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Effect of stifle flexion angle on the repeatability of real-time patellar ligament elastosonography in medium to large breed dogs

Introduction

Thickening of the patellar ligament is a common radiographic finding following tibial plateau levelling osteotomy¹⁻⁵ and tibial tuberosity advancement^{6,7} in dogs. Ultrasonographic assessment of the patellar ligament in dogs following tibial tuberosity advancement has revealed marked thickening at six weeks post-operatively, with subsequent reduction in ligament thickness observed at sixteen weeks⁶ and six months post-operatively⁷.

Patellar ligament thickening is thought to occur in response to inflammation as a result of cranial cruciate ligament rupture and/or stifle surgery. It is generally considered to be an incidental finding^{4,6,7} although it is clinically challenging to differentiate incidental thickening from desmitis in clinical patients with persistent lameness following osteotomy procedures for cranial cruciate ligament rupture. Typically, patients with patellar desmitis will show signs of pain during palpation of the affected patellar ligament, however this is a subjective assessment which could be influenced by the nature of the dog and other causes of pain following stifle surgery. Post-operative diagnosis of patellar desmitis is challenging, as the ultrasonographic and radiographic appearance of the patellar ligament is impacted by surgical trauma^{6,7}.

Multiple imaging modalities are available for the patellar ligament in humans and dogs including computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound^{8,9}. B-mode ultrasound has been described as a method of diagnosing desmitis, with typical patterns including thickening, fibre disruption and reduced echogenicity which alter depending on the chronicity of the lesion⁸. Ultrasound examination is a non-invasive and

relatively inexpensive imaging modality which can provide several advantages over MRI for imaging the patellar ligament in dogs. These include the ability to assess the ligament in a dynamic manner^{7,8}, comparing the changes observed with the associated joint held in several positions and also permits evaluation of multiple regions of the patellar ligament individually⁶. Ultrasound examination of the patellar ligament is operator dependent and requires good knowledge of ultrasonographic anatomy.

A major limitation of B-mode ultrasound is that it does not allow elasticity of tissues to be evaluated¹⁰. B-mode ultrasound has been shown to be a poor indicator of patellar ligament pathology in humans¹⁰, with acute tendinopathies often demonstrating a normal fibre pattern¹¹, prior to tendon thickening and fibre disruption occurring¹⁰. Elastasonography is an ultrasound-based imaging technique which provides information about the elasticity of tissue by assigning different chromatic patterns, according to different tissue elasticity responses¹². On real-time elastasonography an elastosonogram is collected providing qualitative information about the densities of the tissues. Post processing techniques enable a semi-quantitative evaluation of the elastosonographic images, to determine a ratio of softness or hardness depending on the characteristics of the lesion¹³. Elastasonography has been reported in human sports medicine for imaging of the patellar ligament¹⁴⁻¹⁷ and Achilles tendon^{11,18,14}, however the 'normal' and 'pathological' findings are variable and poorly defined¹⁷.

Pathological conditions will result in a change in the tissue elasticity¹⁹ where pathological changes such as patellar desmitis are typically represented by increased stiffness scores²⁰.

Elastasonography has recently become available for use in small animal veterinary patients, with applications described for imaging of the liver, spleen, kidneys and prostate²¹ and for differentiating benign and malignant neoplasia within lymph nodes²² and subcutaneous mass lesions¹³. Application of elastasonography for the assessment of the patellar ligament in healthy dogs has been described, with the technique considered to be feasible and

reproducible²³. In this study the elastosonography was performed with the stifle joint in full passive flexion. Reports in the human literature describe this technique being performed with the stifle in full flexion, extension¹⁰ and in 20-30 degrees of flexion¹⁴. The emphasis on positioning of the stifle during elastosonographic evaluation suggests that positioning is a key aspect for obtaining meaningful results.

The aims of this study were:

- 1) To describe the elastosonographic findings of the patellar ligament in healthy, medium to large breed dogs in four different positions (lateral recumbency with the stifle in full passive flexion, full passive extension, 135 degrees extension, and with the patient standing with the stifle at a natural standing angle),
- 2) To determine the most appropriate stifle angle to perform elastosonography of the patellar ligament,
- 3) To correlate the B-mode ultrasonographic appearance of the patellar ligament with the histological appearance of a ligament

Materials and Methods

The study protocol was approved by the by the Veterinary Ethical Review Committee at the Royal (Dick) School of Veterinary Studies, University of Edinburgh (VERC approval 1.19). Eighteen University of Edinburgh veterinary staff and student-owned dogs deemed clinically healthy on the basis of history, clinical and orthopaedic examination findings performed by (JM), weighing at least 15kg, were prospectively recruited. Owners provided informed written consent for their dog to be enrolled in the study.

The standing angle of each dog's left stifle joint was measured using a goniometer. The left stifle was arbitrarily selected for comparison of the stifle flexion angle between dogs. The

hair directly overlying both patellar ligaments was clipped and ultrasound gel applied. All examinations were performed by the same operator (ML) without sedation. B-mode ultrasound and real-time elastosonography of both patellar ligaments was performed using an 8 to 18MHz linear electronic array ultrasound transducer (LA435 - MyLab Twice, Esaote, Genova, Italy) with the dog standing or in lateral recumbency depending on the position applied. Each patellar ligament was evaluated with B-mode ultrasound in the sagittal plane to assess the organisation of the fibres and to rule out any pathological changes within the ligament. The thickness of each patellar ligament was also measured at the midpoint between the patella and the tibial tuberosity to allow any significant change in ligament thickness between the first and second scans, which could potentially affect the elastosonography readings, to be identified. The patellar ligament was considered normal when the ligament fibres were homogenous and parallel.

Rhythmic, compressive and retractile movements of the probe were performed with the same machine and probe, using post-processing software (ElaXto, Elastosonography, Esaote) over the patellar ligament in the sagittal plane as reported previously²³. The examinations were performed with the stifle in four positions which included: the dog in a 'normal' standing position; lateral recumbency with the limb of interest uppermost and the stifle positioned at 135 degrees (standing angle), in full passive flexion and finally in full extension (Figure one). During the standing angle position scan, a goniometer was used to ensure that the stifle was positioned in 135 degrees of flexion. Dogs were excluded from the study if they did not tolerate the examination or if there was evidence of ligament fibre disruption or scar tissue.

Appropriate elastosonography image quality was confirmed by the absence of artefacts, when the elastogram and B-mode ultrasound images matched and a green spring was produced, representing a real-time feedback of accurate image acquisition as previously reported^{23,24}.

The elastosonography images were evaluated post-processing and the borders of the patellar ligament were manually drawn in the B-mode image, whilst being simultaneously copied onto the elastosonography image (Figure two), thus making the visible portion of the patellar ligament the region of interest. The percentage of hard and soft areas in the region of interest was calculated by the commercial software. The percentage of hardness for each patellar ligament was recorded by the operator (ML), for purposes of data analysis, with the combined percentage of hardness and softness for each patellar ligament was 100 (ie. Percentage softness = 100 – percentage hardness, and vice versa). The images were reviewed by the supervising board-certified veterinary radiologist (TL) for adequacy.

The B-mode ultrasound and elastosonography assessments were repeated two weeks later (second scan) which was a nominally selected period of time to assess the accuracy and repeatability of the measurement. The generated images were assessed using the same protocol.

Two patellar ligaments were harvested from a five-year old, female (intact) American bulldog which was euthanised for reasons unrelated to the study (the body was donated for research purposes and its use was approved by the Veterinary Ethical Review Committee (VERC approval 1.19)). The ligaments were fixed in 10% neutral buffered formalin prior to histological evaluation. Longitudinal and transverse sections were processed and embedded in paraffin-wax. 5µm sections were stained with haematoxylin and eosin and Masson's trichrome.

Statistical analysis

Data were checked for normality, and presented as means (+/- standard deviations) or medians (and ranges) as appropriate. Statistical analysis was performed using commercially available statistical software (Minitab 18, Minitab LTD, Coventry, UK). For the purposes of

data analysis, the percentage hardness of the patellar ligament was selected as the authors anticipated that any chronic or fibrotic change within the ligament would increase the value²⁰ and was therefore considered more intuitive. Analyses of variance (ANOVA) were performed on: the percentage hardness elastosonography fitting the effects of stifle position, limb (left or right side), scan (first or second) and dog as factors; and the thickness of the patellar ligament on B-mode fitting the effects of ultrasound limb (left or right side), scan (first or second) and dog as factors. The normality of the data was checked using residuals plots. Post-hoc Tukey t-tests were used to compare the elastosonography readings for each pair of four stifle positions. Additionally, in order to compare the variability of measurements from different stifle positions, an ANOVA was performed separately for each stifle position, fitting the effects of the dog, limb (side) and scan (first or second).

Results

Eighteen dogs were enrolled in the study. Dog breeds included German Shepherd (n=4), Border collie (n=4), Labrador retriever (n=2), Springer spaniel (n=2), cross breed (n=3) and one each of: Boxer; Cocker spaniel and; Griffon. There were 10 female (2 entire, 8 neutered) and 8 male (1 entire, 7 neutered) dogs. The mean dog weight was 23.82kg (± 6.60), and the mean dog age was 6.28 years (± 3.55). The mean measured standing stifle angles were 131.1° (± 7.28). No dogs were excluded from the study.

On B-mode ultrasound, all dogs had homogenous patellar ligaments with parallel ligament fibres and no evidence of fibre pattern disruption, fluid accumulation or core lesions. The ultrasonographic appearance of all patellar ligaments was trilaminar (Figure three) whereby the superficial outlines of the ligaments were thin, well demarcated and hyperechoic, with the central portion being homogenous with parallel ligament fibres heterogeneously hypoechoic.

The patellar ligament thickness for scan one (2.76mm, ± 0.08) was not significantly different to those of scan two (2.89mm, ± 0.03) ($P=0.121$).

The elastosonography readings were statistically significantly lower when the examination was performed with the dog standing ($18.81 \pm 10.01\%$), compared to examination with the dog in lateral recumbency and at 135° ($27.69 \pm 13.63\%$), full extension ($34.64 \pm 14.66\%$) or full flexion ($27.52 \pm 11.90\%$) ($P < 0.005$) (Figure four). The limb used (right or left) and scan (first or second) had no significant effect on the elastosonography reading.

The variability (residual mean square value) in the elastosonography readings on the same dog and side, was lowest when the dog was standing: 89.32%. By comparison the variability in scan readings on the same dog and side for: stifle angles of 135° , full passive extension and flexion were 179.79%, 199.4% and 113.83% respectively. The influence of limb used, or scan number had no significant effect on the elastosonography reading in any stifle position ($P > 0.05$).

Histology of the patellar ligament in the transverse and longitudinal plane stained with Masson's trichrome showed dense collagen bundles of the patellar ligament surrounded by loose fibro-fatty connective tissue, representing the periligament (Figure five). This correlated with the findings of B-mode ultrasound where the patellar ligament had a trilaminar appearance, representing the periligament on either side of the patellar ligament.

Discussion

The results of this study show that the elasticity of the normal canine patellar ligament can be evaluated using elastosonography with the stifle in four different positions, however the measured stiffness varied with position. Normal canine patellar ligaments produce a highly soft elastogram²³ which is represented by the low percentage hardness values (percentage hardness = $100 - \text{percentage softness}$, and vice versa) obtained in this study. The

lowest readings of percentage hardness, indicating the position where the patellar ligament was most soft were obtained with the patient standing. This position also had the least variability between readings of all the stifle positions. These findings suggest that the standing position is the most appropriate and reliable position to use when performing elastosonography of the canine patellar ligament. This may be due to the stifle joint and patellar ligament being in their most normal physiological state, both in terms of loading and angle, in this position and therefore providing the most accurate representation of the elastic properties of the patellar ligament.

Elastosonography has been described in humans for evaluation of the elasticity of normal and abnormal patellar ligaments^{10,14,15,17,20,25}. These evaluations were performed with the stifle in 20-30 degrees of flexion^{10,14}, 30 degrees of flexion¹⁵ and 30 and 90 degrees of flexion plus full passive extension²⁵. One veterinary study reports elastosonography to be a feasible and repeatable technique for evaluating the elasticity of patellar ligaments in normal dogs measured with the stifles positioned in full passive flexion²³. The stifle position was based on the findings of a human study¹⁴. Following evaluation of the elastosonographic properties of the patellar ligament in humans, using multiple stifle positions, Berko²⁵ demonstrated that the elasticity of the patellar ligament varied with position and therefore recommended that elastosonographic evaluations of this structure be performed with the stifle in a standardised position. The present study demonstrates that the elastosonographic findings of the canine patellar ligament vary according to stifle position. The softness of the patellar ligament was greatest with the dog standing and this position also provided the lowest variability in readings between limb used, dog and repeatability. This finding is likely beneficial for evaluating dogs with pathology of the patellar ligament as, in a human study significant flexion of the stifle joint often resulted in marked pain in patients with patellar ligament

pathology¹⁰, and therefore, performing elastosonography in a standing position is likely to be better tolerated than with the stifle in full passive flexion.

Piccionello and colleagues²³ recommended performing patellar ligament elastosonography with the stifle in full passive flexion, however did not evaluate the elastosonography readings for any other stifle position and therefore did not establish the optimal position for performing patellar ligament elastosonography. The flexed stifle position used by Piccionello and colleagues²³ was extrapolated from a human study¹⁴. However, given the different standing stifle angle and the postural differences between humans and dogs, the authors do not believe that the most appropriate position for performing elastosonography of the canine patellar ligament should be based on human studies. Moreover, there is particular emphasis on the importance of stifle angle when performing patellar ligament elastosonography in human patients^{10,14,25} which the present study has demonstrated to also be the case in dogs. Our results show particularly that full passive flexion in lateral recumbency resulted in wide variation between results for elastosonography of the patellar ligament when compared with other stifle positions. The standing stifle angle varied between dogs (as measured using goniometry) and therefore performing patellar ligament elastosonography with the dog standing should provide a more physiological representation of the patellar ligament properties. As the variability in the results was less for this position compared with lateral recumbency with the stifle angle of 135°, the dynamic loading of the ligament may be the most important influence on the degree of variability in this position.

B-mode ultrasound findings of the patellar ligament were consistent with previous reports where the patellar ligament had a trilaminar appearance^{26,27}. The correlation between the ultrasound and histopathology findings in present study has confirmed the cranial and caudal hyperechoic borders of the patellar ligament to be loose fibrofatty connective tissue consistent with the periligament⁴.

Measuring patellar ligament thickness on B-mode ultrasound was a repeatable technique, with no significant difference identified between measurements of the first and second ultrasound scans. Ultrasonographic evaluation of the patellar ligament has been shown to be superior to radiography for investigation of patellar ligament thickening in dogs following tibial plateau levelling osteotomy⁴ and tibial tuberosity transposition^{6,7}. In humans, ultrasound and MRI are the preferred imaging modalities for the patellar ligament⁸, however ultrasound evaluation provides the key benefit of facilitating dynamic studies to be performed, with the stifle in different positions and with different stresses applied to it⁸. The thickness of human patellar ligaments on ultrasound evaluation has been shown to be influenced by body mass index and the strength of the quadriceps musculature¹⁶. Atrophy of the quadriceps musculature is a common finding following cranial cruciate ligament rupture²⁸⁻³⁰ and in the initial post-operative period following tibial plateau levelling osteotomy for management of cranial cruciate ligament rupture³¹. This concurs with the finding in humans that quadriceps muscle strength influences the patellar ligament thickness¹⁶; the measured thickness of the patellar ligament with B-mode ultrasound may reduce in response to quadriceps muscle atrophy, so having a pre-operative reading of patellar ligament thickness for comparison may not provide particularly valuable information on the physiological status of the patellar ligament in response to tibial plateau levelling osteotomy alone.

A limitation of this study is that it only included dogs >15kg. Whilst this was to ensure that the ultrasound probe could be placed longitudinally along the patellar ligament, it remains unclear whether elastosonography is a technically feasible method for evaluating the patellar ligament in dogs <15kg. Additionally, the ultrasound scans were repeated a nominal period of two weeks between the studies. The range of maximum passive extension and flexion, and the standing stifle angle will differ between dogs and, as already indicated, the standing stifle

angle varied between dogs. The individual differences between the range of motion of the stifle joints is likely to influence the variation between the elastosonography readings.

In conclusion, elastosonography is a feasible technique for evaluating the elasticity of the normal canine patellar ligament in dogs >15kg and would be a useful technique for investigating the mechanical changes within the patellar ligament following stifle surgery. Elastosonography of the patellar ligament should be performed with the dog standing to provide the most accurate elastograms with lowest variability between readings.

Figure legends:

Figure 1

Figure demonstrating each of the stifle positions with a dog in lateral recumbency with the limb of interest uppermost. The stifle is shown in full passive flexion (A), full passive extension (B), in 135° of flexion (C) and in with the dog standing (D). The ultrasound probe is placed longitudinally along the patellar ligament.

Figure 2

A: Longitudinal B-mode ultrasound image of the patellar ligament (*) with well organised and homogeneous fibres

B: corresponding elastosonogram image of the patella ligament predominantly red (head arrow). The green spring (arrow) confirms accuracy of the elastosonogram. The green spring icon in the bottom right of the image confirms accuracy of the elastosonogram.

Figure 3

Longitudinal B-mode ultrasound image of the patellar ligament (represented by the star symbol). The cranial and caudal borders of the patellar ligament are demarcated by + symbols. The patellar ligament is homogenous and hypo-echoic with hyper-echoic cranial and caudal borders representing the periligament, creating a trilaminar appearance. The scale numbers indicate centimetre measurements.

Figure 4

Box and whisker plots demonstrating the median percentage stiffness (and interquartile range) of the patellar ligament in each of the four stifle positions. The whiskers represent the variability. The percentage hardness and variability are the lowest when the examination is performed with the dog standing.

Figure 5

A: Transverse section of the mid-portion of the patellar ligament stained with Masson's Trichrome stain viewed at 2x magnification. The scale bar represents 1000µm. Cranial is orientated at the top of the image. The image shows dense collagen bundles of the patellar ligament (2) with surrounding loose fibrofatty connective tissue of the periligament (1 and 3).

B: Longitudinal B-mode ultrasound image showing the patellar ligament (cranial and caudal extremities indicated by the +) and the periligament (arrow heads) which corresponds with the histopathology findings.

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