# Prevalence and relevance of antibodies to type-I and -II collagen in synovial fluid of dogs with cranial cruciate ligament damage

Hilde de Rooster, DVM, MS; Eric Cox, DVM, PhD; Henri van Bree, DVM, PhD

**Objective**—To measure and compare synovial fluid antibody titers to type-I and -II collagen in stifle joints with instability caused by complete or partial cranial cruciate ligament (CCL) rupture and joints with osteoarthrosis secondary to other pathologic changes in dogs.

**Animals**—82 dogs with diseased stifle joints.

**Procedure**—Synovial fluid samples were collected from 7 dogs with clinically normal stifles (control group) and 82 dogs with diseased joints (50 stifle joints with complete rupture of the CCL, 20 with partial damage of the CCL, and 12 joints with radiographic signs of osteoarthritis secondary to other arthropathies). Synovial fluid samples were tested for autoantibodies to type-I and -II collagen by an ELISA.

**Results**—In dogs with complete and partial CCL rupture, synovial fluid antibody titers to type-I and -II collagen were significantly increased, compared with control dogs. Forty-eight percent (24/50) of samples from dogs with complete CCL rupture and 35% (7/20) of samples from dogs with partial CCL rupture had antibody titers to type-I collagen that were greater than the mean plus 2 standard deviations of the control group titers. Synovial fluid antibody titers to type-II collagen were high in 40% of the dogs with partial or (8/20) complete (20/50) CCL rupture. Dogs with osteoarthrosis secondary to other pathologic changes had significantly increased synovial fluid antibodies to type-I and -II collagen, compared with control dogs.

**Conclusion**—Increases in autoantibodies to collagen in synovial fluid are not specific for the type of joint disorder. It is unlikely that the anticollagen antibodies play an active role in the initiation of weakening of the CCL. (*Am J Vet Res* 2000;61:1456–1461)

In humans, rupture of the cruciate ligament is mostly a purely traumatic event to the knee joint.[1] By contrast, in dogs, this structure may be damaged or ruptured without a history of vigorous trauma.[2] It is defined as a spontaneous **cranial cruciate ligament (CCL)** rupture if it occurs during movements and activities that normally should not injure this ligamentous structure. Various predisposing factors such as aging of the ligament,[3] lack of exercise,[4] conformational abnormalities,[5] or **osteoarthritis (OA)** within the stifle joint[3] are associated with damage to the CCL. In dogs, the exact cause and pathogenesis of this disease (excluding the acute traumatic form) still is unclear. Many veterinarians and scientists perform studies on canine CCL specimens to better understand the pathophysiologic mechanisms of this disease. The challenge to unravel the multifactorial etiopathogenesis of CCL disease remains. It is intriguing that 2 main clinical entities of canine CCL disease seem to exist. Ruptures occur in elderly small- and medium-sized dogs, and also in young large-breed dogs.[6]

The study presented here on the involvement of synovial fluid anticollagen antibodies in CCL disease in dogs was undertaken because of the inability to explain the high occurrence of clinical CCL rupture in dogs in the absence of severe trauma. Different types of collagen exist. Type-I collagen is the main constituent of skin, bone, tendon, and synovial tissue.[7] The canine meniscus also mainly contains collagen type I.[8] Type-II collagen is restricted to the articular cartilage, the intervertebral discs, and the vitreous humor.[7,9] The concept that chondrocytes in osteoarthritic cartilage switch their collagen synthesis from the characteristic type II to type I is no longer supported.[10] Potential relationships in dogs between anticollagen antibody titer in synovial fluid from stifle joints with CCL disease, the degree of degenerative joint disease, and duration of clinical signs were explored.

## Materials and Methods

**CCL-group dogs**—Dogs admitted to the University of Ghent Veterinary School Department of Diagnostic Imaging between Feb 1996 and Jan 1998 for surgical stabilization of stifle joints with CCL disease were studied. Fifty dogs had a complete rupture of the CCL, whereas in another 20 dogs, a diagnosis of partial rupture was made during surgery. Breed, age at time of hospital admission, body weight, and sex were recorded. Data on the duration of the clinical signs of lameness were available for all but 4 dogs. Radiographic views taken before surgery were evaluated to determine the degree of arthrosis before surgery. The grading (grade 0 to 3) was done according to a modification of the criteria of Brünnberg.[11] The grade of OA could not be determined in 4 dogs, because no radiographs were available. During surgery, synovial fluid was aspirated from the affected stifle joint just prior to the stab incision. The joint was held sufficiently flexed to cause tension of the capsule, and the needle was passed lateral to the straight patellar ligament, directed obliquely and caudally.

**OA-group dogs**—Twelve dogs without CCL rupture that had orthopaedic joint disorder in 1 joint were screened in a similar manner. Unfortunately, duration of clinical signs of lameness of the affected limb was inconsistently recorded. In all dogs, the degree of radiographic OA was determined. Synovial fluid samples were collected from the following diseased joints: 2 stifle joints, 3 shoulder joints, 6 elbow joints,

Received Aug 30, 1999.
Accepted Oct 11, 1999.
From the Department of Diagnostic Imaging of Domestic Animals (de Rooster, van Bree), and the Laboratory of Veterinary Immunology, Department of Virology, Parasitology and Immunology (Cox), Faculty of Veterinary Medicine, University of Ghent, Salisburylaan 133, B-9820 Merelbeke, Belgium.

and 1 tarsal joint. Synovial fluid samples were collected from joints other than the stifle prior to installing an arthroscopic portal.

**Control-group dogs**—Seven dogs that were euthanatized for nonorthopaedic reasons by IV administration of a drug[a] were used as a source of synovial fluid from clinically normal stifles. Prior to euthanasia, results of clinical and radiographic assessment indicated no swelling of the stifle joints. Synovial fluid was collected by aseptic percutaneous arthrocentesis. At necropsy, the absence of macroscopic signs of degenerative joint disease was confirmed. In addition, the presence of intact CCL was established.

**Sample storage**—Synovial fluid samples were centrifuged to separate contaminating blood, cells, and debris. Subsequently, the purified sample was stored at –18 C until testing.

**ELISA for anticollagen antibody measurement**—Synovial fluid from the dogs was tested for autoantibodies against type-I (human) and -II (bovine) collagen. An ELISA was used to determine antibody concentrations. Polysorb 96-microwell plates[b] were coated with highly purified human type-I collagen[c] or bovine type-II collagen.[c] Both antigens were diluted in 50 m*M* carbonate/bicarbonate coating buffer (pH 9.4) to a concentration of 30 µg/ml. One hundred microliters of coating solution was added to each well and incubated for 24 hours at 4 C.

The collagen-coated wells were then washed at least 4 times with PBS solution supplemented with 0.05% (vol/vol) Tween 20.[d] Subsequently, 2-fold dilutions of inactivated synovial fluid samples (30 minutes at 56 C) in washing solution supplemented with 3% (wt/vol) BSA (dilution buffer) were added to the wells. As a blank, collagen-coated wells were incubated with 100 µl of the diluent only. Also, negative and positive control samples were assayed in each assay. The samples were incubated at room temperature (approx 25 C) for 1.5 hours.

Bound antibodies were detected by adding 100 µl of an appropriate dilution of affinity isolated rabbit antidog IgG (whole molecule) conjugated to horseradish peroxidase.[e] The conjugate was incubated for 2 hours at 37 C. Between each step, the wells were washed 4 times with the washing solution. Binding of conjugate was observed by adding 50 µl of 2,2'-Azino-di-[3-ethylbenzthiazoline sulfonate (6)] diammonium salt crystals substrate. Absorbance at 405 nm was measured after 60 minutes of incubation at 37 C, using an ELISA reader.[f]

**Antibody titers**—The anticollagen-specific antibody titer of a sample is the highest dilution of that sample with an **optical density (OD)** just above the cutoff value. For each collagen type, the cutoff value was determined by calculating the sum of the mean OD of all synovial fluid samples with negative results for the collagen type and the corresponding 3 standard deviations ($P < 0.01$). For each sample, the significance limit represents the mean OD of synovial fluids from control-group dogs plus 2 standard deviations.

**Statistical analysis**—Mann-Whitney rank sum tests were used to compare data such as age, body weight, degree of OA, and duration of the clinical signs between dogs with complete CCL ruptures and dogs with partial CCL ruptures. The titers were $\log_2$ transformed to meet normal distribution (Wilk-Sharpiro/Rankit Plot). One-tailed 2-sample *t*-tests (for equal or unequal variances as applicable) were used to compare data from a clinically affected group with the control group, whereas a paired *t*-test was used to compare the titers to type-I collagen with those to type-II collagen for samples from all different groups.

Simple regression analyses were used to explore the potential relationships between the titer of antibodies against type-I or -II collagen and the degree of OA in the OA-group dogs. For the CCL-group dogs, multiple regression analyses were performed separately for dogs with complete or partial rupture to assess potential relationships between the titer and the type of anticollagen antibodies (as dependent variables) and the age of dogs, their body weight, the degree of OA on the radiographic views before surgery, and the duration of the clinical signs (as independent variables). Stepwise regression was performed to find the best-fitting model relating the prevalence of anticollagen antibodies to the independent variables. Furthermore, the relationships between these variables and autoantibodies to type-I and -II collagen were assessed by Mann-Whitney rank sum tests. The same statistical test was used to compare the titer and type of anticollagen antibodies between the dogs with meniscal damage and those without. Hypotheses tested were accepted if the *P* value was $< 0.05$.

## Results

Of the 70 dogs in the CCL group, 7 were considered to be mixed-breed dogs. Purebred dogs included Rottweilers (n = 17), Boxers (5), Yorkshire terriers (5), Bernese Mountain Dogs (3), and Golden Retrievers (3). There were 2 each of the following breeds: Beauceron, Braque, Doberman Pinscher, English Bulldog, Labrador Retriever, Neapolitan Mastiff, Nizinny, and Poodle. Furthermore, 1 of each of the following were included: American Cocker Spaniel, American Staffordshire Terrier, Bichon Frise, Bullmastiff, Cairn Terrier, English Springer Spaniel, French Mastiff, Giant Schnauzer, Maltese, Newfoundland, Saarloos Wolfhound, Saint Bernard, Siberian Husky, and Whippet. Twenty dogs had a partial CCL tear, of which 5 also had a medial meniscal tear (**Table 1**). The remainder (50/70) had complete ruptures; in 33 of them, the medial meniscus was also damaged.

The mean age of CCL-group dogs was 5 years old (range, 0.5 to 12 years old) with a mean body weight of 32.8 kg (range, 3 to 72 kg). The group of dogs with

Table 1—Mean (range) distribution of dogs by age, body weight, degree of osteoarthritis, and duration of clinical signs

| Groups | Number of dogs | Age (y) | Body weight (kg) | Degree of OA | Duration of signs (m) |
|---|---|---|---|---|---|
| CCL | 70 | 5.0 (0.5–12) | 32.8 (3–72) | 2 (0–3) | 2.4 (0.2–24) |
| cCCL | 50 | 5.4 (1–11.5) | 31.0 (3–72) | 2 (0–3) | 1.3 (0.2–7) |
| pCCL | 20 | 3.8 (0.5–12) | 37.2 (18–60) | 2 (1–3) | 4.5 (0.2–24) |
| OA | 12 | 2.7 (0.5–15.5) | 33.2 (9–50) | 1 (0–3) | NA |
| Control | 7 | 5.3 (0.5–14) | 22.6 (10-35) | 0 (0–0) | 0 (0–0) |

CCL = Cranial cruciate ligament rupture. cCCL = Complete rupture of the cranial cruciate ligament. pCCL = Partial rupture of the cranial cruciate ligament. OA = Osteoarthritis secondary to pathologic changes other than CCL rupture. Control = Clinically normal. NA = Not available.

Table 2—Synovial fluid anticollagen antibodies in canine arthropathies

| Groups | Number of samples | Anticollagen antibody titers in $log_2$ Type I | Type II |
|---|---|---|---|
| CCL | 70 | 1.3857 ± 1.5064† | 1.9857 ± 0.7559† |
| cCCL | 50 | 1.5200 ± 1.5810† | 2.1400 ± 1.5651† |
| pCCL | 20 | 1.0500 ± 1.2763* | 1.6000 ± 1.5009* |
| OA | 12 | 1.6667 ± 1.3707† | 3.5833 ± 1.4434‡ |
| Control | 7 | 0.2857 ± 0.4880 | 0.7143 ± 0.7559 |

Mean titers significantly different from the control group value as follows: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.
*See* Table 1 for key.

partial rupture of the CCL was significantly younger than the dogs with complete ruptures ($P = 0.04$), whereas there was no significant difference found in body weight between groups. The period between the first clinical signs and repair of the ruptured CCL varied greatly, ranging from < 1 week to > 2 years old. In dogs with partial CCL rupture, the lameness had been present for a significantly longer time, compared with the dogs with complete CCL rupture ($P = 0.02$).

The 12 dogs in the OA group were of similar breeds and sizes as those in the CCL group. Only 1 mixed-breed dog was screened. Fifty percent of the OA dogs were Rottweilers (6). Of the other breeds, there was 1 each of the following: Bernese Mountain dog, Doberman Pinscher, Newfoundland, Poodle, and Saint Bernard. Most dogs in the OA group were young (mean, 2.7 years old; range, 0.5 to 15.5 years old). The mean body weight was 33.2 kg (range, 9 to 50 kg).

In the control group, 7 dogs belonged to the following breeds: Beagle (2), Belgian Malinois (1), German Shepherd Dog (2), and Labrador Retriever (1). One mixed-breed dog was included. These dogs ranged in age from 0.5 to 14 years old (mean, 5.3 years old), and their body weight varied from 10 to 35 kg (mean, 22.6 kg).

There was a significant difference in mean titer of antitype-I collagen antibodies between the CCL-group dogs with complete or partial ruptures and the control group ($t = -4.26$, $P < 0.001$ and $t = -2.25$, $P = 0.02$, respectively; **Table 2**). Also, the antitype-II collagen antibodies in synovial fluid of CCL-group dogs with complete or partial ruptures were significantly higher than those in synovial fluid samples from clinically normal dogs ($t = -3.94$, $P < 0.001$ and $t = -2.01$, $P = 0.03$, respectively). The mean titer of antitype-I and -II collagen antibodies were significantly different between OA group and the control-group dogs ($t = -3.16$, $P = 0.003$, and $t = -4.85$, $P < 0.001$, respectively).

Twenty-one of the 50 dogs with complete CCL rupture (42%) and 7 of the 20 dogs with partial CCL ruptures (35%) had an antitype-I antibody titer of < 10 (OD < 0.163; **Fig 1**). Most of the CCL-group dogs had synovial fluid values that were within the range of control-group dogs. Antibody titers to type-I collagen were greater than the significance limit in 48% (24 of 50) of the dogs with complete CCL rupture and 35% (7 of 20) of the dogs with partial CCL rupture. Ten of 50 dogs with complete CCL rupture (20%) and 7 of 20 dogs with partial CCL rupture (35%) had an antitype-II antibody titer of < 10 (OD < 0.221; **Fig 2**). Antibody titers to type-II collagen were greater than the significance limit in 40% (20 of 50) of the dogs with complete CCL rupture and in 40% (8 of 20) of the dogs with partial CCL rupture.

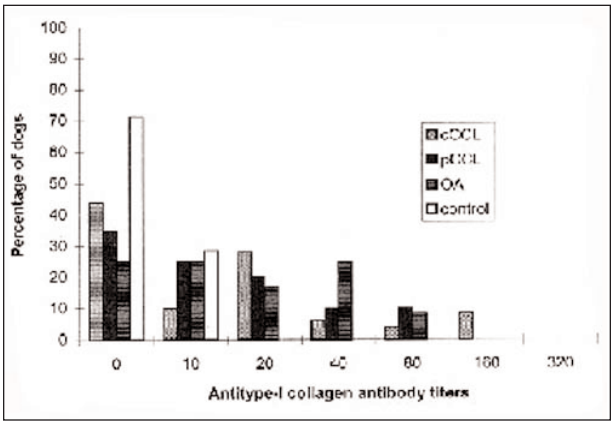


Figure 1—Antibody titers to type-I collagen in canine synovial fluids. cCCL = Complete rupture of the cranial cruciate ligament. pCCL = Partial rupture of the cranial cruciate ligament. OA = Osteoarthritis secondary to pathologic changes other than CCL rupture. Control = Clinically normal.

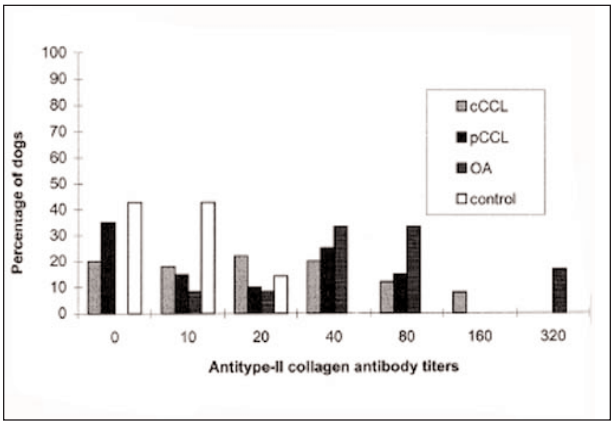


Figure 2—Antibody titers to type-II collagen in canine synovial fluids. *See* Figure 1 for key.

Synovial fluid prevalence of antitype-I and -II collagen antibodies did not correlate in any of the dog groups. Paired analyses revealed a significant difference between the titers of antibody to both collagen types in the CCL-group dogs ($t = -4.64$, $P < 0.001$) and the OA-group dogs ($t = -3.15$, $P = 0.009$). Titers against type-II collagen were higher than titers against type-I collagen. In the control group, no difference was found between antibody titers. For dogs with complete CCL

rupture and dogs with partial CCL rupture, the mean titer of autoantibodies to type-I or -II collagen did not differ statistically between the group with meniscal damage and the dogs with intact menisci ($\alpha = 0.05$).

In CCL-group dogs, multiple regression analysis was used to indicate associations between anticollagen antibody titers and the variables age, body weight, degree of radiographic OA, and duration of clinical signs. In dogs with complete CCL rupture, there was a weak negative relationship between $\log_2$ antibody titers against collagen type I and body weight ($R^2 = 18\%$, $P = 0.003$). The age, duration of clinical signs, nor the degree of OA were significant variables for the model. For the dogs with partial tearing of the CCL, the only association found was a weak positive relationship between antitype-I collagen antibody titers and the dog age ($R^2 = 22\%$, $P = 0.03$). Significant relationships between antibody titers to type-II collagen and any of the variables were not found in the dogs with complete ruptures. In contrast, regression analysis for dogs with partial CCL ruptures indicated an association of antitype-II collagen antibodies with the variable degree of OA ($R^2 = 22\%$, $P = 0.03$). When the degree of OA and the age were expressed in a single regression model, 42% of the variance was covered ($P = 0.04$).

When data from CCL-group dogs was subdivided into those with samples with negative autoantibody results and those with samples with positive autoantibody results (irrespective of the titer of antibodies to collagen), the same variables were reassessed. In dogs with complete CCL rupture, the only variables that differed significantly were the body weight ($P = 0.03$) and the age ($P = 0.04$) of dogs between samples with positive and negative results for antitype-I collagen antibodies. The mean body weight for dogs with positive antitype-I results was lower, whereas their mean age was higher, compared with dogs with negative results. In dogs with partial CCL rupture, samples with positive results for autoantibodies to type-I collagen also had a higher mean age ($P = 0.02$), compared with dogs with negative results. None of the other variables differed between dogs with positive and negative results. The use of simple regression analysis failed to reveal any association between antitype-I or -II collagen antibodies and the degree of OA in the OA group ($\alpha = 0.05$).

## Discussion

Results of our study indicate that there is a significant increase in synovial fluid antibody titers to antitype-I collagen and -II collagen in canine joint disorders. The presence of these anticollagen antibodies in synovial fluid of dogs with OA was not a new finding and was in agreement with previous studies.[12-14] In our study, tests on synovial fluid samples from stifle joints with CCL disease and from joints in which the OA was unrelated to CCL disease were simultaneously conducted for antitype-I and -II antibodies. To our knowledge, this is the first report on autoantibodies to collagen in synovial fluid of dogs with CCL rupture that differentiates between partial and complete damage.

The group of dogs with a partially ruptured CCL was generally younger but did not differ in body weight from the dogs with complete CCL ruptures. Most dogs with partial rupture were lame for a more extended period of time before they were admitted for surgery.

It is widely accepted that immunologic processes are involved in the pathogenesis of many joint disorders in dogs. Degenerative joint disease is no longer considered a purely noninflammatory process. In dogs with naturally occurring rupture of the CCL, high immunoglobulin depositions are found in the synovial membrane,[15] and lymphocytic-plasmacytic synovitis has been associated with damage to the CCL.[16-18] The latter finding has been confirmed after experimental section of the CCL, where discrete cell aggregates of small lymphocytes and plasma cells, suggestive of immune-mediated disorders, were found in the synovium of dogs 2 months after surgical section of the CCL.[19] These studies only provided histologic and immunohistopathologic data. Experimental transsection of the CCL in dogs is well known as the Pond-Nuki model.[20] The resulting joint instability initiates a sequence of events to the affected stifle joint and is a key factor in the production of degenerative joint disease. Experimental models can provide extremely useful information about natural processes. However, results of a study on synovial fluid mediators[21] did not establish an identical pathogenesis for degenerative changes, secondary to spontaneous CCL damage or after experimental transsection of the CCL.

Abnormal loading of the unstable stifle joint during weight bearing causes mechanical damage to the joint cartilage, which in turn may release antigenic material and expose it to the immune system. Autoantibodies can be formed in response to prolonged and abundant presentation of this antigen immunologically recognized as foreign substance.[13,22] Because collagen type II is the major constituent of cartilage,[7,9] a high antibody-titer against collagen type II may be expected in synovial fluid from OA joints. Results of our study indicate that there is a weak association between the degree of radiographic OA and the antitype-II antibody titer in the subgroup of dogs with partial CCL rupture. In the other dog groups (dogs with complete CCL rupture and OA-group dogs), the degree of radiographic OA was not correlated with the amount of detected autoantibodies. This is in accordance with the findings of Arican et al[14] who failed to relate the release of cartilage breakdown products such as glycoaminoglycans and keratan sulfate to anticollagen autoantibodies. Consideration should also be given to the fact that the radiographic degree of OA does not always reflect the exact amount of osteophytosis found during arthrotomy.

Stifle joint instability does not only cause damage to the cartilage but also increases the risk of medial meniscal damage. The menisci as well as the intraarticular CCL are mainly composed of type-I collagen fibers tissue.[7,8] Damage to the CCL or to the meniscus can, therefore, expose this collagen type as an antigenic target. In our study, damage to the medial meniscus did not influence the titer of antibodies to type-I collagen in the CCL-group dogs. Forty-four percent of the dogs with CCL rupture (48% of dogs with complete rupture and 35% of dogs with partial ruptures) in our study did

have high antitype-I antibody titer in the synovial fluid. The dogs with complete or partial rupture of the CCL in which antitype-I antibody positivity was observed were older than the dogs with samples with negative results. The detected titers of autoantibodies in synovial fluid of dogs with partial CCL rupture were slightly low, compared with samples from dogs with complete tearing. Conversely, in other joints with pathologic changes, positive antitype-I antibody titer results could also be detected in half of the dogs.

Observations of the titers of autoantibodies to collagen in the synovial fluid of dogs with CCL disease (complete and partial ruptures) were not in line with some reported data. Niebauer et al[12] found similar titers of anticollagen antibodies for collagen type I and II. In our study, no correlations were found between antitype-I and -II collagen antibody titers in the synovial fluid of individual dog and joint disease, irrespective of the joint problem. Nevertheless, the mean titer of antitype-I collagen antibodies in both subgroups of CCL disease dogs was increased, compared with the control group, and the same was true for the antitype-II titer. A possible explanation could be the origin of the collagen used in the ELISA. Results of inhibition experiments indicate weak cross-reactivity between collagen type I and II.[12,13,22] Commercially available highly purified antigenic solutions of human type-I and bovine type-II collagen were used as antigenic targets. Apart from the availability, the choice of coating collagen was inspired by a publication of Bari et al.[13] Results of their research revealed an equal test reaction of canine serum with bovine type-II collagen as with canine type-II collagen. However, testing of canine synovial fluids preliminary to our study in which bovine type-I and human type-I collagen were compared as antigenic targets consistently resulted in high nonspecific reactions when bovine collagen was used (data not shown).

Niebauer et al[12] suggested that high autoantibody titers are observed more often in dogs with signs of acute arthritis (rupture up to a few weeks before), although no statistical evidence for this statement was presented. In our study, we investigated whether the prevalence of autoantibodies in dogs with CCL disease was correlated directly with the duration of the signs. Results of our study revealed no correlation between the dogs with complete and partial CCL rupture. The history is, of course, only an approximation of the real duration of signs. The lameness may only be subtle and the real onset initially unnoticed by the owner. Measurement of anticollagen antibodies in the dogs' sera was reported to be less diagnostic for particular joint diseases,[23,24] so this line of investigation was not pursued in our study.

Our data does not support the idea that autoantibodies to collagen play an active role in the initiation of CCL rupture. Several findings suggest that antitype-I and -II collagen antibodies only play a role in the perpetuation of joint inflammation in general. Differences in the anticollagen antibody titers between dogs with acute and chronic disease could not be established. Autoantibodies to type-I collagen did not seem to be specific for dogs with damage to the CCL. Moreover, antitype-II collagen antibody titers were generally higher than those of antitype I. Furthermore, in humans, rupture of the anterior cruciate ligament is primarily a traumatic event. One may expect, however, that formation of autoantibodies to collagens is also occurring in human knee joints of OA patients, but spontaneous rupture of the anterior cruciate ligament in humans is not reported. The presence of anticollagen autoantibodies does not necessarily mean that CCL rupture is an immune-mediated event in dogs.

---

[a] $T_{61}$, Hoechst Roussel Vet GmbH, Unterschleissheim, Germany.
[b] Polysorb 96-microwell plates, NEN Science Products, Zaventem, Belgium.
[c] Collagen, Southern Biotechnology Associates Inc, Birmingham, Ala.
[d] Tween 20, Merck-Schuchardt, Hohenbrunn, Germany.
[e] Horseradish peroxidase, Sigma Biosciences, St Louis, Mo.
[f] Tecan Spectra Flour, Sercolab Systems, Mechelen, Belgium.

---

## References


1. Drapé J, Ghitalla S, Autefage A. Rupture du ligament croisé antérieur (L.C.A.) chez le chien: pathologie traumatique ou dégénérative? *Point Vét* 1990;22:573–580.
2. Arnoczky SP. Surgery of the stifle—the cruciate ligaments (Part I). *Compend Contin Educ Pract Vet* 1980;2:106–116.
3. Zahm H. Die Ligamenta decussata in gesunden und arthrotischen Kniegelenk des Hundes. *Kleintierpraxis* 1965;10: 38–47.
4. Narama I, Masuoka-Nishiyama M, Matsuura T, et al. Morphogenesis of degenerative changes predisposing dogs to rupture of the cranial cruciate ligament. *J Vet Med Sci* 1996;58: 1091–1097.
5. Hohn RB, Newton CD. Surgical repair of ligamentous structures of the stifle joint. In: Bojrab MJ, ed. *Current techniques in small animal surgery.* Philadelphia: Lea & Febiger, 1975;470–479.
6. Bennett D, Tennant D, Lewis DG, et al. A reappraisal of anterior cruciate ligament disease in the dog. *J Small Anim Pract* 1988;29:275–297.
7. Morgan K, Clague RB, Collins I, et al. Incidence of antibodies to native and denaturated cartilage collagens (types II, IX, and XI) and to type I collagen in rheumatoid arthritis. *Ann Rheum Dis* 1987;46:902–907.
8. Wildey GM, McDevitt CA. Matrix protein mRNA levels in canine meniscus cells in vitro. *Arch Biochem Biophys* 1998; 353:10–15.
9. Stuart JM, Cremer MA, Townes AS, et al. Type II collagen-induced arthritis in rats. Passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis. *J Exp Med* 1982; 155:1–16.
10. Hui-Chou CS, Lust G. The type of collagen made by the articular cartilage in joints of dogs with degenerative joint disease. *Coll Relat Res* 1982;2:245–256.
11. Brünnberg L, Rieger I, Hesse EM. Sieben Jahre Erfahrung mit einer modifizierten "over-the-top"-Kreuzbandplastik beim Hund. *Kleintierpraxis* 1992;37:735–746.
12. Niebauer GW, Wolf B, Bashey RI, et al. Antibodies to canine collagen types I and II in dogs with spontaneous cruciate ligament rupture and osteoarthritis. *Arthritis Rheum* 1987;30: 319–327.
13. Bari AS, Carter SD, Bell SC, et al. Anti-type II collagen antibody in naturally occurring canine joint diseases. *Brit J Rheumatol* 1989;28:480–486.
14. Arican M, Carter SD, Bennett D, et al. Measurement of glycosaminoglycans and keratan sulphate in canine arthropathies. *Res Vet Sci* 1994;56:290–297.
15. Lawrence D, Bao S, Canfield PJ, et al. Elevation of immunoglobulin deposition in the synovial membrane of dogs with cranial cruciate ligament rupture. *Vet Immunol Immunopathol* 1998;65:89–96.
16. Tirgari M. The surgical significance of the blood supply of the canine stifle joint. *J Small Anim Pract* 1978;19:451–462.
17. Pendersen NC, Pool RR, Morgan JP. Joint diseases in dogs

and cats. Meniscal disorders and cruciate ligament rupture. In: Ettinger SJ, ed. *Textbook of veterinary internal medicine. Diseases of the dog and cat*. London: WB Saunders Co, 1983;2197–2198.

18. Galloway RH, Lester SJ. Histopathological evaluation of canine stifle joint synovial membrane collected at the time of repair of cranial cruciate ligament rupture. *J Am Anim Hosp Assoc* 1995;31:289–294.

19. Lipowitz AJ, Wong PL, Stevens JB. Synovial membrane changes after experimental transection of the cranial cruciate ligament in dogs. *Am J Vet Res* 1985;46:1166–1170.

20. Pond MJ, Nuki G. Experimentally-induced osteoarthritis in the dog. *Ann Rheum Dis* 1973;32:387–388.

21. Hay CW, Chu Q, Budsberg SC, et al. Synovial fluid interleukin 6, tumor necrosis factor, and nitric oxide values in dogs with osteoarthritis secondary to cranial cruciate ligament rupture. *Am J Vet Res* 1997;58:1027–1032.

22. Stuart JM, Huffstutter EH, Townes AS, et al. Incidence and specificity of antibodies to types I, II, III, IV, and V collagen in rheumatoid arthritis and other rheumatic diseases as measured by $^{125}$I-radioimmunoassay. *Arthritis Rheum* 1983;26:832–840.

23. Niebauer GW, Wolf B, Yarmush M, et al. Evaluation of immune complexes and collagen type-specific antibodies in sera and synovial fluids of horses with secondary osteoarthritis. *Am J Vet Res* 1988;49:1223–1227.

24. Osborne AC, Carter SD, May SA, et al. Anti-collagen antibodies and immune complexes in equine joint diseases. *Vet Immunol Immunopathol* 1995;45:19–30.