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Use of C-reactive protein as a marker for Lyme disease in Bernese Mountain Dog: A preliminary study

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Summary

The aim of the study was to determine the correlation between serum C-reactive protein (CRP) levels, the presence of antibodies to *B. burgdorferi*, and the occurrence of clinical signs of Lyme borreliosis in Bernese Mountain Dogs (BMDs). The study included 54 BMDs divided into three groups. The first group (n = 29) consisted of animals with signs suggestive of Lyme disease and rapid tests showing the presence of antibodies to *B. burgdorferi* in their serum. The second group (n = 15) included dogs that did not show signs of Lyme disease and had antibodies to spirochetes in their serum. The third group consisted of BMDs (n = 10) that showed no signs of disease and in whose serum no antibodies to *B. burgdorferi* were detected by rapid tests. Of the 29 dogs in the first group, 18 had elevated CRP levels. These animals showed signs of arthritis and renal failure. After treatment with doxycycline, the clinical signs of the disease disappeared, and the CRP concentration decreased to normal levels in 16 dogs. In the second group, 2 animals had elevated CRP levels, while in the third group, no antibodies to spirochetes were detected in the serum of any of the dogs, and CRP levels remained within the physiologically normal range. Our own observations indicate that C-reactive protein is a promising marker for monitoring the effectiveness of Lyme disease therapy in BMDs, as well as for distinguishing asymptomatic positive seroreagents from dogs of this breed that are actually suffering from the disease. However, to confirm its usefulness, further research is needed on representatives of this breed, especially those suffering from a chronic form of the disease.

Keywords: Bernese Mountain Dogs, borreliosis, CRP, serology test

Lyme disease is a multi-organ disease caused by an increased immune response of the organism to *Borrelia burgdorferi sensu lato* (18, 19). These bacteria are transmitted by *Ixodes* ticks (5, 22, 23). Despite numerous monitoring and prevention programs, the disease is still the most commonly diagnosed tick-borne infection in humans and animals in the Northern Hemisphere (16). Over the past decades, there has been an increasing number of patients with Lyme borreliosis and first cases in areas previously considered free of this disease. The probable cause is global warming, which results in a short, mild winter and early spring. Even slight increases in the ambient temperature allow ticks to colonize areas located higher above sea level, into which Lyme disease is introduced along with arachnids (16).

There are at least 52 species of *Borrelia*, of which 21 belong to the group *B. burgdorferi* sensu lato (LB), whose representatives are responsible for Lyme borreliosis, 29 are classified as *Borrelia*, causing recurrent fever, while the pathogenicity of the other two spirochete species has yet to be definitively established.

For spirochete transmission to occur, infected ticks (in central Europe, *Ixodes ricinus*) must feed on the host for at least 48 hours. During this time, the bacteria multiply in the arachnid intestines, penetrate to the haemolymph, which enables them to reach the salivary glands. *Borrelia* initially multiply locally in the skin, at the site of inoculation, and then spread to other tissues, including joints.

An important element in the pathogenesis of the disease is that the spirochetes persist extracellularly: they multiply in the intercellular spaces in the skin. The clinical signs of the disease are related to the increased inflammatory reaction that takes place in the body in the course of the disease. In this way, flagellin, one of the most immunogenic proteins, stimulates the production of antibodies that bind to axons of nerve cells, which may result in the development of neurological symptoms in humans. Increased expression of Il-8 in the synovial membranes is the main cause of polyarthritis, while strong general inflammatory reactions (fever, increased heart and respiration rates) may be associated with the release of increased quantities of cytokines, TNF, Il-6, and Il-8.

Two forms of the disease are distinguished in dogs: articular and renal. The articular form is characterized by fever and shift lameness. The pathological changes in joints are generally progressive, and chronic polyarthritis may persist despite treatment.

The renal form is a consequence of glomerulonephritis, leading to renal failure with proteinuria, uraemia, and peripheral oedema.

Clinical observations made by many authors indicate that the definition of Lyme disease as a multi-organ disease, or even a multi-system disease, is absolutely correct. Although the most common disorders occurring in its course are related to the locomotor system, there are also signs from the circulatory system and the skin (1, 4, 7, 9).

It should be noted, however, that not every contact with the pathogen leads to the development of the disease. This is evidenced by the positive results of serological tests for Lyme disease in animals and humans that do not give rise to signs of the disease (2, 11-13). Serological screening is extremely helpful in diagnosing asymptomatic infections and in determining the epizootic status regarding Lyme disease in a given area. Most often, immunofluorescence and ELISA tests are used for this purpose. Rapid serological tests may also be helpful (2, 8, 10).

Another aspect is the problem of Lyme disease in Bernese Mountain Dogs. Our own observations, literature data, and reports from veterinarians indicate that dogs of this breed often test positive for antibodies against *Borrelia burgdorferi* in rapid diagnostic tests without showing any clinical signs of the disease. Also, quantitative determination of the immunoglobulin level for spirochetes indicates that Bernese Mountain Dogs may have an increased hereditary susceptibility to *Borrelia* infections.

Due to the challenges of diagnosing Lyme disease in BMDs, there is a need to search for alternative methods to diagnose this disease in this breed. The aim of this study was to determine the correlation between serum C-reactive protein (CRP) levels, the presence of antibodies to *B. burgdorferi*, and the occurrence of clinical signs typical of Lyme disease in BMDs.

Material and methods

The study included 54 BMDs aged six months to eight years, divided into three groups. The first group (n = 29) consisted of animals with signs suggestive of Lyme disease (shifting lameness n = 20, nephritis n = 9) and serum antibodies to *Borrelia burgdorferi* detected by rapid tests. The second group (n = 15) included dogs without signs of Lyme disease, but with serum antibodies to spirochetes. The third group consisted of Bernese Mountain Dogs (n = 10) that showed no signs of disease and had no serum antibodies to *Borrelia burgdorferi* according to rapid tests.

Blood was collected from the antecubital vein of all dogs for haematological tests (Mythic 5Vet PRO CORMAY), determination of basic serum biochemical parameters (AST, ALT, urea, creatinine) (ACCENT S120 CORMAY), rapid serological tests for *B. burgdorferi*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, *Dirofilaria immitis*, and rapid tests for serum CRP concentration.

Serological testing for selected tick-borne diseases. Serological testing for *B. burgdorferi*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Dirofilaria immitis* was performed using CaniV4 rapid tests (VetExpert). The test material was whole blood collected into EDTA tubes. 20 µl of blood was applied to the assay and flooded with 3 drops of buffer. Results were read after 15 minutes.

Serum CRP in dogs. C-reactive protein concentrations were measured with a BIONOTE Vcheck Analyzer V200 (Vet Expert) using Canine CRP 2.0 assays (Vet Expert). 5 µl of serum or plasma was applied to the assay, flooded with coagulate, and then analyzed. In the animals of the first group, CRP levels were measured twice: on admission to the clinic and at the end of six weeks of doxycycline therapy at a dose of 10 mg/kg.

Statistical analysis. The results obtained were analyzed using the Shapiro-Wilk test to determine the normality of the distribution. Since the distribution in the first and second groups was not normal, the Kruskal-Wallis test, followed by the post-hoc Dunn's test with Bonferroni correction, was used for statistical analysis of all groups. Values with a probability of $p < 0.05$ were considered statistically significant. Correlations between the parameters studied were calculated by the Spearman method.

Results and discussion

Of the 29 dogs in the first group, whose serum showed the presence of antibodies to *Borrelia*, 18 dogs had elevated CRP levels. These animals developed signs of arthritis (n = 20) and renal failure (n = 9). Haematological and biochemical abnormalities found

in these animals were leukocytosis (n = 23), thrombopenia (n = 10), elevated ALT activity (n = 22), elevated AST activity (n = 17), elevated urea (n = 10), and elevated creatinine (n = 9). Following treatment with doxycycline, clinical signs of the disease resolved in all 29 animals in this group, while CRP levels decreased to normal levels in 16 of the 18 dogs that initially had elevated levels of this parameter (Tab. 1).

In the second group of dogs, which showed no signs of disease, but whose serum contained antibodies to *Borrelia*, 2 animals had elevated CRP levels. Other haematological and biochemical abnormalities found in these animals were leukocytosis (n = 2), thrombopenia (n = 2), elevated ALT activity (n = 5), elevated AST activity (n = 2), elevated urea (n = 3), and elevated creatinine (n = 2) (Tab. 2).

In the third group, none of the dogs had serum antibodies to *Borrelia*, and CRP levels remained within the physiologically normal range. The other haematological and biochemical parameters also showed no deviation from normal in those animals (Tab. 3).

Statistical analysis of the study groups showed a statistically significant increase in the CRP concentration in the first group compared to the second group ($p = 0.0037$) and the third group ($p = 0.00234$). There was also a positive and very high correlation ($r = 0.9059$) between elevated CRP levels and the presence of clinical signs of Lyme disease (Fig. 1). All dogs

Tab. 1. Results of serological (CaniV-4 test) and haematological blood tests, CRP concentrations, and serum biochemical parameters and the occurrence of clinical signs in the dogs from the first group

No.	Presence of antibodies to Bb in the CaniV-4 test	CRP concentration (mg/l)	WBC (10^3/litre)	PLT (10^3/litre)	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)	Clinical signs	CRP concentration (mg/l) after 6 weeks of treatment
1	+	200.0	50.4	260	158.0	33.0	44.2	–	shifting lameness, fever	16.5
2	+	32.5	22.0	160	100.5	60.8	114.2	2.2	kidney pain, oliguria, fever	10.0
3	+	32.5	40.2	120	106.9	85.0	86.9	2.5	shifting lameness, fever	10.5
4	+	75.5	45.0	220	113.4	107.5	322.6	1.8	kidney pain, oliguria, fever	12.5
5	+	121.0	40.6	112	448.4	741.7	39.4	0.62	shifting lameness, fever	13.5
6	+	75.5	32.4	100	110.7	48.2	38.2	1.23	shifting lameness, fever	10.5
7	+	150.5	36.4	111	84.0	65.2	314.2	3.3	kidney pain, oliguria, fever	12.5
8	+	159.0	29.4	185	112.4	82.5	280.2	2.1	kidney pain, oliguria, fever	50.5
9	+	159.0	25.6	160	80.6	–	42.5	–	shifting lameness, fever	12.0
10	+	48.5	41.2	121	44.0	62.0	41.1	0.7	shifting lameness, fever	10.0
11	+	50.5	33.1	114	160.2	76.2	36.1	1.2	shifting lameness, fever	12.0
12	+	39.5	26.6	160	143.8	60.7	44.1	0.8	shifting lameness, fever	10.0
13	+	157.5	31.6	139	215.6	50.9	32.5	1.4	shifting lameness, fever	13.5
14	+	150.5	21.0	112	150.7	53.4	185.6	3.0	kidney pain, oliguria, fever	12.5
15	+	150.5	37.2	222	187.2	98.7	111.2	2.4	kidney pain, oliguria, fever	11.5
16	+	39.5	42.5	255	224.5	460.8	40.7	0.9	shifting lameness, fever	10.5
17	+	18.5	33.6	170	66.0	44.0	42.3	0.8	shifting lameness, fever	10.0
18	+	112.5	50.2	185	116.8	60.2	39.9	1.2	shifting lameness, fever	11.5
19	+	10.0	32.5	114	340.5	56.7	112.9	3.4	kidney pain, oliguria, fever	10.0
20	+	200.0	40.6	312	289.3	89.0	40.4	1.3	shifting lameness, fever	75.5
21	+	10.0	11.7	112	86.2	66.0	33.7	0.9	shifting lameness, fever	10.5
22	+	16.5	19.52	302	173.3	26.7	32.8	0.92	shifting lameness, fever	10.0
23	+	10.0	17.32	85	19.9	27.5	24.8	0.72	shifting lameness, fever	10.5
24	+	10.0	15.26	224	–	–	143.1	2.62	kidney pain, oliguria, fever	10.0
25	+	16.5	23.1	777	17.9	51.7	18.7	0.27	shifting lameness, fever	11.5
26	+	10.0	10.18	420	43.0	8.0	214.2	2.45	kidney pain, oliguria, fever	11.0
27	+	16.5	14.3	220	76.7	24.3	31.6	1.02	shifting lameness, fever	10.5
28	+	16.5	9.24	73	13.0	25.5	15.9	0.62	shifting lameness, fever	11.5
29	+	16.5	8.74	254	163.1	25.7	30.8	0.61	shifting lameness, fever	10.0
Reference ranges		> 30 abnormal	6.0-17.0	117-460	3.0-50.0	1.0-37.0	20.0-45.0	1.0-1.7		> 30 abnormal

Tab. 2. Results of serological (CaniV-4 test) and haematological blood tests. CRP concentration and serum biochemical parameters in the dogs from the second group

No.	Presence of antibodies to Bb in the CaniV-4 test	CRP concentration (mg/l)	WBC (10^3/litre)	PLT (10^3/litre)	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)	Clinical signs
1	+	11.0	5.4	320	42.1	32.6	76.2	2.12	none
2	+	10.5	6.2	198	22.6	28.7	44.1	1.55	none
3	+	13.5	15.1	148	58.1	34.8	28.4	1.15	none
4	+	10.5	11.8	324	14.1	28.7	28.1	1.22	none
5	+	12.0	14.0	121	18.2	14.1	27.2	1.41	none
6	+	11.0	12.3	140	13.2	28.7	29.6	1.32	none
7	+	10.0	22.9	307	14.8	26.2	21.2	1.37	none
8	+	185.0	18.4	212	48.1	25.4	64.5	2.10	none
9	+	200.0	17.8	276	62.1	38.2	18.1	1.23	none
10	+	10.0	16.8	402	75.3	35.2	40.1	1.62	none
11	+	11.0	14.2	328	40.7	28.9	26.4	1.56	none
12	+	10.0	16.1	170	77.1	12.9	33.6	1.05	none
13	+	10.5	12.8	450	18.8	32.1	42.8	1.34	none
14	+	11.0	8.1	298	86.1	40.2	32.4	1.51	none
15	+	10.0	15.8	362	21.4	8.2	112.3	1.21	none
Reference ranges		> 30 abnormal	6.0-17.0	117-460	3.0-50.0	1.0-37.0	20.0-45.0	1.0-1.7	

Tab. 3. Results of serological (CaniV-4 test) and haematological blood tests. CRP concentration and serum biochemical parameters in the dogs from the third group

No.	Presence of antibodies to Bb in the CaniV-4 test	CRP concentration (mg/l)	WBC (10^3/litre)	PLT (10^3/litre)	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)	Clinical signs
1	–	10.0	16.2	120	42.2	1.2	21.2	1.02	none
2	–	13.0	12.1	224	46.7	22.2	19.5	1.22	none
3	–	11.5	13.4	321	32.1	30.2	24.3	1.15	none
4	–	11.5	13.0	140	13.2	2.0	30.1	1.22	none
5	–	10.0	16.1	360	24.2	22.8	33.2	1.27	none
6	–	10.5	14.6	380	36.8	30.1	28.5	1.34	none
7	–	10.0	15.5	222	38.7	34.2	27.2	1.49	none
8	–	13.5	16.2	430	22.2	28.2	40.1	1.52	none
9	–	10.5	14.3	420	40.1	24.1	42.8	1.33	none
10	–	12.5	13.8	380	33.2	3.2	20.6	1.54	none
Reference ranges		> 30 abnormal	6.0-17.0	117-460	3.0-50.0	1.0-37.0	20.0-45.0	1.0-1.7	

with clinical signs in the first group were also found to have antibodies against spirochetes. In contrast, the analysis of the second group showed a high correlation (r = 0.8259) between the presence of antibodies and the absence of an elevated CRP concentration in dogs showing no signs of Lyme disease.

C-reactive protein is the major acute phase protein in dogs. Its concentration increases in many diseases and pathological conditions, although it has a poor correlation with total white blood cell count and a negative correlation with serum albumin concentration (14). Long-term elevated CRP levels have been observed in conditions such as hidradenitis suppurativa, polyarthritis, and pancreatitis. Interestingly, elevated levels of this protein have not been found in metabolic disorders, such as hypothyroidism, diabetes, or epilepsy. Persistently elevated levels of acute phase proteins in dogs are seen in the course of infectious and invasive diseases. In animals experimentally infected with *Ehrlichia canis* rickettsia, a fourfold increase in CRP concentrations was observed, peaking during the period of increased pathogen replication in the body (17). An increase in serum CRP levels has also been reported in dogs with babesiosis, and its concentration in plasma has been shown to correlate with the severity of the disease process (3). A similar relationship was observed in the course of leishmaniasis, in which CRP levels increased 30-60-fold in infected dogs (accompa-

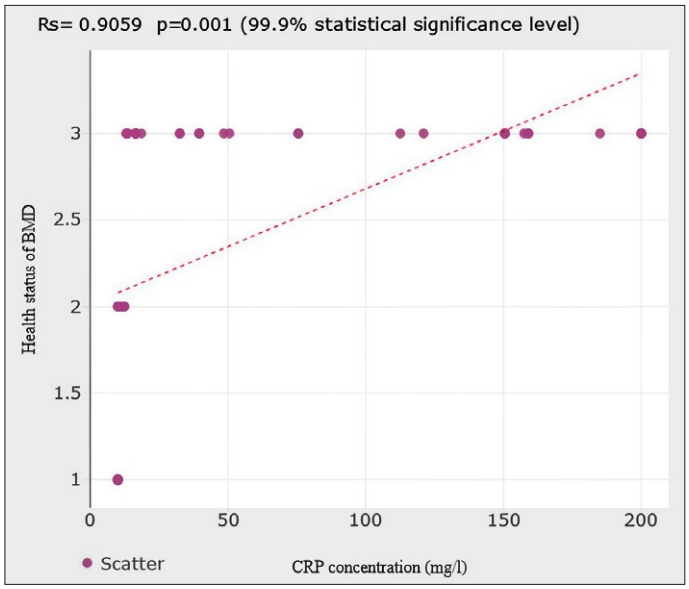

Fig. 1. Correlation between elevated CRP levels and the presence of clinical signs of Lyme disease

nied by increases in HP and CP levels). According to the authors' study, there was a positive and very high correlation between elevated CRP concentrations and the presence of clinical signs of Lyme disease, with a maximum CRP concentration of 200 mg/l, almost seven times as high as normal. However, the presence of antibodies to *Borrelia* in dogs was not shown to be the only factor influencing the increase in their serum CRP concentrations. In the first group, the presence of IgG to spirochetes was shown in all animals, while an increase in CRP concentration was shown in 18 dogs. In animals in this group, a positive correlation between CRP and the presence of serum antibodies could be seen, but it should be borne in mind that all dogs showed clinical signs of Lyme disease, which explained this correlation. Therefore, this positive correlation was due to the presence of clinical signs rather than the presence of serum antibodies. This was confirmed by the results of the second group, where only 2 of the 15 dogs with antibodies to spirochetes in their blood had elevated CRP levels, and none showed clinical signs of Lyme disease. As shown by observations in human medicine, elevated CRP levels are found in humans with Lyme disease early in the course of the infection (20), whereas levels of this marker decrease as the disease process becomes chronic. In our study, all dogs in the first group showed signs of early Lyme disease (no myocarditis, chronic arthritis, or nephritis), and the fact that CRP concentrations were elevated in these dogs may suggest that the findings in this regard are consistent with those obtained in humans. It should also be noted that CRP concentrations of the dogs in the first group decreased after treatment to normal levels in 16 of the 18 dogs. This suggests that C-reactive protein could be used as a marker of the efficacy of Lyme borreliosis therapy. Similar observations have been made in humans (15), where it has been shown that CRP can be useful in the differential diagnosis of patients with suspected Lyme arthritis and that the concentration of this marker decreases significantly after therapy. It can therefore be concluded that CRP is a useful marker of full-blown acute Lyme disease in Bernese Mountain Shepherd Dogs and that changes in its concentration reflect the efficacy of therapy in patients with Lyme disease.

This marker has a further application in the diagnosis of Lyme disease in BMDs. This breed is known to be predisposed to Lyme disease, and many of the dogs have elevated titers of antibodies to *B. burgdorferi* without having been exposed to the spirochete. This finding was provisionally confirmed by the results of studies by Gerber et al. (6). These authors tested 160 Bernese Mountain Dogs and 62 large-breed control dogs (all animals had similar fur and were kept under similar conditions) for the presence of antibodies against *B. burgdorferi*. Serological examination was performed using ELISA and Western blotting. The presence of elevated antibody titres was found in 58% of the Bernese Mountain Dogs and in only 15% of the control dogs. The authors were unable to determine the cause of such a large discrepancy between the groups, suspecting that it might be due to breed predisposition in Bernese Mountain Dogs. Similar conclusions were also reached by German researchers who showed the presence of antibodies against *B. burgdorferi* in the serum of 43.3% of Bernese Mountain Dogs and in only 24.6% of control dogs (21). Yet another Bavarian study with a dog population living in the same geographic area found antibodies against spirochetes in 92% of Bernese Mountain Dogs (12 out of 13 dogs were seropositive) and only 7% of other breeds (13 out of 187 dogs were seropositive). Since it was excluded that these differences were due to increased exposure of Bernese Mountain Dogs to ticks (all animals used in the study came from the same geographical area), it was hypothesized that Bernese Mountain Dogs may be genetically predisposed to *B. burgdorferi* infections, which should be taken into account in clinical practice.

A low serum CRP concentration in dogs with antibodies to the spirochete, compared with a high concentration of this protein in dogs with signs of Lyme disease and the presence of serum antibodies to the bacteria, may, therefore, be considered a marker to distinguish animals with actual Lyme disease from positive healthy seroreagents.

In conclusion, C-reactive protein is a promising marker for monitoring the efficacy of Lyme disease therapy in BMDs and for distinguishing asymptomatic positive seroreagents from dogs of this breed that are actually suffering from the disease. However, further studies in this breed, especially in dogs with chronic disease, are required to confirm the utility of C-reactive protein.

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