, but this modality has low sensitivity for detecting collateral ligament pathology. False negatives are common, as pathology may only be radiographically visible in 9.4% of horses that have osseous lesions related to the collateral ligament with MRI. Radiographically visible pathology may include remodeling of the collateral fossa in the distal phalanx or ossification of the collateral cartilages.
Similarly, nuclear scintigraphy can identify increased radiopharmaceutical uptake near the collateral ligaments, but has low specificity for desmopathy. The solar margin image is the most diagnostic view for this lesion. Although negative findings do not rule out severe injury, nuclear scintigraphy can be used to prioritize MRI findings if multiple lesions are identified.

Ultrasonography can be used to evaluate the proximal portion of the collateral ligaments of the distal interphalangeal joint, but interference of the hoof capsule typically prevents visualization of the distal 50% of the ligament. One can compensate for this interference by increasing the obliquity of the ultrasound probe orientation distally, but this is of limited value in horses with narrow, upright hooves. Transverse images are the most diagnostic, as the narrow probe footprint in this alignment allows for more versatility than the wider, longitudinal orientation. The cross sectional area in the transverse plane is similar for the medial (0.63 ± 0.05 cm²) and lateral (0.62 ± 0.04 cm²) collateral ligaments of the distal interphalangeal joint. Ultrasonography tends to underestimate the frequency of collateral ligament desmopathy: only 27% of lesions identified with MRI were found with ultrasonographic exam.

Computed tomography has been shown to have similar visibility scores for the collateral ligament of the distal interphalangeal joint compared to low field MRI. Contrast enhancement did not affect the visualization scores for these ligaments.

**Clinical Significance and Treatment of Collateral Ligament Desmopathy**

Only slightly less frequent than deep digital flexor tendonitis, desmopathy of the collateral ligaments of the distal interphalangeal joint is the second most common MRI diagnosis for lameness in the horse. The lesion is most commonly a unilateral fore limb condition affecting the insertion of the medial collateral ligament; however injury may occur in other portions of the collateral ligament. The presence of additional soft tissue injuries may complicate diagnosis of desmopathy of the collateral ligaments. Most MR examinations of the equine digit identify abnormalities in three or more structures. Concurrent injuries of the collateral ligaments of the distal interphalangeal joint and deep digital flexor tendon have been identified in 43% of lame horses examined with MRI. Extensively ossified collateral cartilages of the hoof have been correlated to injury of the collateral ligament, but the presence of osseous injury does not appear to influence recovery rates.
No controlled studies have specifically evaluated treatment efficacy. Corrective shoeing and controlled exercise are the mainstays of treatment, with 44% of successful outcomes associated with athletic rest for greater than 18 months. Other published treatment options include cast immobilization and extracorporeal shockwave therapy. Despite treatment, only 28-60% of horses are able to return to full work. Although variation in reported treatments makes comparisons difficult, the difference in response to treatment is likely due to the presence or absence of additional soft tissue injuries within the hoof. This documented poor response to treatment is the impetus for developing more effective treatment options.

**Review of Regenerative Therapies**

In general, tendons and ligaments have low metabolic rates, which may result in slower healing rates. Injured tendon and ligament heals with scar tissue, without restoration of normal biomechanical properties of the structure, presenting a risk for reinjury. Regenerative therapy strives to restore normal structure and function to damaged tissues. The three main components required for regeneration include scaffolds, growth factors, and cells. Although the ideal treatment protocol has not been determined, a multi-modal approach is likely required to obtain repair tissue that is biologically, chemically, and mechanically similar to normal tissue.

**Biologic Scaffolds**

Biologic scaffolds provide a tissue substrate for proliferation of cells during the regenerative process. Urinary bladder matrix (UBM) is a scaffold-based therapy consisting of basement membrane and tunica propria layers of porcine bladder. This product is designed to provide a scaffold for tissue healing and neovascularization, however peer-reviewed evidence of efficacy is lacking. UBM is delivered via intraleisonal injection and may cause significant inflammation. In order to reduce the incidence of post-treatment inflammation, it has been recommended to perform low volume injection and delay treatment until after the acute inflammatory phase of injury has passed. Though used for suspensory ligament injuries, this treatment option has not been critically evaluated for use in treatment of desmopathy of the collateral ligaments of the coffin joints. It is possible that the inflammation incited by injection of this product could cause significant discomfort since the confines of the hoof capsule limit space for swelling at the insertion of the collateral ligament. Platelet rich plasma (PRP) is an
autologous alternative that not only acts as a scaffold, but also provides a rich supply of growth factors.\textsuperscript{53}

\textit{Growth Factors}

Several growth factors have been evaluated for use in stimulating cell proliferation, extracellular matrix production, and neovascularization. These protein-signaling molecules include insulin-like growth factor-I (IGF-I), platelet-derived growth factor (PDGF), bone morphogenetic protein-12 (BMP-12), transforming growth factor-\(\beta\) (TGF-\(\beta\)), vascular endothelial growth factor (VEGF), growth and differentiation factor (GDF-5), and basic fibroblastic growth factor (bFGF). Purified proteins, bone marrow aspirate, or platelet-rich plasma are all methods of providing growth factors for intralesional therapy. TGF-\(\beta\) treatment is no longer popular due to enlargement of treated tendons and a high rate of contralateral limb tendonitis. Repeat injection of human, recombinant IGF-1 has been recommended at days 1, 4, 7, and 10 in horses with superficial digital flexor tendonitis. This treatment has failed to improve prognosis compared to reported success with conservative therapy.\textsuperscript{8}

Platelet rich plasma (PRP) is a way to provide multiple growth factors in one product, which is processed by concentrating and activating platelets from autologous, peripheral blood. Platelets naturally contain PDGF, TGF-\(\beta\), and VEGF and these factors are released upon platelet degranulation.\textsuperscript{8} One study demonstrated that PRP contains the highest concentration of anabolic growth factors and enhances tendon matrix gene expression \textit{in vitro} without activating catabolic cytokines,\textsuperscript{54} while another study found the anabolic activity to be slightly less than that of acellular bone marrow in a suspensory ligament explant model.\textsuperscript{55} PRP also acts as a scaffold since it clots after exposure to the basement membrane of damaged cells.\textsuperscript{8} The fibrin clot matrix of activated PRP not only provides a binding site for platelets, but also for other cells associated with healing such as endothelial cells, smooth muscle cells, fibroblasts, and stem cells. Additionally, platelets were shown to be analgesic, although the mechanism is unknown.\textsuperscript{50}

Injection of PRP one week after surgically induced superficial digital flexor tendonitis resulted in enhanced tendon repair compared to a saline treated control as evaluated by biochemical, biomechanical, and histological examination.\textsuperscript{56} PRP-treated tendons contained higher collagen, glycosaminoglycan, and DNA content. There was higher strength at failure and elastic modulus in the PRP-treated tendon. Additionally, the treated tendon had a more normal
arrangement of the collagen fibrils compared to the untreated control. These findings were evaluated at 24 weeks in an in vivo model. Another study evaluating intralesional injection of PRP activated with bovine thrombin for treatment of suspensory ligament desmitis reported return of Standardbred racehorses to full performance with analysis out to three years, but the number of starts in the third year after injury and amount of winnings in the first year were lower than a control group of horses without suspensory desmitis.9

Arguments against use of PRP include its lack of a cell source and potential to stimulate scar tissue formation versus tissue regeneration.8 Additionally, it has not been determined whether the presence of leukocytes in PRP preparations is beneficial. The release of reactive oxygen species and expression of matrix metalloproteinase 8 and 9 by neutrophils suggest inclusion of leukocytes in the preparation could lead to negative impacts on the tissues.50 Several commercial systems are available for PRP processing, but no equine specific metric has been determined to assess the quality of these products and optimize the preparation for therapeutic use.53

Cell Therapy

The third aim of regenerative therapy is to provide cells that may allow formation of new tissue biologically similar to the tissue present before injury. The potential therapeutic mechanisms for cell therapy include differentiation into tissue-specific cell phenotypes, trophic effects via bioactive protein production, and anti-inflammatory activity.51 The most common cell sources in equine regenerative medicine include mesenchymal stem cells (MSCs) from adipose (A-MSC) and bone marrow (BM-MSC);8,57 however use of embryonic58 and amniotic59 cells has recently been described. A-MSCs are obtained by processing fat collected in the tail head region of the horse to concentrate A-MSCs. This supplies an unknown but large number of MSCs with a 48-hour delay from collection to delivery. Differentiation assays have shown A-MSC to have inferior performance for tissue regeneration,8 and they may be more appropriate for anti-inflammatory indications.57

Stems cells from bone marrow have been shown to out perform cells from other sites in terms of differentiation, and they can be obtained with minimal donor site morbidity.51 Bone marrow can be collected from either the sternum or tuber coxae and processed in three main ways prior to intralesional delivery: 1) immediate use of bone marrow aspirate, 2) concentration
of bone marrow by centrifugation, or 3) culturing and expanding of cells. Immediate use of bone marrow aspirate is rapid, inexpensive, and simple, but carries the risk of tissue mineralization and delivers a small number of stem cells. By centrifuging the aspirate for 14 minutes, the number of MSCs can be concentrated 12-fold, but this still delivers only approximately 10,000-50,000 stem cells depending on the patient. This is a rapid procedure that can be performed at the time of diagnosis and provides the three main factors for regeneration: scaffold, growth factors, and cells. Laboratory processing can expand and select stem cells to provide 10 x 10^6 cells for delivery into sites of injury. Additionally, this technique removes other components of bone marrow, such as bone spicules, which may not be beneficial to healing when present. The ideal carrier for these cells remains to be determined, but options include platelet-rich plasma, serum, or bone marrow supernatant. The optimum timing for injection also remains to be determined. Allowing time for granulation and angiogenesis might produce a tissue environment more likely to support MSC growth compared to the hemorrhagic, inflamed tissue present immediately after injury. Processing of autologous BM-MSCs requires a 2-3 weeks window, so point of injury injection is only possible if cells have been collected previously and stored.

Intralesional injection of MSCs shows promising results for treatment of superficial digital flexor tendonitis, with fewer reports in the literature evaluating effects on ligament healing. Implantation of autologous mesenchymal stem cells was found to have no adverse effects, cause no tissue aberrations on histologic examination. Improved return to performance and less reinjury was seen with MSC implantation within one month of injury compared to other treatments in a study evaluating 141 clinical cases. Later implantation of cells appears to have an increased reinjury rate, possibly due to the formation of fibrous tissue at the injury site, so injection is recommended in the first month after injury. This has not yet been evaluated for desmopathy of the collateral ligaments of the distal interphalangeal joint, and future studies are necessary to optimize timing of treatment in this region and to critically assess the benefit.

Intralesional Delivery Methods

Limited reports describe intralesional injection for the treatment of desmopathy of the collateral ligaments of the distal interphalangeal joint. One peer-reviewed study includes a clinical case treated with intralesional injection of urinary bladder matrix performed with ultrasonographic guidance. That report recognizes that lesions in this proximal portion of the
ligament are uncommon, precluding widespread use of ultrasonographic guidance of intralesional therapy. The large portion of the collateral ligament located deep to the hoof capsule would not be visible with this technique. Radiographic guidance of injection into the collateral ligament has been described. The needle is directed based on identification of bony landmarks to approximate the location of the collateral ligament of the distal interphalangeal joint. Alternatively, an injection technique using MRI guidance would show the entire course of the ligament and lesion when MRI-compatible needles are utilized, greatly facilitating intralesional injection. Standard needles are made from ferromagnetic metals that create interference in the magnetic field with severe distortion of the image and areas of zero signal. Conversely, MRI-compatible needles made from alloys such as nitinol (nickel, titanium) have low susceptibility to the magnetic field and produce minimal artifact. Although MRI guidance has been described in human medicine for localization of breast cancer or hepatic lesions, MRI guided injection of the collateral ligament of the distal interphalangeal joint has not been previously described.

Conclusions
Desmopathy of the collateral ligaments of the distal interphalangeal joint is not only a common cause of equine lameness, but also carries a poor prognosis for return to athletic function. This may result in costly losses if the horse has economic value and emotional frustration for the owners of affected cases. Treatments described in case series have been largely ineffective and inconsistent. Intralesional delivery of regenerative therapy may provide a future treatment route with improved efficacy, but treatment protocols are lacking. Since desmopathy most commonly affects the insertion of the distal interphalangeal joint collateral ligaments, injection techniques must allow accurate targeting of this area. Alignment of the injection along the entire length of the ligament would ensure maximal deposition of the regenerative therapy.

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Chapter 2

Comparison Of Radiographic Guidance To Magnetic Resonance Imaging Guidance For Injection Of The Collateral Ligaments Of The Distal Interphalangeal Joint In An Equine Cadaver Model

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Abstract

Desmopathy of the collateral ligament of the distal interphalangeal joint is a common cause of lameness in the horse and carries a poor prognosis for soundness. Intraliesional treatment has been suggested as a way to improve outcome; however, limited reports describe methods for injecting this ligament. This study compares the accuracy of injecting the collateral ligament of the distal interphalangeal joint using magnetic resonance imaging (MRI) versus radiographic control. Equine cadaver digit pairs (n=10) were divided by random assignment to injection of the ligament by either technique and assessed using post-injection MRI or gross sections. Images from the proximal, middle, and distal portions of the ligament were blindly evaluated for successful injection. McNemar’s test was performed to determine statistical difference between injection techniques. Fisher’s exact test was used to evaluate number of injection attempts and injection of the medial or lateral collateral ligament. MRI-guided injection was successful more frequently than radiographic-guided injection on post-injection MRI (24 of 30 versus 9 of 30; p=0.0006) and gross sections (26 of 30 versus 13 of 30; p=0.0008). At each level of the ligament (proximal, middle, and distal), MRI-guided injection resulted in more successful injections than radiographic guidance. Statistical significance occurred at the proximal aspect of the collateral ligament based on post-injection MRI (p=0.0143) and the middle portion of the ligament based on gross sections (p=0.0253). Based on these results, injection guided by standing, low-field MRI should be considered an option for delivering intraliesional regenerative therapy to horses with desmopathy of these collateral ligaments.

Introduction

Desmopathy of the collateral ligament of the distal interphalangeal joint (CL) is the second most common diagnosis for equine lameness based on magnetic resonance imaging (MRI) of the hoof. Injury of this ligament affects equine athletes in a wide variety of disciplines including jumping, racing, and dressage. Lateral motion and medial rotation occur during the stance phase of the inner limb as a horse moves on a circle, and such motion may lead to collateral ligament injury, especially when exacerbated by unbalanced hooves or uneven footing. Most commonly, this is a unilateral forelimb condition affecting the insertion of the medial collateral ligament; however, injury may occur in other portions of the ligament.

The collateral ligaments of the distal interphalangeal joint are symmetrically located on the dorsomedial and dorsolateral side of the limb, spanning from the collateral fossa of the
middle phalanx to the collateral fossa of the distal phalanx, dorsal to the ungular cartilage. The majority of the collateral ligament lies deep to the hoof capsule, making both diagnosis and treatment of desmopathy more challenging than other collateral ligaments. Horses with collateral ligament desmopathy may have changes such as remodeling of the collateral fossa in the distal phalanx or ossification of the collateral cartilages detected radiographically, but this modality has low sensitivity for detecting collateral ligament pathology. Similarly, nuclear scintigraphy can identify increased radiopharmaceutical uptake near the collateral ligaments, but has low specificity for collateral ligament desmopathy. Ultrasonography has good sensitivity and specificity for lesions in the proximal portion of the collateral ligament, but interference of the hoof capsule typically prevents visualization of the distal half of the ligament. MRI is considered the gold standard for diagnosing desmopathy of the collateral ligament of the distal interphalangeal joint, since it has the highest sensitivity and specificity. The increasing availability of equipment to perform cross sectional imaging in the standing, sedated patient (Hallmarq Veterinary Imaging, Guildford, Surrey, UK) makes MRI a practical and effective tool for diagnosing lameness isolated to the hoof. Previous reports have shown variable, poor response to treatment, with 28-60% of horses returning to their previous level of exercise. Intraleosional injection of regenerative therapeutics has been recommended for improved healing of tendon and ligament injuries, although limited studies have evaluated delivery to structures within the hoof. A recent report describes injection of the collateral ligament of the distal interphalangeal joint using radiographic guidance after MRI or ultrasonographic diagnosis of desmopathy. The needle is directed based on identification of bony landmarks, but the collateral ligament is not visible during radiographic guidance. Alternatively, MRI-guided injection of platelet rich plasma or mesenchymal stem cells has been performed by the authors to treat clinical cases affected by collateral ligament desmopathy. The entire course of the ligament is visible with this technique when MRI-compatible needles are utilized. The present study describes the technique for MRI-guided injection into the collateral ligament of the distal interphalangeal joint and compares the accuracy of this technique to the previously described radiographic guidance in a cadaver model. It was hypothesized that 1) MRI-guided injection of collateral ligament would be possible using non-ferromagnetic needles in a low-field magnet 2) MRI-guided injection would be more accurate than the previously reported radiographic-guided injection technique, and 3) MRI-
guided injection would be more accurate than radiographic-guided injection at each of three levels (proximal, mid and distal) within the ligament.

Methods

Experimental Design

Equine cadaver digits were randomly assigned to guided injection technique using a paired study design (Figure 1). MRI guidance was compared to radiographic guidance, and the same investigator (MML) performed all injections. Injections were performed on the left side of each specimen to control for handedness and to randomize targeting of the lateral or medial collateral ligament. The investigator responsible for injection had limited prior experience with either technique. The accuracy of each injection was evaluated using the following markers: the position of a wire visualized with MRI and the observation of injected dye in gross sections. These images were assigned unique codes and evaluated by a blinded investigator (NAW) to prevent correlation of scores.

Specimens

Paired thoracic limb digits were collected from 10 horses euthanatized for reasons other than this study and without a history of lameness isolated to the hoof. The sample size was chosen based on a study evaluating a technique for injection of the deep digital flexor tendon within the hoof.23 Included horses were adult (median 14 years, range 4-21 years,) geldings (n=6) or mares (n=4). Represented breeds included Thoroughbred (n=6), Warmblood or Warmblood cross (n=3), and Tennessee Walking Horse (n=1). Digits were amputated at the

Figure 1: Organizational flow-chart illustrating experimental design.
metacarpophalangeal joint, labeled, and frozen at −20°C. Each digit was prepared by clipping all hair and obtaining a dorso60proximal-palmarodistal oblique radiographic projection (Eklin Medical Systems Inc., Santa Clara, California, USA) to guide removal of any radiopaque foreign material that would cause artifacts during MRI acquisition. Specimens were thawed at 4°C for 24-36 hours prior to further imaging and injection. Baseline transverse and frontal MRI studies were performed in all digits to verify no pathology was present in the collateral ligaments prior to enrollment into the study.

**Pre-injection Magnetic Resonance Imaging**

MRI studies were obtained using T1-weighted, three-dimensional, gradient echo (T1W GE) sequences for all specimens with an open 0.27 Tesla permanent magnet and extremity radiofrequency coil (Hallmarq Veterinary Imaging, Guildford, Surrey, UK). Transverse MRI sequences were aligned with slices parallel to the sole and frontal images aligned with slices perpendicular to the sole. The following pulse sequence parameters applied throughout the study: repetition time (TR) 23 ms, echo time (TE) 7 ms, flip angle 40°, number of acquisitions (NEX) 1, slice thickness 3.0 mm, gap 0.0 mm, field of view 16.9 cm, matrix size 256 x 256.

**Injection Techniques**

For both MRI and radiographic guidance, a maximum of four attempts for correct needle placement were allowed prior to injection. Both techniques utilized repositionable needles designed for preoperative marking of human breast cancer lesions. Similar to the stylet of a spinal needle, these needles contain a non-ferromagnetic metallic marker wire that can pass through the lumen of the needle. Once deployed beyond the needle bevel, a hook or barb at the wire tip engages the tissue and maintains the location of the wire as the needle is withdrawn. Non-ferromagnetic needles were used for MRI-guided injections, while virtually identical ferromagnetic needles were used for radiographic guidance. The non-ferromagnetic needles create minimal signal void artifact during MRI acquisition, allowing for greater anatomic detail during needle placement compared to standard needles (Figure 2).
MRI-Guided Injection: The specimen was centered in a dedicated hoof radiofrequency coil simulating the position of a horse’s left thoracic limb during MRI examination under standing sedation (Figure 3). Standard, three-plane pilot images were obtained to ensure placement of the digit in the isocenter of the magnet and to facilitate sequence planning. A 20-gauge x 9 cm, non-ferromagnetic, repositionable needle (MRI TULOC® Localization System, Somatex Medical Technologies, Teltow, Brandenburg, Germany) was then inserted approximately 1 cm through the skin at the origin of the collateral ligament. This location was selected by palpating the space between the common digital extensor tendon and the dorsal edge

Figure 2A and 2B: Transverse T1-weighted, three-dimensional, gradient echo (T1W GE) MR images showing the difference in the amount of anatomic detail obscured by the signal void associated with (A) a ferromagnetic catheter (Abbocath®, Hospira, Inc., Lake Forest, Illinois, USA.) and (B) a non-ferromagnetic needle (MRI TULOC® Localization System, Somatex Medical Technologies, Teltow, Brandenburg, Germany).
of the ungular cartilage just distal to the narrowest portion of the middle phalanx. The needle was directed perpendicular to the solar surface of the hoof. Frontal and transverse T1-weighted, three-dimensional, gradient echo (T1W GE) MR sequences were obtained to determine if needle position was optimal. Identification of the small focal signal void caused by the needle was used to guide the angle of the injection while attempting to place the needle within the collateral ligament of the distal interphalangeal joint from its origin to the insertion. Scans were repeated after each positioning attempt. When needle position was considered optimal (or after the maximum of four needle-positioning attempts), one mL of new methylene blue dye was injected using a luer-lock syringe. The marker wire was then deployed into the tissue and the needle was withdrawn.

Figure 3: Palmar view of a specimen positioned within the MRI unit during MRI-guided injection. The needle was inserted in the space between the common digital extensor tendon and the dorsal edge of the ungular cartilage just distal to the narrowest portion of the middle phalanx.

**Radiograph-Guided Injection:** Radiograph-guided injection was performed according to the previously published technique. Briefly, a 20-gauge x 10 cm, repositionable needle (RPLN™ Repositional Breast Localization Needle, CP Medical, Inc., Portland, Oregon, USA) was inserted approximately 1 cm through the skin above the coronary band, midway along the width of the middle phalanx, and angled distodorsally toward the collateral fossa of the distal phalanx. This position was slightly more palmar than the injection location used for MRI-guided injection and the angle approximated the long axis of the proximal phalanx to enter the insertion.
of the ligament at a different angle than the more vertical alignment of the other technique. Sequential horizontal dorsopalmar and lateromedial radiographic projections were obtained as the needle was advanced or redirected at a correct angle to enter the collateral fossa of the distal phalanx (Figure 4). After a maximum of four attempts or when position was considered ideal, injection of dye and insertion of a metallic marker wire were performed as described for MRI guidance.

Figure 4A and 4B: Radiograph-guided injection as seen in a horizontal dorsopalmar (A) and lateromedial (B) projection. Gross analysis confirmed this injection to be in the collateral ligament of the distal interphalangeal joint at the proximal, middle, and distal portions of the ligament.
**Evaluation of Accuracy**

**Marker Wire Position:** Immediately following injection by either technique, frontal and transverse T1-weighted, three-dimensional, gradient echo (T1W GE) sequences were obtained for all specimens with a marker wire in place to identify location of the needle tract relative to the collateral ligament at each of three levels. Prior to beginning the study, test specimens used to validate the study design and methods showed that identification of the metallic marker wire in T1-weighted, three-dimensional, gradient echo (T1W GE) images provided an accurate and reproducible method to analyze injection location in cadaver limbs, while short tau inversion recovery (STIR) MR images did not allow reliable identification of fluid signal in cadaver specimens. Thus, T1-weighted, three-dimensional, gradient echo (T1W GE) sequences and the metallic marker wire were used for the post-injection MRI analysis for the study.

The blinded post-injection images contained no identifying information revealing which technique was used. For each specimen, location of the marker wire with respect to the collateral ligament of the distal interphalangeal joint was evaluated in three transverse images, each taken from three standardized locations corresponding to the proximal, middle and distal aspects of the ligament (Figure 5). Specifically, these areas were defined as the level of 1) the middle phalanx collateral fossa, 2) the condyles of the middle phalanx, and 3) the collateral fossa of the distal phalanx, respectively.

![Figure 5A, 5B, and 5C: Transverse T1-weighted, three-dimensional, gradient echo (T1W GE) images showing the marker wire within the collateral ligament of the distal interphalangeal joint at the (A) proximal, (B) middle, and (C) distal aspect of the ligament. Note the marker wire tip splits into a double arch configuration, causing a different shape of the signal void (C) at the tip compared (A, B) to the marker shaft.](image-url)
Placement of the marker within the body or margin of the collateral ligament was considered successful whereas placement of the marker outside the ligament was considered unsuccessful. When the marker wire was visible within the synovial pouches of the distal interphalangeal joint, it was recorded. Frontal images were reviewed to confirm marker location, but were not separately scored.

**Dye Location:** After post-injection MRI, all specimens were frozen at −20°C, sectioned transversely with a band saw at 1 cm intervals parallel to the coronary band intersecting the proximal, middle and distal regions of the collateral ligament of the distal interphalangeal joint as defined above. Three digital photographs were obtained of each specimen: one each from proximal, middle and distal collateral ligament (Figure 6). Dye located within the body or margin of the collateral ligament was considered successful whereas dye outside the ligament was considered unsuccessful. In all limbs, dye was visible along the entire proximal to distal length of the needle track. Specimens with dye visible within the synovial pouches of the distal interphalangeal joint were recorded.

![Figure 6A, 6B, and 6C: Gross specimens showing new methylene blue dye within the collateral ligament of the distal interphalangeal joint in the (A) proximal, (B) middle, (C) and distal aspects of the ligament.](image)

**Statistical Analysis**

Accuracy grades for MRI-guided and radiograph-guided techniques were compared using McNemar’s test. Post-injection MRI and gross section grades were analyzed separately for all comparisons. To evaluate overall injection success rate, the number of successful injections was compared to the number unsuccessful injections for MRI-guided and radiograph-guided techniques. Additionally, injection success in the proximal, middle, or distal aspect of the
ligament was evaluated separately. The rate of distal interphalangeal joint penetration was compared using McNemar’s test, as well. A Fisher’s exact test was performed to detect difference between (1) number of injection attempts, and (2) injection of the medial or lateral CL of the distal interphalangeal joint. Statistical significance was set to \( p \leq 0.05 \). All statistical tests were selected by a statistician (SRW) and performed using SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina, USA).

**Results**

Evaluation of injection accuracy using post-injection MR images is summarized in Table 1, and using gross section images is summarized in Table 2. Successful marker placement within the body or margin of the collateral ligament of the distal interphalangeal joint was seen in 80.0% (24/30) of images from the MRI-guided injection group compared to 30.0% (9/30) from radiograph-guided injections based on post-injection MRI \( (p = 0.0006; \text{Table 1}) \). Gross section images showed successful dye placement in 86.7% (26/30) images in the MRI-guided group compared to 43.3% (13/30) images of the radiograph-guided injections \( (p = 0.0008; \text{Table 2}) \).

MRI-guidance was significantly more successful at the middle portion of the collateral ligament \( (9/10, 90\%) \) than radiograph-guidance \( (4/10, 40\%) \) based on gross examination \( (p = 0.0253) \). Using post-injection MRI evaluation, MRI guidance was more frequently successful than radiographic guidance at the proximal portion of the collateral ligament \( (6/10 \text{ v. } 0/10; p = 0.0143) \).

At other levels of the collateral ligament, no statistical difference was detected; however, MRI-guidance always had more successful injections than radiographic guidance, and a trend toward significance \( (p \leq 0.10) \) was noted at every level of the collateral ligament, regardless of evaluation method. MRI guidance was more frequently successful in the distal \( (9/10 \text{ v. } 4/10, p = 0.0588) \) and middle \( (9/10 \text{ v. } 5/10, p = 0.1025) \) portions of the ligament compared to radiographic guidance based on evaluation of the post-injection MR images. MRI guidance led to successful injection of the collateral ligament more frequently at its proximal \( (8/10 \text{ v. } 3/10, p = 0.0588) \) and distal portions \( (9/10 \text{ v. } 6/10, p = 0.0833) \) than radiographic guidance based on gross examination.

There was no significant difference in penetration of the distal interphalangeal joint between techniques regardless of the evaluation method \( (\text{Table 3}; p = 0.5637) \). All injections were performed after three or four positioning attempts. There was no difference between groups for
either number of attempts (p=0.69) or injection of the medial or lateral collateral ligament of the distal interphalangeal joint (p=0.75).

**Discussion**

This study describes an MRI-guided technique for injecting the collateral ligament of the distal interphalangeal joint and demonstrates its accuracy compared to a previously reported technique. The overall accuracy of MRI-guided injection of the collateral ligament of the distal interphalangeal joint was significantly better than radiograph-guided injection when evaluated by post-injection MRI and gross examination. When separately evaluating injection success at the three different levels of the ligament, statistical significance was maintained at the middle portion of the ligament when scored based on identification of dye within the ligament (p=0.0253), and proximal portion of the ligament when scored based on marker wire location (p=0.0143). The trend toward statistical significance at other levels of the collateral ligament would possibly reach significance with a larger sample size.

Overall, the clinical relevance of this study is that MRI-guided injection may enable successful delivery of regenerative therapeutics to the collateral ligament of the distal interphalangeal joint with at least 80% accuracy at each level. Prior to employing either injection technique in clinical cases, practicing the technique on cadaver limbs is recommended. Since obtaining the results of this study, MRI sequence timing has been developed allowing for real-time visualization as the needle is being placed in the ligament. The authors suggest altering the MRI sequences in this manner to further refine MRI-guided injection accuracy.

The ferromagnetic nature of standard needles severely limits their utility for injection with MRI guidance as they create large signal voids (Fig. 2), so non-ferromagnetic needles (MRI guidance) and ferromagnetic needles (radiographic guidance) of similar size, bevel type and length were used for each injection technique. It is possible that 18-gauge spinal needles, as used in the previous report of the technique, would yield more accurate results for radiographic guidance; however, the needles utilized in this study were selected to minimize variation between groups and are consistent with the gauge used to treat other ligaments in our clinical cases.

Iatrogenic trauma is expected to be less with smaller gauge needles as long as they are stiff enough to maintain a straight trajectory during insertion. Repositioning the needles proved difficult in equine cadaver limbs, possibly due to the small gauge used in this study. Increasing
number of attempts did not always subjectively lead to increased accuracy due to over-correction, deflection into a previous tract, or bending of the needle. Despite needle placement appearing ideal with radiographic guidance, the marker and dye were frequently located just outside and parallel to the collateral ligament during post-injection examination. This is considered a flaw of radiograph-guided technique, since it relies on the identification of landmarks instead of direct visualization of the ligament. It was subjectively easier to refine needle placement with MRI guidance, and this is supported by the higher accuracy of this technique.

The two post-injection evaluation methods (MRI with guide wire and gross sections with dye) were chosen to provide the most comprehensive evaluation of injection accuracy. Since sectioning artifacts or smearing of dye from within the joint can complicate gross examination, it was decided to perform cross sectional imaging prior to processing specimens with the band saw to guarantee data collection. T1-weighted MRI sequences have been used in human mammography in order to document location of marker wire position after it is deployed into the tissue, as performed in this study. Marker wires and collateral ligaments were readily identified on transverse T1-weighted, three-dimensional, gradient echo (T1W GE) sequences of all specimens (Fig. 5). The hooked end of the marker wire limits post-injection migration in the tissue, but the depth of the wire was not confirmed to be the same as the full depth of needle penetration. It is possible, therefore, that false negatives occurred in the distal portion of the ligament during MRI analysis. Dye was readily identified at all sectioned levels in all specimens, but additional sectioning was required in several cases to compensate for sectioning inaccuracy. Since analysis was performed identically for all limbs regardless of injection technique, any effect of additional sectioning should be even between groups. It is interesting to note that the volume of injected fluid (one mL) was not adequately recognized in cadaver specimens using short tau inversion recovery, fast spin-echo (STIR FSE) sequences, possibly due to the high water content of thawed specimens, post-mortem changes, lack of compliance of the normal collateral ligaments, or diffusion of fluid into the distal interphalangeal joint. It is the authors’ impression based on clinical cases that evaluation of injected fluid on short tau inversion recovery, fast spin-echo (STIR FSE) sequences is useful in the live animal.

When the injections were evaluated individually at each of three regions in the ligament (proximal, middle, and distal), the statistical advantage of MRI guidance over radiographic
control was not seen at the distal portion of the ligament. This was not due to increased inaccuracy of MRI-guided injection, but was due to improved accuracy of radiograph-guided technique. Evaluation based on post-injection MRI showed MRI guidance to be superior at the middle portion of the ligament, while MRI guidance was found to be superior at the proximal portion of the collateral ligament based on gross examination. Radiographic guidance was not accurate at the proximal portion of the ligament since needle insertion was started palmar to the ligament origin. This was dictated by the palmaroproximal-dorsodistal angle of needle insertion as described by the previous report of the technique. The clinical significance of differing accuracy at one level compared to another has not been determined. Some clinicians suggest that injection should target the insertion, where lesions are more common, and avoid penetration of the remainder of the ligament to avoid iatrogenic trauma. Conversely, it is the authors’ opinion that injection throughout the length of the ligament provides more area for therapeutic effect, thus the ideal injection technique would follow the collateral ligament from origin to insertion. Injection through the entire length of the ligament may be more easily performed in clinical cases affected by desmopathy, especially if the cross sectional area of the ligament is enlarged.

Injection of the proximal portion of the ligament could theoretically be performed with ultrasonographic guidance; however, the large portion of the collateral ligament located deep to the hoof capsule would not be visible with this technique. Only one peer-reviewed study includes a clinical case treated with intralesional injection in the collateral ligament of the distal interphalangeal joint proximal to the coronary band using ultrasonographic guidance. That report recognizes that lesions in this location are uncommon, precluding widespread use of ultrasonographic guidance of intralesional therapy. Ultrasonographic guidance was not evaluated in this study because the authors routinely perform MRI-guided collateral ligament injection and intended to compare accuracy of this technique to the only other published technique, namely radiographic guidance. Potentially, ultrasonography could be used to increase the accuracy of radiograph-guided injection by locating the proximal portion of the CL. Future study may be warranted to evaluate a combination of ultrasonographic and radiographic guidance, as well as real-time MRI guidance.

It is important to note that both radiograph-guided and MRI-guided injection techniques commonly led to penetration of the distal interphalangeal joint. The collateral ligament is thin and embedded in the joint capsule, so accurate injections within the ligament may lead to
penetration of the joint. Administration of therapeutic medications into the joint may be of clinical benefit, so joint penetration is not considered a failure of the injection techniques in this study. It should, however, be emphasized that injections in vivo must be performed with strict adherence to aseptic technique and that potentially irritating substances, such as sclerosing agents, should be avoided.

In conclusion, MRI guidance can be used to accurately inject the collateral ligament of the distal interphalangeal joint and the frequency of successful injection is significantly higher than with radiographic control (80.0-86.7% v. 30.0-43.3%). The recent development of timing sequences that provide real-time MRI guidance of injection (Hallmarq Veterinary Imaging, Guildford, Surrey, UK) make this technique increasingly practical in the standing, sedated patient. Future investigations should evaluate outcome after injection in clinical cases to determine if intralesional therapy provides a benefit for desmopathy of the collateral ligament of the distal interphalangeal joint.

Acknowledgements

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### Tables

Table 1: Post-Injection MRI Analysis for MRI-Guided and Radiograph-Guided Injection within the Collateral Ligament of the Distal Interphalangeal Joint

<table>
<thead>
<tr>
<th>CL Evaluation</th>
<th>Injection Guidance</th>
<th>Successful (%)</th>
<th>Unsuccessful (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (n=30)</td>
<td>MRI</td>
<td>24 (80.0)</td>
<td>6 (20.0)</td>
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<td></td>
<td>Radiograph</td>
<td>9 (30.0)</td>
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<td>6 (60.0)</td>
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<td>9 (90.0)</td>
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<td></td>
<td>Radiograph</td>
<td>4 (40.0)</td>
<td>6 (60.0)</td>
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*Significant difference using McNemar’s test.

**Key:** CL = collateral ligament of the distal interphalangeal joint, Successful = injection within the body or margin of the collateral ligament; Unsuccessful = injection outside the ligament.
Table 2: Gross Sectioning Analysis for MRI-Guided and Radiograph-Guided Injection within the Collateral Ligament of the Distal Interphalangeal Joint

<table>
<thead>
<tr>
<th>CL Evaluation</th>
<th>Injection Guidance</th>
<th>Successful (%)</th>
<th>Unsuccessful (%)</th>
<th>P Value</th>
</tr>
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<td>Overall (n=30)</td>
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</tr>
</tbody>
</table>

*Significant difference using McNemar’s test (P ≤ 0.05)

**Key:** CL = collateral ligament of the distal interphalangeal joint, Successful = injection within the body or margin of the collateral ligament; Unsuccessful = injection outside the ligament
<table>
<thead>
<tr>
<th>Post-Injection Analysis</th>
<th>Injection Guidance</th>
<th>In the Joint (%)</th>
<th>Not in the Joint (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI</td>
<td>MRI</td>
<td>3 (30.0)</td>
<td>7 (70.0)</td>
<td>0.5637</td>
</tr>
<tr>
<td></td>
<td>Radiograph</td>
<td>2 (20.0)</td>
<td>8 (80.0)</td>
<td></td>
</tr>
<tr>
<td>Gross Section</td>
<td>MRI</td>
<td>5 (50.0)</td>
<td>5 (50.0)</td>
<td>0.5637</td>
</tr>
<tr>
<td></td>
<td>Radiograph</td>
<td>4 (40.0)</td>
<td>6 (60.0)</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 3: Conclusions

Desmopathy of the distal interphalangeal joint collateral ligaments is a potentially career ending condition affecting the equine athlete. There is a paucity of literature regarding effective treatment modalities that maximize healing of this ligament. Regenerative therapy, such as injection with platelet rich plasma or stem cells, has led to improved recovery in horses with damage to other ligaments and tendons, so it is possible that similar results could be expected with treatment of the collateral ligaments of the distal interphalangeal joint. The location of the ligaments within the hoof capsule presents a challenge for delivering local therapy compared to treatment of ligaments that are more readily accessible. Radiographic guidance is the only previously described technique, but the accuracy of this method has not been reported. MRI was presented as an alternative technique for targeting injection of the collateral ligament. Using an equine cadaver model and paired study design, MRI-guided injections were more frequently successful than injections performed with radiograph guidance. MRI-guided injection may provide a means to deliver regenerative treatments within the hoof and to improve long-term outcome for performance horses affected my desmopathy of the collateral ligaments of the distal interphalangeal joints. Future studies should document the feasibility, accuracy, and effectiveness of injection in vivo.