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Research paper

Cruciate ligament degeneration and stifle joint synovitis in 56 dogs with intact cranial cruciate ligaments: Correlation of histological findings and numbers and phenotypes of inflammatory cells with age, body weight and breed

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ABSTRACT

The majority of dogs with ruptured cranial cruciate ligaments (crCLs) have inflammatory changes of the stifle joint suggesting that synovitis is an important factor which is involved in the development of degenerative ligament changes. Detailed information is not available concerning the possible occurrence of inflammatory changes in the stifle joint synovium of dogs with macroscopically intact crCLs and its correlation with presence and severity of degenerative changes of the crCLs. Therefore, the purpose of this study was to examine post mortem tissue samples of 56 dogs with non-ruptured crCLs histologically for the presence of inflammatory and degenerative changes of the stifle joint synovium and cruciate ligaments, respectively. In 25/56 (44.6%) dogs, histology showed that both lymphoplasmacytic synovitis and degenerative alterations of the crCLs were present. In these dogs, there was a significant positive statistical correlation between the severity of synovitis and degenerative crCL lesions. The results suggest that synovitis in the stifle joints of dogs with non-ruptured crCLs is a frequent event and probably is involved in the development of degenerative lesions occurring in canine crCLs. Also, the severity of crCL degeneration in these 25 dogs was significantly correlated with their age and body weight. In 2/56 (3.6%) cases, only lymphoplasmacytic synovitis was found in the absence of degenerative crCL lesions. In 15/56 (26.8%) dogs, only degenerative lesions of the crCLs without synovitis were present. Statistically, a significant correlation was found between the severity of degenerative alterations and age and body weight of these dogs. Phenotyping of inflammatory cells by immunohistochemistry showed that the synovium of dogs which histologically had lymphoplasmacytic synovitis was infiltrated with CD3+ T lymphocytes, CD79+ B lymphocytes, major histocompatibility class II antigen (MHC class II)+ cells and macrophages expressing CD163 or S100/A8/S100A9 (calprotectin), while tartrate-resistant acid phosphatase (TRAP)+ cells were absent. Quantification and statistical evaluation of inflammatory cell types in the inflamed synovium revealed that the numbers of lymphocytic cells and macrophages were significantly correlated with the severity of synovitis. These findings indicate that, besides T and B lymphocytes, both pro- and anti-inflammatory macrophages play a role in the development of degenerative crCL alterations.

1. Introduction

Spontaneous rupture of the cranial cruciate ligament (crCL) is one of the most important causes of hindlimb lameness in dogs (Doom et al., 2008). The majority of adult dogs rupture their crCL often during normal daily activities due to degenerative changes within the ligament (Doom et al., 2008; Hayashi et al., 2004). The results of several investigations implicate that multiple factors, such as age, body weight, breed predisposition, sex hormones, genetics, stifle morphology and crCL matrix composition, contribute to the degenerative process (Adams et al., 2011; Doom et al., 2008; Taylor-Brown et al., 2015; Vasseur et al., 1985; Whitehair et al., 1993). It has been suggested that immunopathological mechanisms, such as autoimmune reactions to collagen antigens released from damaged ligaments and cartilage, are likely to be involved in the development of degenerative crCL lesions (Doom et al., 2008; Hayashi et al., 2004).

At the time of surgical treatment of spontaneous canine crCL rupture, 51–67% of dogs have a lymphoplasmacytic synovitis of the diseased stifle joint (Erne et al., 2009; Galloway and Lester, 1995; Lemburg et al., 2004). Immunohistochemical phenotyping of

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inflammatory cells in the stifle joint synovium of such dogs revealed the presence of B and T lymphocytes, plasma cells and MHC class II –positive cells with dendritic morphology (Galloway and Lester, 1995; Lemburg et al., 2004). Furthermore, CD11b+/CD18+ mononuclear inflammatory cells and macrophage-like cells expressing tartrate-resistant acid phosphatase (TRAP) have been detected in the inflamed synovium and also in cruciate ligament tissue of dogs with ruptured crCLs (Barrett et al., 2005; Klocke et al., 2005; Muir et al., 2002, 2005).

Arthroscopical and histological examinations in dogs with unilateral crCL rupture showed that inflammatory changes were present in the synovium of the stable contralateral stifle joint, suggesting that synovitis is an early feature of canine crCL disease (Bleedorn et al., 2011; Muir et al., 2011). In the literature, two hypotheses have been expressed regarding the mechanisms responsible for the development of cruciate fibre damage resulting in eventual crCL rupture and the role of synovitis (Bleedorn et al., 2011; Muir et al., 2011). The first hypothesis is that synovitis is a primary event inducing progressive fibre disruption in the crCL. The second hypothesis says that specific factors such as ageing, genetics, body weight, sex hormone status, and stifle morphology may induce minor fibre rupture subsequently leading to development of synovitis by ligament matrix components such as neoepitopes of collagen (Bleedorn et al., 2011; Muir et al., 2011).

Several studies described the histological findings in intact and ruptured canine crCLs (Hayashi et al., 2003; Ichinohe et al., 2015; Vasseur et al., 1985). In one of these previous reports, in which the histological changes of non-ruptured crCLs in dogs of different age and body weight were characterised, the presence of few mononuclear inflammatory cells in the stifle joint synovium of dogs with degenerative crCL lesions was mentioned (Vasseur et al., 1985). To date, detailed information about the possible occurrence of inflammatory changes in the stifle joint synovium of dogs with macroscopically intact crCLs and its correlation with presence and severity of degenerative changes of the crCLs is not available. The aims of this study were: (i) to determine the incidence of stifle joint synovitis and degenerative crCL lesions in post mortem tissue samples from dogs without clinical history of joint diseases and non-ruptured crCLs, (ii) to characterise the synovial inflammatory cell types immunohistochemically, and (iii) to correlate the histological and immunohistochemical data, i.e. presence and severity of histological lesions and the numbers of macrophages and T and B lymphocytes, with age, body weight and breed of the examined dogs.

2. Materials and methods

2.1. Dogs

The 56 dogs in this study from which post mortem tissue samples were collected were either purebred or crossbred dogs (Table 1). Their ages ranged between 0.17 and 16.5 years (mean: 5.13 years) and their body weight varied from 8.9 to 98 kg (mean: 34.73 kg). The 56 dogs, with consent of the owners, had been routinely submitted by the Small Animal Clinic to the Department of Pathology, both at the University of Veterinary Medicine Hannover, Germany. The dogs had been euthanased for diseases unrelated to the joints due to poor prognosis and were not part of an animal experiment in accordance with the Animal Welfare Act. A full necropsy was carried out on 41 out of 56. From 15 dogs, only the stifle joints were collected.

2.2. Macroscopical examination of joints and collection of samples

A total of 112 cranial cruciate ligaments (crCLs), 85 caudal cruciate ligaments (cdCLs) and a total of 560 synovial membrane samples were collected from both stifle joints of 56 dogs between 2012 and 2014. Collection of tissue samples from the stifle joints was performed as described elsewhere (Susta et al., 2012). Unopened joints were separated from the proximal and distal bones by sawing. Thereafter, 2–10 mL of 10% neutral buffered formalin (NBF) was injected into the femoro-tibial joint through an incision in the joint capsule followed by immersion of the whole joints in 10% NBF. After 72 h the joints were opened and examined macroscopically for intactness of the cranial and caudal CL as well as of the menisci. Arthrotic lesions (osteophytes and cartilage alterations) were evaluated according to criteria given by other authors (Verset et al., 2013). From all 56 dogs both crCLs and from 44 dogs also one or both (n = 85) cdCLs were severed at their attachments. From each dog, synovial tissue was collected from 5 different synovial regions. One sample was taken from the femoro-patellar joint (region 1: lateral parapatellar area) and 4 samples were taken from the femoro-tibial joint: 2 samples from the lateral pouch (region 2: cranial area of the lateral pouch; region 3: area of the sulcus extensorius of the lateral pouch) and 2 samples from the medial pouch (region 4: cranial area of the medial pouch; region 5: caudal area of the medial pouch). Formalin-fixed CLs and synovial tissue samples were embedded in paraffin wax. During embedment CLs were oriented, ensuring that longitudinal sections were obtained. Paraffin sections (2–4 µm) of all tissue samples were stained with haematoxylin and eosin (H&E). On selected sections, special stains for demonstrating acid glycosaminoglycans (Alcian blue stain) and calcification (Von-Kossa stain) were performed.

2.3. Microscopical evaluation of cruciate ligament and synovial sections

Histological changes on H&E stained sections of CLs and synovial membranes were evaluated by light microscopy. Degenerative lesions of crCLs and cdCLs were graded according to criteria described in the literature (Vasseur et al., 1985). Degenerative alterations comprised loss of fibrocytes and of the normal bundling pattern of collagen fibres, acellular areas, chondroid metaplasia and mineralisation. Sections of ligaments without histological alterations were given a grade of 0. Sections of ligaments with small single or multiple areas of degenerative alterations were graded as having mild lesions (grade 1). If moderate degenerative changes affecting up to half of the diameter of the ligament were present grade 2 was given and if severe lesions affecting more than half of the diameter of the ligament were found grade 3 was assigned.

The severity of inflammatory changes on H&E sections of synovial membrane samples was graded on a 0–3 scale. In H&E stained sections of the 5 synovial samples of each dog the total numbers of inflammatory cells (lymphocytes, plasma cells, macrophages) within the subintimal tissue were counted using a light microscope (Zeiss Axioplan, Zeiss, Oberkochen, Germany) at 400x magnification. Sections without inflammation were graded as 0. Sections with mild inflammatory changes (up to 10 inflammatory cells per section) were given grade 1. Sections with moderate inflammatory cell infiltration (>10 and ≤ 150 cells) were given grade 2. In cases of severe infiltration (more than 150 cells per section) grade 3 was assigned. Histological examination and grading were performed in a blinded fashion by one investigator (AKD). For ensuring consistency in quantitation randomly selected sections were also evaluated by a second investigator (MHT).

2.4. Immunohistochemical phenotyping of inflammatory cells in synovial tissue samples

Histological and statistical evaluation of inflammatory cell numbers had shown that synovial samples from regions 4 and 5 had the highest degree of synovitis. Therefore, phenotyping of inflammatory cells was performed on sections of these two synovial membrane regions of all 56 dogs. Serial paraffin sections (2–4 µm) from the synovial tissue samples of all 5 synovial regions from the 56 dogs were subjected to immunohistochemistry with primary antibodies (Table 2) detecting T and B lymphocytes, MHC class II antigens and macrophages using the avidin-biotin-peroxidase (ABC) method. After dewaxing (Roti®-Histol, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) the sections were rehydrated and incubated in 70% ethanol with 0.5% hydrogen peroxide