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Serum C-reactive protein concentrations in Nova Scotia Duck Tolling Retrievers with immune-mediated rheumatic disease

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Abstract

Nova Scotia Duck Tolling Retrievers (NSDTRs) are a dog breed often affected by immune-mediated rheumatic disease (IMRD), a disorder characterised by chronic stiffness and joint pain. Most, but not all, dogs with IMRD, have antinuclear antibodies (ANA), which are also commonly present in the autoimmune disease systemic lupus erythematosus (SLE). The clinical and diagnostic findings of IMRD indicate that it is an SLE-related disorder. C-reactive protein (CRP), an acute phase protein, is a quantitative marker of inflammation for many diseases and is used for diagnosing and monitoring systemic inflammation in both humans and dogs. However, in human SLE, CRP concentrations are often elevated but correlate poorly with disease activity; they can be low in individual patients with active disease. The aim of the study was to investigate CRP in a group of NSDTRs with the SLE-related disorder IMRD. The hypothesis was that CRP concentrations would be increased in dogs with IMRD compared to healthy dogs, but that the increase would be mild. Serum CRP concentrations were measured in 18 IMRD-affected NSDTRs and 19 healthy control NSDTRs using two different canine-specific CRP assays. Dogs with IMRD and ANA had higher CRP concentrations than the control dogs, but the concentrations were below the clinical decision limit for systemic inflammation for most of the IMRD dogs. These results indicate that CRP concentrations were increased in dogs with IMRD and ANA, but the increase was mild, similar to what has been observed in human SLE.

Keywords: C-reactive protein, Canine, Immune-mediated rheumatic disease, Nova Scotia Duck Tolling Retriever, Systemic lupus erythematosus

Findings

Nova Scotia Duck Tolling Retriever (NSDTR) dogs are, compared to other dog breeds, at increased risk of autoimmune disorders and particularly immune-mediated rheumatic disease (IMRD) [1, 2]. Dogs affected by IMRD have a clinical picture indicative of chronic non-erosive polyarthritis with stiffness and pain from multiple joints, sometimes in conjunction with other clinical manifestations. Most, but not all, IMRD cases display antinuclear antibodies (ANA) on indirect immunofluorescence (IIF) [2]. Antinuclear antibodies and non-erosive polyarthritis are also commonly present in the systemic autoimmune

inflammatory disease, systemic lupus erythematosus (SLE), in humans as well as in dogs [3–5] and IMRD is considered an SLE-related disorder [2, 6].

Measurement of C-reactive protein (CRP), an acute phase protein produced in the liver during systemic inflammation, is used for diagnosing and monitoring inflammatory diseases in both humans and dogs [7–9]. CRP is a quantitative marker of inflammation [10], and its measurement is used to monitor disease activity in dogs with immune-mediated polyarthritis [11, 12]. Despite being a reliable marker of systemic inflammation, CRP concentrations in human SLE correlate inconsistently with disease activity. CRP concentrations are often elevated, but individual patients with active disease can have low CRP concentrations [13, 14]. In the present study, we investigated the CRP concentrations in a group of NSDTRs with the SLE-related disorder IMRD. The

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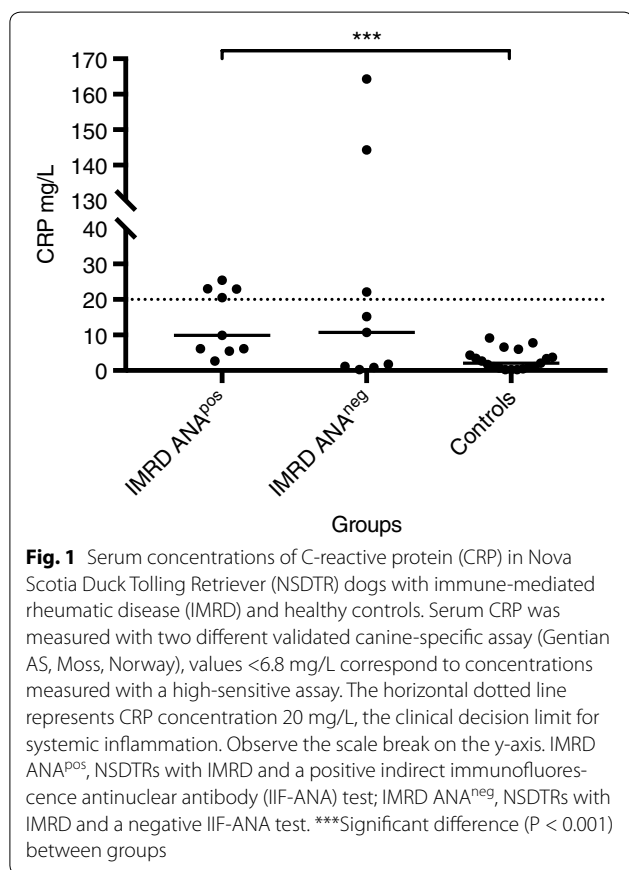
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Table 1 Age, sex and ANA test result for the 37 dogs included in the study

Number	Group	Median age in years (range)	Female/male	ANA test result
9	IMRD	5 (3–8)	8/1	Positive
9	IMRD	7 (2–9)	3/6	Negative
19	Control	4 (1–8)	10/9	Negative

ANA antinuclear antibodies, IMRD immune-mediated rheumatic disease, Control healthy control dogs



hypothesis was that the serum CRP concentrations in these dogs would be higher than in the healthy controls, but that the elevation would be milder than in immune-mediated polyarthritides in other breeds [11, 15, 16].

Serum CRP concentrations were measured in healthy and IMRD-affected NSDTRs. Serum samples from IMRD dogs had been collected in connection with other studies [2, 17, 18] and previously tested for the presence of ANA with IIF [19]. Samples were considered positive at a titre of $\geq 1:100$. The study was approved by the Uppsala Animal Ethical Committee and Swedish Board of Agriculture (C417/12). In total, sera from 37 NSDTRs were included of which 18 had signs of IMRD and 19 were

healthy controls (Table 1). Of the 18 sera from dogs with IMRD, nine were positive (ANA^{pos}) and nine were negative (ANA^{neg}) for the IIF-ANA test. All IMRD dogs had displayed clinical signs consistent with IMRD upon physical examination, including lameness or pain from two or more joints on manipulation [2]. The signs had been apparent for at least 14 days before sampling and no other causes than IMRD were suspected. None of the dogs had been treated with anti-inflammatory drugs at the time of sampling. The control dogs were considered healthy, based on normal physical examination and owner interview at the time of sampling and during a followup interview after 4 weeks. All the blood samples were collected by venipuncture into tubes with clot activator, after written owner consent. The blood samples from the IMRD dogs were either sent by post, or collected at, the University Animal Hospital, Swedish University of Agricultural Sciences, Uppsala, Sweden, where the samples were stored and analysed. The sera had been stored at -80°C for a maximum of 8 years before the CRP analysis in the current study. Some of the samples had undergone up to four freeze–thaw cycles. The samples from control dogs had been collected at the University Animal Hospital and stored at -80°C for a maximum of 3 months before analysis.

For the current study, the serum samples were thawed and the serum CRP was measured in duplicate with a validated canine-specific immunoturbidimetric assay (Gentian cCRP, Gentian AS, Moss, Norway) [20], in a single run. For samples with CRP concentrations <6.8 mg/L, i.e., the assay's limit of quantification (LOQ), re-analysis was performed in duplicate with a second, high-sensitivity canine-specific CRP assay (Gentian hsCRP) [21] on the same occasion. Analyses were performed on a fully automated, open-system clinical chemistry/immunoassay analyser (Abbott Architect c4000, Abbott Park, IL, USA). Statistical analyses were performed using software R V3.0.2. To compare the CRP concentrations between the three groups, Welch ANOVA was performed followed by pair-wise comparisons with Welch two-sample *t* test. Calculations were performed on log-transformed data. The significance level was set to $P < 0.017$ using Bonferroni adjustment for multiple testing. CRP concentrations <0.50 mg/L, corresponding to the LOQ of the hsCRP assay, were assigned the value 0.25 mg/L in the statistical analyses.

Dogs with IMRD and ANA had higher CRP concentrations than the healthy control dogs ($P < 0.001$), but no significant difference was observed between the IMRD ANA^{neg} and control dogs. The CRP concentrations in individual dogs, in each of the three groups, are presented in Fig. 1. The median CRP concentration and range were 9.9 (2.7–25.4) mg/L in the IMRD ANA^{pos}

dogs, 10.8 (<0.5–164.3) mg/L in the IMRD ANA^{neg} dogs and 2.1 (<0.5–9.2) mg/L in the control dogs.

Despite the statistically significant difference in CRP concentration between the IMRD ANA^{pos} and healthy dogs, several of the IMRD dogs in both the ANA^{pos} and the ANA^{neg} groups had concentrations similar to those of the healthy dogs. This resembles the CRP alterations in human SLE, where the CRP concentrations are often mildly elevated, but can also be low in individual patients with active disease [13]. In most of the IMRD dogs, the increase in CRP concentration was less than in immune-mediated polyarthritis in other dog breeds [11, 15, 16], but higher than concentrations reported for e.g., canine osteoarthritis [22]. A CRP of 20 mg/L has previously been established at our laboratory as the decision limit for clinical diagnosis of systemic inflammation. This decision limit was determined by performing an ROC curve analysis of CRP data from dogs with and without known systemic inflammatory disease (data not shown), similarly to what has been previously described [23]. In the present study, most of the IMRD dogs had CRP concentrations below or just above the clinical decision limit. Measurement of CRP concentrations below the clinical decision limit is necessary to detect smaller changes between healthy and diseased dogs, as shown in the present study.

There was no significant difference between the IMRD ANA^{neg} dogs and healthy controls, but two dogs had markedly elevated CRP concentrations (>100 mg/L). These two had typical clinical signs of IMRD, but the diagnosis of IMRD can be challenging, especially in the absence of ANA. It is also possible that these dogs suffered from disorders other than, or in addition to, IMRD that induced systemic inflammation, with resulting high CRP concentrations. The CRP concentrations in these two dogs were of similar magnitude as those reported in other studies of immune-mediated polyarthritis [11, 15, 16]. In a study by Hillström et al. [16], CRP was measured with the same assay as here and the median CRP concentration in dogs with immune-mediated arthritis was 118.0 mg/L, which is considerably higher than the medians in the present study. Unfortunately, direct comparison of CRP concentrations between other studies is not feasible because of the lack of standardization of CRP measurements.

Different veterinarians examined the dogs, which is a limitation of the study. Another limitation is that handling and storage of the samples were not standardised. However, CRP is stable at room temperature [20], and in freezer storage [24] and is unaffected by repeated freeze–thaw cycles [20]. The results of the present study indicate that CRP concentrations increased in NSDTRs with IMRD, but the concentrations varied and were often

below the clinical decision limit for systemic inflammation. Two of the individual IMRD ANA^{neg} dogs had high CRP concentrations which might indicate that they suffered from concurrent or disorders other than IMRD. The CRP alterations in IMRD ANA^{pos} dogs are similar to those reported in humans with SLE [13]. Whether CRP has a potential clinical value for diagnosing and monitoring IMRD in individual dogs, warrants further investigations.

Abbreviations

ANA: antinuclear antibodies; ANA^{pos}: antinuclear antibody positive; ANA^{neg}: antinuclear antibody negative; CRP: C-reactive protein; hsCRP: high-sensitivity CRP; IIF: indirect immunofluorescence; IMRD: immune-mediated rheumatic disease; LOQ: limit of quantification; NSDTR: Nova Scotia Duck Tolling Retriever; SLE: systemic lupus erythematosus.

Authors' contributions

All the authors conceived and designed the study together. HDB, MK and HHA performed sampling and clinical evaluation of the dogs. AH performed the laboratory analyses and HDB the statistical analyses. HDB and AH drafted the manuscript. All authors participated in preparation of the manuscript. All authors read and approved the final manuscript.

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Competing interests

HHA has been deputy editor of *Acta Veterinaria Scandinavica*, but has not in any way been involved in or interacted with the review process or editorial decision making. AH's Ph.D. project, led by RH, at the Swedish University of Agricultural Sciences (SLU) was partly financed by Gentian AS, Moss, Norway, the company manufacturing the CRP assays in this study. The other authors declare that they have no competing interests.

Availability of data and materials

The dataset is available upon reasonable request.

Consent for publication

All dog owners gave their informed consent before participation.

Ethics approval and consent to participate

The study was approved by the Uppsala Animal Ethical Committee and Swedish Board of Agriculture (C417/12).

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References

- Bremer HD, Wilson A, Bonnett BN, Hansson-Hamlin H. Disease patterns and incidence of immune-mediated disease in insured Swedish Nova Scotia Duck Tolling Retrievers. *Vet Rec.* 2015;177:74.
- Hansson-Hamlin H, Lilliehöök I. A possible systemic rheumatic disorder in the Nova Scotia duck tolling retriever. *Acta Vet Scand.* 2009;51:16.
- Monier JC, Ritter J, Caux C, Chabanne L, Fournel C, Venet C, Rigal D. Canine systemic lupus-erythematosus. 2. antinuclear antibodies. *Lupus.* 1992;1:287–93.
- Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ. The 1982 revised criteria for the classification of systemic lupus-erythematosus. *Arthritis Rheum.* 1982;25:1271–7.
- Fournel C, Chabanne L, Caux C, Faure JR, Rigal D, Magnol JP, Monier JC. Canine systemic lupus erythematosus. I: a study of 75 cases. *Lupus.* 1992;1:133–9.
- Hansson-Hamlin H, Lilliehöök I, Trowald-Wigh G. Subgroups of canine antinuclear antibodies in relation to laboratory and clinical findings in immune-mediated disease. *Vet Clin Pathol.* 2006;35:397–404.
- Nakamura M, Takahashi M, Ohno K, Koshino A, Nakashima K, Setoguchi A, Fujino Y, Tsujimoto H. C-reactive protein concentration in dogs with various diseases. *J Vet Med Sci.* 2008;70:127–31.
- Lowrie M, Penderis J, Eckersall PD, McLaughlin M, Mellor D, Anderson TJ. The role of acute phase proteins in diagnosis and management of steroid-responsive meningitis arteritis in dogs. *Vet J.* 2009;182:125–30.
- Gabay C, Kushner I. Mechanisms of disease: acute-phase proteins and other systemic responses to inflammation. *N Eng J Med.* 1999;340:448–54.
- Kjelgaard-Hansen M, Strom H, Mikkelsen LF, Eriksen T, Jensen AL, Luntang-Jensen M. Canine serum C-reactive protein as a quantitative marker of the inflammatory stimulus of aseptic elective soft tissue surgery. *Vet Clin Pathol.* 2013;42:342–5.
- Foster JD, Sample S, Kohler R, Watson K, Muir P, Trepanier LA. Serum biomarkers of clinical and cytologic response in dogs with idiopathic immune-mediated polyarthropathy. *J Vet Intern Med.* 2014;28:905–11.
- Kjelgaard-Hansen M, Jensen AL, Houser GA, Jessen LR, Kristensen AT. Use of serum C-reactive protein as an early marker of inflammatory activity in canine type II immune-mediated polyarthritis: case report. *Acta Vet Scand.* 2006;48:9.
- Pepys MB, Lanham JG, Debeer FC. C-reactive protein in SLE. *Clin Rheum Dis.* 1982;8:91–103.
- Becker GJ, Waldburger M, Hughes GRV, Pepys MB. Value of serum C-reactive protein measurement in the investigation of fever in systemic lupus erythematosus. *Ann Rheum Dis.* 1980;39:50–2.
- Ohno K, Yokoyama Y, Nakashima K, Setoguchi A, Fujino Y, Tsujimoto H. C-reactive protein concentration in canine idiopathic polyarthritis. *J Vet Med Sci.* 2006;68:1275–9.
- Hillström A, Bylin J, Hagman R, Bjorhall K, Tvedten H, Konigsson K, Fall T, Kjelgaard-Hansen M. Measurement of serum C-reactive protein concentration for discriminating between suppurative arthritis and osteoarthritis in dogs. *BMC Vet Res.* 2016;12:240.
- Wilbe M, Kozyrev SV, Farias FH, Bremer HD, Hedlund A, Pielberg GR, et al. Multiple changes of gene expression and function reveal genomic and phenotypic complexity in SLE-like disease. *PLoS Genet.* 2015;11:e1005248.
- Wilbe M, Jokinen P, Truve K, Seppala EH, Karlsson EK, Biagi T, et al. Genome-wide association mapping identifies multiple loci for a canine SLE-related disease complex. *Nat Genet.* 2010;42:250–4.
- Hansson H, Trowald-Wigh G, Karlsson-Parra A. Detection of antinuclear antibodies by indirect immunofluorescence in dog sera: comparison of rat liver tissue and human epithelial-2 cells as antigenic substrate. *J Vet Intern Med.* 1996;10:199–203.
- Hillström A, Hagman R, Tvedten H, Kjelgaard-Hansen M. Validation of a commercially available automated canine-specific immunoturbidimetric method for measuring canine C-reactive protein. *Vet Clin Pathol.* 2014;43:235–43.
- Hillström A, Hagman R, Söder J, Häggstrom J, Ljungvall I, Kjelgaard-Hansen M. Validation and application of a canine-specific automated high-sensitivity C-reactive protein assay. *J Vet Diagn Invest.* 2015;27:182–90.
- Bennett D, Eckersall PD, Waterston M, Marchetti V, Rota A, McCulloch E, Sbrana S. The effect of robenacoxib on the concentration of C-reactive protein in synovial fluid from dogs with osteoarthritis. *BMC Vet Res.* 2013;9:42.
- Christensen MB, Langhorn R, Goddard A, Andreasen EB, Moldal E, Tvarijonavičute A, et al. Comparison of serum amyloid A and C-reactive protein as diagnostic markers of systemic inflammation in dogs. *Can Vet J.* 2014;55:161–8.
- Doumatey AP, Zhou J, Adeyemo A, Rotimi C. High sensitivity C-reactive protein (hs-CRP) remains highly stable in long-term archived human serum. *Clin Biochem.* 2014;47:315–8.

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