Effects of transection of the cranial arm of the medial glenohumeral ligament on shoulder stability in adult Beagles

Y. Fujita1; S. Yamaguchi2; K. A. Agnello3; M. Muto1

1Laboratory of Surgery II, School of Veterinary Medicine, Azabu University, Chuo-ku, Sagamihara-shi, Kanagawa, Japan; 2Tokyo Animal Orthopaedic Surgery Hospital, Yamaguchi Pet Clinic, Koto-ku, Tokyo, Japan; 3Department of Clinical Studies - Philadelphia, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Keywords
Shoulder instability, arthroscopy

Summary
Objective: To assess the effects of arthroscopic transection of the cranial arm of the medial glenohumeral ligament on shoulder stability.

Animals: Six adult Beagles.

Procedures: After transection, the effects were compared with baseline values by orthopaedic and radiographic examinations, by synovial fluid analysis at two, four, and six weeks, and by arthroscopic evaluation at six weeks. The articular surfaces of the glenoid cavity and humeral head were evaluated radiographically and arthroscopically for evidence of arthritis, and five intra-articular regions were examined arthroscopically for villus reactions and vascularisation.

Results: According to orthopaedic examinations (including measurement of the abduction angle), radiography, and synovial fluid analyses, there were no abnormal findings. Arthroscopically, the articular surfaces of the glenoid cavity and humeral head showed no signs of degeneration, but the cranio medial and caudal joint capsules had significant villus reactions and the subscapularis tendon and medial glenohumeral ligament had significant vascularisation. The biceps tendon was unchanged.

Conclusions: Transection of the cranial arm of the medial glenohumeral ligament in normal Beagles did not appear to affect shoulder stability. However, villus reactions and vascularisation in the medial compartment suggest that damage to the medial glenohumeral ligament may trigger a process in which inflammation can lead to enzymatic breakdown of cartilage. Exacerbated by weight bearing and repetitive motion, this may result in shoulder instability over time.

Introduction
Shoulder instability, defined as an abnormal translation of the humeral head within the glenoid fossa – or the inability of the humeral head to stay centred in the glenoid cavity – is one cause of forelimb lameness in dogs (1, 2). Although medial shoulder instability is the most common form of shoulder instability to be reported, its pathophysiology is unclear (1).

Because the glenoid cavity is relatively small and shallow, shoulder stability depends on a complex interaction between the articular surfaces and the surrounding ligaments, tendons, muscles, and joint capsules (1). Disruption in just one of these soft tissue structures can cause discomfort and dysfunction (2). For example, total luxation has been reported to occur after transection of the medial glenohumeral ligament, a passive stabiliser (3). Also, a retrospective clinical study found distention, tearing, or thickening of the medial glenohumeral ligament in 31 (66%) of 47 unstable shoulders (1).

Physical and orthopaedic examinations, including manipulation of the glenohumeral joint and measurement of the abduction angle, are considered to be essential for the diagnosis of medial shoulder instability (4). Bardet also recommended using the cranio-caudal and mediolateral drawer tests, but they are difficult to perform consistently in all dogs (1, 5).

Although described in the veterinary literature, instability is not easily diagnosed. Often clinical signs of excessive range-of-motion and laxity are inconclusive and have many possible explanations. Moreover, conventional diagnostic imaging cannot substantiate clinical findings (1, 6-9). For example, ultrasonography is able to display the cranial, lateral, and caudal portions of the shoulder, but not the medial component, and while radiographic evaluation can detect secondary osteoarthritic changes, it has minimal benefit in diagnosing soft tissue pathology (10).

The use of arthroscopy and magnetic resonance imaging offer important advances in objectively demonstrating and diagnosing soft tissue abnormalities (11-15). Our study focused on arthroscopy because it has been shown that dogs can have moderate to severe degenerative changes in the shoulder joint even when...
clinical signs are mild and synovial fluid and radiological changes are mild or absent (5).

Arthroscopy can also help clarify the role of the glenohumeral abduction angle in evaluating medial shoulder instability. One study found that the mean abduction angle was 53.7 ± 4.7° in unstable shoulders and 32.6 ± 2° in unaffected shoulders. The arthroscopic evidence suggests that the abduction angle is increased when there are pathological changes in multiple structures, such as partial tears or separation of fibres in the joint capsules, tendons or ligaments; synovial hypertrophy or hyperplasia; and fibrillation of the medial glenoid rim (4, 11, 16).

To our knowledge, there are no clinical or experimental data on abduction measurements in dogs with pathology in just one structure of the shoulder joint. In addition, none of the studies cited above examined the presence of underlying disease or an initial event that may have precipitated medial shoulder instability.

The medial glenohumeral ligament is shaped like a Y (Figure 1), and while its role in shoulder stability has been described, the respective contributions of its cranial and caudal arms have not been fully studied. We hypothesised that a lesion in the medial glenohumeral ligament is one cause of medial shoulder instability in dogs, and that instability can be detected by measurement of the abduction angle, and arthroscopy, but not by orthopaedic examination, plain shoulder radiography, or synovial fluid analysis. To test this hypothesis, we transected the cranial arm of the medial glenohumeral ligament arthroscopically and observed the effects over a six-week period using these diagnostic modalities.

**Materials and methods**

**Inclusion criteria**

Six adult Beagles (females; mean age, 51.3 months, range: 50 – 52 months; mean body weight, 8.1 kg, range: 7.3 – 8.7 kg) with no history of orthopaedic disease were enrolled. All of the dogs were judged to be healthy based on the results of a routine physical examination, complete blood count and serum biochemistry analyses, and orthopaedic, neurologic, and radiographic examinations of both shoulders.

**Animal protection**

This study was conducted at the School of Veterinary Medicine, Azabu University (Sagamihara, Japan), in compliance with the university’s Animal Experiment Guidelines 2007 (based on the Guidelines for Animal Experimentation, 1987, of the Japanese Association for Laboratory Animal Science). It was approved by the Azabu University Animal Experiment Committee (protocol #090605-1). The dogs belonged to the University.

**Arthroscopy**

All procedures were performed by one investigator (YF). Each dog was positioned in lateral recumbency with the left shoulder uppermost. A 2.3 mm 30º oblique scope with a digital camera to capture still images was introduced via a caudolateral portal approximately 1 cm caudal and slightly distal to the acromion of the scapula (6). A cranialateral portal directly distal to the acromion of the scapula was established for the insertion of a small joint hook tip probe and arthroscopic scissors (6).

Before transection and at six weeks postoperatively, the surfaces of the glenoid cavity and the humeral head of each left shoulder were assessed for cartilage damage based on the modified Outerbridge grading system; the cranio-medi- al and caudal joint capsules were examined macroscopically for villus reactions (synovitis); and the biceps tendon, subscapularis tendon, and medial glenohumeral ligament were evaluated for vascularisation (17).

Villus reactions in the joint capsules were graded on a four-point scale of 0 to 3, largely based on the following: normal, mild (congested synovium), moderate (synovial villi in a broad area), or severe (tall synovial villi across the joint, with some bleeding) (18). Vascularisation of the tendons and the medial glenohumeral ligament was subjectively graded as 0 (none), 1 = mild (at the periphery of the structure), 2 = moderate (up to 50% of the surface), or 3 = severe (more than 50% of the surface).

For these procedures, the dogs were premedicated with atropine sulfate (0.025 mg/kg, SC), carprofen (4.4 mg/kg, SC), and butorphanol tartrate (0.1 mg/kg, IV). Anaesthesia was induced with thiopental sodium (25 mg/kg, IV, to effect) and maintained with isoflurane via an endotracheal tube. The joint was extended and irrigated with lactated Ringer’s solution.

Immediately after the preoperative examination, the intact structure of the medial glenohumeral ligament was confirmed by probing, and the cranial arm of the medial glenohumeral ligament was then transected.

**Orthopaedic examination**

Before transection and at two, four, and six weeks postoperatively, the dogs underwent an orthopaedic examination, with a subjective lameness evaluation and assessment of signs of pain on palpation, swelling, and range-of-motion of the target joint by go-
Table 1  The mean ± standard deviation (º) for the extension, flexion and abduction angles of the left shoulder joint following arthroscopically assisted transection of the cranial arm of the medial glenohumeral ligament. For all angles, there were no significant differences across time points compared with baseline by analysis of variance.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (Mean ± SD)*</th>
<th>Postoperative (weeks) (Mean ± SD)*</th>
<th>p-value (ANOVA)</th>
</tr>
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<tbody>
<tr>
<td>Extension (º)</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>158.8 ± 1.6 (157.1 – 160.4)</td>
<td>157.3 ± 2.0 (155.2 – 159.4)</td>
<td>0.26</td>
</tr>
<tr>
<td>4</td>
<td>157.2 ± 1.5 (156.1 – 158.2)</td>
<td>157.3 ± 1.6 (155.6 – 158.9)</td>
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<tr>
<td>6</td>
<td>157.2 ± 1.5 (156.1 – 158.2)</td>
<td></td>
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<tr>
<td>Flexion (º)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2</td>
<td>41.1 ± 1.5 (39.6 – 42.7)</td>
<td>41.3 ± 2.7 (38.5 – 44.1)</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>43.3 ± 1.6 (41.6 – 45.0)</td>
<td>43.0 ± 0.9 (42.1 – 43.9)</td>
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<tr>
<td>6</td>
<td>43.3 ± 1.6 (41.6 – 45.0)</td>
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<td></td>
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<tr>
<td>Abduction (º)</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>30.3 ± 2.3 (27.9 – 32.7)</td>
<td>31.1 ± 0.3 (30.7 – 31.4)</td>
<td>0.62</td>
</tr>
<tr>
<td>4</td>
<td>31.3 ± 0.9 (30.3 – 32.3)</td>
<td>30.9 ± 1.0 (29.8 – 31.9)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>31.3 ± 0.9 (30.3 – 32.3)</td>
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</tbody>
</table>

*All Mean ± SD values are shown with a 95% confidence interval. ANOVA = analysis of variance

Statistical analysis

Results were analysed using statistical software<sup>d</sup>. Mean ± standard deviation was determined, and data were tested for normality using the Kolmogorov-Smirnov test. For the orthopaedic and radiographic examinations and the synovial fluid analyses, an analysis of variance (ANOVA) was used to compare the results with baseline and across all follow-up time points (two, four, and six weeks). A Wilcoxon signed-rank test was used to compare arthroscopic findings immediately before and at six weeks after transection. The level of significance was set at α = 0.05.

**Synovial fluid analysis**

Arthrocentesis of each left shoulder joint was performed with a 3.81 cm 22 gauge needle and syringe immediately before and at six weeks after the procedure under general anaesthesia, and at two and four weeks under sedation. Each analysis involved a visual examination (for colour, turbidity, viscosity and volume) and measurement of the total protein content with a refractometer, the total nucleated cell count with a haemocytometer, and the volume. The colour, turbidity, and viscosity of the fluid were assessed as normal or abnormal. Smears of fluid were prepared with Wright-Giemsa stain. Total protein content and total nucleated cell count were graded as normal (<4.8 g/dL and <3000 cells/µL, respectively) or abnormal, and the percentage of neutrophils, large mononuclear cells (macrophages), and small mononuclear cells (lymphocytes) was determined (20).

Radiography

To assess degenerative changes, two orthogonal radiographic views (caudocranial and mediolateral views) of each target joint were obtained under sedation, as described above, at two and four weeks postoperatively and under general anaesthesia immediately before and six weeks after the procedure. On a scale of 0 to 3, the findings were macroscopically graded as normal, mild, moderate, and severe for irregularity of the joint surface, presence and severity of osteophytosis as well as sclerosis and remodelling of the subchondral bone.

Postoperative care

Following arthroscopy, carprofen (2.2 mg/kg, PO, BID) for seven days was administered to each dog and the dogs were monitored for evidence of discomfort, with additional pain medication administered immediately after the procedure, if needed. Throughout the study, they were exercised outside on a leash twice daily for 15 minutes.

After completion of this study, the dogs were enrolled in an unrelated anatomical study for which they were euthanized with sodium pentobarbital (100 mg/kg, IV). The anatomical study was conducted at Azabu University according to the guidelines specified above.

**Results**

All the dogs recovered from the procedure without any complications and the administration of additional analgesic medication was not required. Healing was uneventful, and the dogs exercised willingly throughout the study.

The orthopaedic examinations at two, four, and six weeks were unremarkable, with no significant changes in the flexion, extension, and abduction angles between measurement points by ANOVA (Table 1). In addition, there was no radiographic evidence of osteoarthritis.

There were no changes in synovial fluid. Colour, turbidity, and viscosity appeared normal in all of the collected samples. Mean baseline values were 0.13 mL for volume, 2.2 g/dL for total protein, and 252.2 mg/dL for total nucleated cell count, and none of these components changed significantly from baseline or between time points by ANOVA (p = 0.10, 0.60, and 0.35, respectively). Moreover, mean values for total protein and total nucleated cell count remained well below the upper limit of normal (20). Mean percentages for neutrophils, large mononuclear cells, and small mononuclear cells were 2%, 26%, and 73%, respectively, at baseline, and likewise, there were no significant changes between time points or for each time point compared with baseline by ANOVA (p = 0.63, 0.99, and 0.99, respectively).

<sup>d</sup> PASW Statistics 18.0: IBM, Tokyo, Japan
Six weeks after transection, arthroscopy revealed that the cranial arm of the medial glenohumeral ligament remained separated, as confirmed by probing. The articular surfaces of the glenoid cavity and the humeral head showed no changes compared with baseline (grade of '0' for all observations), but there was significant evidence of inflammation, as indicated by villus reactions in the craniomedial and caudal regions of the joint capsules and by vascularisation in the subscapularis tendon and medial glenohumeral ligament in comparison to baseline (Table 2). There was no change in the biceps tendon. The craniomedical region of the joint capsule showed signs of mild (1 dog) to moderate (5 dogs) inflammation and the subscapularis tendon also showed signs of mild (2 dogs) to moderate (4 dogs) inflammation, with scores ranging from 1 to 2 for each (Figure 2). For the medial glenohumeral ligament and the caudal region of the joint capsule, there was mild inflammation in all the dogs. Macroscopically, villus reactions were more obvious in the craniomedical than in the caudal regions of the joint capsule, and vascularisation was more apparent in the subscapularis tendon than in the medial glenohumeral ligament. No signs of severe inflammation were observed in any of the structures evaluated.

### Discussion

Medial shoulder instability in dogs is a relatively common condition that can lead to forelimb lameness. Although the exact cause is unknown, dogs diagnosed with shoulder instability often have injury to multiple periarticular tissues (1, 4, 11, 15, 21, 22). Pathology of the subscapularis tendon, an active stabiliser, has been detected in 57% - 86% of cases, and damage to the medial glenohumeral ligament has been reported in 66% of unstable shoulders (1, 11, 16).

During the six-week period of our study, there were not any signs of instability or forelimb lameness detectable by physical or orthopaedic examination, possibly because the active stabilisers, such as the subscapularis tendon, which play important roles in shoulder stability, were still intact and able to compensate for damage to the medial glenohumeral ligament (2). However, since clinical examinations are largely subjective, we may have failed to detect subtle signs of instability, such as slight lameness. Manipulation can be similarly unreliable in determining subtle joint laxity (23). Therefore, although the abduction angle in our study (Table 1) fell within the normal range, there may have been subtle laxity that escaped our notice. Concerning the abduction angle, it is also important to note that the ‘normal’ range of 32.6° ± 2° is based on observations in large breed dogs (body weight more than approximately 20 kg), and so may differ by breed and size (16). Moreover, arthroscopic evidence suggests that the abduction angle is increased in the presence of multiple periarticular injuries, but not when just one structure is affected, as in our study (4, 11).

Synovial fluid analysis is also essential to the clinical evaluation of primary joint diseases and systemic diseases affecting the joints (24). Our analyses did not detect any significant differences compared with baseline for any component of the fluid at any of the time points. This ruled out inflammatory arthritis, but not localised inflammation or degenerative arthritis.

While there is no characteristic radiographic marker of shoulder instability, we...
looked for degenerative changes secondary to joint injury. No signs of subchondral sclerosis, irregularity of joint surfaces, or osteophytes were detected, which could indicate that instability, if present, was insufficient to produce changes easily recognised by radiology (4). It is also possible that radiology may be unable to detect degenerative changes visible on arthroscopic examination (5).

Six weeks after transection, arthroscopic examination of the medial compartment revealed significant villus reactions in both joint capsules evaluated and vascularisation in the subscapularis tendon and medial glenohumeral ligament, even though synovial fluid analyses showed no changes from baseline, and orthopaedic examinations were likewise unremarkable. This is consistent with a report in which dogs that were clinically normal had notable damage to the medial stabilisers of the shoulder joint on arthroscopic examination (25).

To explain these arthroscopic findings, evidence points to a process triggered by articular injury in which chondrocytes, synoviocytes, and inflammatory cells release inflammatory cytokines (e.g., interleukins, tumour necrosis factor) and cartilage-degrading enzymes (e.g., matrix metalloproteinases) into the joint space (26-28). Over time, increased inflammatory cytokine activity and synthesis of matrix metalloproteinases, together with the cumulative effects of weight bearing and repetitive motion, may result in a self-perpetuating cycle of synovial inflammation, cartilage breakdown, and mechanical dysfunction.

Although we saw no signs of dysfunction during our six-week study, arthroscopic evidence of inflammation in the periarticular structures of the shoulder suggests that the undue burden of compensating for injury to the medial glenohumeral ligament caused more widespread damage that can lead to cartilage breakdown and shoulder instability over time.

While the vascularisation that we observed in soft tissue structures may represent an angiogenic response to injury that has been associated with inflammation in a rabbit model, caution is needed in applying these results from one species to another (29).

Our study has several limitations. They include duration, since six weeks may not be enough for the development of orthopaedic, radiographic, and synovial fluid abnormalities. Furthermore, there was lack of a control group and also the outcome assessors were not blinded to the intervention. In addition, because the proximity of lateral portals to anatomical structures can make it difficult to observe lateral intra-articular structures, the use of dorsal recumbency with lateral and craniomedial portals may have facilitated more complete inspection of the shoulder joint (21). Additional limitations of our study include the assessment of inflammation by just one investigator, and a reliance on still images alone to evaluate arthroscopic findings, since our equipment lacked a video camera.

To assess the effects of transection from a clinical practice perspective, we performed orthopaedic and radiographic examinations. However, force plate analysis provides a more objective assessment of lameness, and in vitro biomechanical studies are needed to determine if instability is induced by the experimental procedure.

Since medial shoulder instability has generally been diagnosed in large breed dogs, different breeds and body sizes should be studied (4, 5, 11). Future areas of investigation might also include transection of the caudal arm, total transection of the medial glenohumeral ligament, transection of the subscapularis tendon, and the effects of experimentally induced versus naturally occurring injury. Further study of the inflammatory process is also needed, such as microscopic analysis of the villus tissue to characterise and quantify the expression of inflammatory markers, the design and validation of scoring systems for canine soft tissue inflammation without evidence of osteoarthritis (we found none in our literature search), and arthroscopy at more frequent intervals to trace the development of inflammation and statistically analyse the extent to which it is a measure of the disease process itself, and not the intervention (30).

Conclusion

Our results suggest that a partial tear in the medial glenohumeral ligament, induced by transection of the cranial arm, could be one cause of medial shoulder instability in dogs. Although transection did not affect gross shoulder stability in the experimental period based on the evaluation methods used, arthroscopic findings revealed inflammation in the medial compartment at six weeks. From this we infer that transection triggered a cycle in which inflammation leads to enzymatic breakdown of cartilage, exacerbation by weight bearing and repetitive motion, and shoulder instability.

Because synovial fluid analyses were normal throughout the study, we also conclude that inflammation could have been detected earlier only by arthroscopy.

Conflict of interest

None declared.

References

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