FULL PAPER *Clinical Pathology*

C-Reactive Protein Concentration in Dogs with Various Diseases

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ABSTRACT. To investigate the clinical utility of C-reactive protein (CRP) determination in dogs, its plasma concentration was measured by a laser nephelometric method in 928 dogs with various diseases, and was compared with other inflammatory parameters. CRP concentration was elevated in various inflammatory diseases, this was most frequently observed in cases with neoplastic and immune-mediated diseases. All cases of pyometra, panniculitis, acute pancreatitis, polyarthritis, and hemangiosarcoma showed significantly elevated CRP concentrations. On the other hand, the CRP concentration was elevated only in few cases of neurological diseases such as epilepsy, meningoencephalitis, and hydrocephalus and endocrine diseases such as hypothyroidism, hyperadrenocorticism, and diabetes mellitus. Only a weak correlation was observed between the CRP concentration and white blood cell (WBC) counts (r=0.44) but no correlation with band neutrophil counts. There was no correlation between the CRP and albumin concentrations, but a weak negative correlation (r=–0.40) when excluding chronic intestinal diseases and nephrotic syndrome, which can cause protein loss. Thus, CRP can be useful to detect inflammations that cannot be detected by WBC and, or band neutrophil counts, suggesting that the examination of CRP concentration is essential as routine diagnostic test.

KEY WORDS: albumin, C-reactive protein (CRP), canine, neutrophil, WBC.

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Acute phase proteins (APPs) constitute a group of proteins whose blood concentration changes rapidly in response to stress such as infection and tissue damage. C-reactive protein (CRP), haptoglobin (Hp), ceruloplasmin (Cp), serum amyloid A (SAA), α-1 acid glycoprotein, and fibrinogen are positive APPs, and their blood concentrations are elevated mainly due to increased production in the liver [3]. On the other hand, albumin and transferin are known as negative APPs [5].

C-reactive protein (CRP) is a representative APP, and it was originally detected in the serum of human pneumonia patients as a protein that precipitates with the C-polysaccharide of pneumococci. The blood concentration of CRP is known to be elevated 100- to 1000-fold within 24–48 hr after inflammatory irritation, and it is broadly used as a sensitive inflammatory marker in human medicine. Similarly, the CRP concentration is known to be elevated in canine patients with inflammatory conditions, and its elevation has been reported in infectious diseases such as leptospirosis, babesiosis, and parvovirus infection [5]; surgical trauma [6]; malignant tumors such as lymphoma and hemangiosarcoma [12, 18]; pyometra [8]; acute pancreatitis [11]; immune-mediated hemolytic anemia; arthritis; glomerulonephritis [13]; and experimental inflammation. However, in veterinary medicine, CRP levels have not been widely examined in routine tests, and there are no thorough studies concerning CRP concentration in disease. Furthermore, comparison between CRP and other inflammatory markers, including white blood cell (WBC) counts, neutrophil counts, and body temperature has only been performed in a limited number of cases [2, 20], and the clinical significance of CRP remains unknown.

Canine CRP has been measured using ELISA and time-resolved immunofluorometric assay (TR-IFMA) [5, 15]; however, these tests require specialized equipment and are time-consuming. In Japan, a laser nephelometric immunoassay (LNIA), for canine CRP, was developed in 1998 [13, 14] and is now commercially available. In this assay, CRP and anti-canine CRP antiserum are mixed in a specific buffer, exposed to a laser beam, and the scattered light is then measured. This equipment enabled us to measure the CRP concentration in plasma or serum easily and rapidly in the clinical setting.

In this study, we evaluated the clinical usefulness of CRP based on the large amount of data accumulated by the routine measurement of canine CRP levels. In this study, we investigated the CRP value in various diseases and compared the CRP concentration in each with other traditional inflammatory markers such as body temperature, WBC counts, neutrophil counts, and band neutrophil counts. We also evaluated the correlation between the concentrations of CRP and albumin, a negative APP.

MATERIALS AND METHODS

Clinical cases: We reviewed the medical records of 928 canine patients that were referred to and diagnosed at the Veterinary Medical Center of the University of Tokyo from 2002 to 2006. Patients with multiple diseases or unconfirmed diagnoses as well as patients that had already been

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treated were excluded from the study.

Laboratory examination: The details obtained from the medical records were age, gender, body temperature, clinical signs, diagnosis, WBC count, segmented neutrophil count, band neutrophil count, albumin concentration, and CRP concentration. The data of the blood examinations obtained on the day of diagnosis were used for analysis. Body temperature was measured via the rectum, and a temperature higher than 39.7°C was considered to indicate pyrexia [1]. WBC counts were obtained using an automatic analyzer (Hematology Analyzer Ac-T diff, Beckman Coulter, CA) and were considered high when the value exceeded 17,500/μl. Band neutrophil and segmented neutrophil counts were determined based on the percentage of WBCs (upper limit of the reference range; 1,000/μl and 11,500/μl, respectively). The plasma albumin concentration was measured by automatic blood chemistry measurement equipment (DRI-CHEM 5500, Fujifilm Medical Co., Japan; reference range, 2.6–4.0 g/d*l*).

CRP measurement: Plasma CRP concentration was measured using a canine CRP measurement kit (Laser CRP-2, Arrows Co., Ltd., Osaka, Japan) according to the manufacturer's instructions. The reference range of the plasma CRP concentration was considered as less than 0.95 mg/d*l* based on the manufacturer's data sheet and a previous report [13] which agree with our laboratory's reference range (data not shown).

Statistical analysis: The correlation of the CRP concentration with body temperature, WBC count, neutrophil count, band neutrophil count, and albumin concentration was analyzed using Spearson's rank correlation coefficient. The CRP concentration in 2 groups with each variable was compared by the Mann-Whitney U test. A P value of less than 0.05 was considered statistically significant.

RESULTS

Representative diseases with a high CRP level: We classified 928 canine patients according to their disease. Table 1 shows the diseases in which the CRP concentration was higher than the reference range (>1 mg/d*l*) in more than 50% of the cases. All cases of sterile nodular panniculitis, pyometra, acute pancreatitis, immune-mediated polyarthritis, and hemamgiosarcoma showed elevated CRP concentrations. Particularly, patients with sterile nodular panniculitis, pyometra, and acute pancreatitis demonstrated a CRP concentration exceeding 10 mg/d*l* in many cases (over 70% of all cases). In this study, cases with neoplastic diseases (hemangiosarcoma, nasal adenocarcinoma, cholangiocellular carcinoma, acute lymphoblastic leukemia, malignant histiocytosis, and lymphoma) and immune-mediated diseases (sterile nodular panniculitis, idiopathic polyarthritis, and immune-mediated hemolytic anemia) were observed most frequently, that is, 30% (n=274) and 10% (n=97), respectively. The CRP concentration was also elevated in lower respiratory diseases such as bronchopneumonia and pneumonia.

Representative diseases without a high CRP level: Table 2 shows the diseases in which the median CRP concentration did not exceed the reference range and the percentage of cases with a CRP level higher than the reference range, which constituted less than 20% (n≥5) of the total cases. In neurological diseases such as epilepsy, atlantoaxial subluxation, hydrocephalus, and necrotizing meningoencephalitis and endocrine diseases such as hypothyroidism, hyperadrenocorticism, and diabetes mellitus, most of the cases did not exhibit elevated CRP concentrations. The most frequently observed respiratory diseases were those causing local inflammation of the upper respiratory tract such as

Table 1. Representative diseases with significantly high plasma CRP levels

Diseases*	(n)	CRP (mg/d*l*)			Number of dogs	
		Median**	Min.	Max.	≥1 mg/d*l* (%)	≥10 mg/d*l* (%)
Pyometra	7	20.0	3.5	>20	7 (100%)	5 (71%)
Sterile nodular panniculitis	7	20.0	10.0	>20	7 (100%)	5 (100%)
Acute pancreatitis	5	15.0	6.1	>20	5 (100%)	3 (60%)
Idiopathic polyarthritis	31	13.0	1.7	>20	31 (100%)	21 (67%)
Hemangiosarcoma	5	7.6	3.1	16	5 (100%)	2 (40%)
Nasal adenocarcinoma	5	6.7	0.1	9	4 (80%)	0 (0%)
Immune-mediated hemolytic anemia	35	6.5	0	>20	30 (86%)	9 (26%)
Cholangiocellular carcinoma	7	6.3	0.1	19	6 (86%)	2 (29%)
Acute lymphoblastic leukemia	6	4.5	0.1	>20	5 (83%)	1 (17%)
Malignant histiocytosis	16	4.3	0.3	>20	11 (69%)	4 (25%)
Lymphoma	127	3.5	0	>20	91 (72%)	26 (21%)
Bronchopneumonia/Pneumonia	16	3.3	0.2	11	12 (75%)	1 (6%)
Malignant mesothelioma	7	2.7	0.7	15	5 (71%)	1 (14%)
Demodicosis	7	2.6	0	7.1	5 (71%)	0 (0%)
Chronic hepatitis	7	1.6	0	9.7	4 (57%)	0 (0%)
Cardiac tamponade	6	1.5	0.7	6.7	3 (50%)	0 (0%)
Myelodysplastic syndrome	5	1.3	0	14	3 (60%)	1 (20%)
Intestinal adenocarcinoma	13	1.0	0	14	8 (62%)	3 (23%)
Immune-mediated thrombocytopenia	7	1.0	0	12	4 (57%)	1 (14%)

* Diseases (n≥5) in which the median of the CRP concentration exceeded the reference range (≥1 mg/d*l*) were shown.
** CRP values higher than the measurement limit (>20 mg/d*l*) were statistically analyzed as 20 mg/d*l*.

Table 2. Representative diseases without increased CRP levels

Diseases*	(n)	CRP (mg/dl)			Number of dogs
		Median	Min.	Max.	≥1 mg/dl (%)
Epilepsy	33	0	0	0.7	0 (0%)
Atlantoaxial subluxation	5	0	0	0.4	0 (0%)
Hypothyroidism	5	0	0	0.5	0 (0%)
Urolithiasis	5	0	0	0.7	0 (0%)
Brain tumor	8	0	0	1.0	1 (13%)
Intervertebral disk protrusion	22	0.1	0	3.0	3 (14%)
Hyperadrenocorticism	11	0.1	0	1.5	1 (9%)
Tracheal collapse	11	0.1	0	0.7	0 (0%)
Rhinitis	8	0.1	0	0.7	0 (0%)
Mitral valve insufficiency	10	0.2	0	2.1	1 (10%)
Necrotizing meningoencephalitis	9	0.2	0	0.8	0 (0%)
Hypoadrenocorticism	7	0.2	0	7.2	1 (14%)
Portosystemic shunt	13	0.3	0	3.0	2 (15%)
Bronchitis	6	0.3	0	1.8	2 (33%)
Megaesophagus	5	0.3	0	0.6	0 (0%)
Hydrocephalus	10	0.3	0	1.1	1 (10%)
Allergic bronchitis	5	0.4	0.1	0.8	0 (0%)
Diabetes mellitus	5	0.4	0.2	0.5	0 (0%)
Leiomyosarcoma	6	0.4	0.2	2.7	1 (17%)

* Diseases (n≥5) in which the median of the CRP concentration did not exceed the reference range (<1 mg/dl) are shown.

rhinitis and allergic rhinitis. In other diseases such as urolithiasis, mitral valve insufficiency, tracheal collapse, and megaesophagus, the elevation of CRP concentration was not evident.

Body temperature and CRP concentration: The correlation between CRP concentration and body temperature was evaluated in 825 dogs. By using Spearson's rank correlation coefficient, no correlation (r=0.213) was observed. On comparison of the CRP concentrations between the group with pyrexia (n=83, median=14.0 mg/dl) and that without pyrexia (n=773, median=0.6 mg/dl), the CRP level was observed to be significantly higher in the group with pyrexia (P<0.001).

WBC counts and CRP concentration: The correlation between CRP concentration and WBC counts is shown in Fig. 1. The CRP concentration was dissociated from WBC counts in many cases, and by using Spearson's rank correlation coefficient, only a weak correlation was observed (r=0.44, P<0.001). When we compared the CRP concentration of the group with high WBC counts (>17,500/μl) (n=385, median=3.8 mg/dl) to that of the group with normal WBC counts (≤17,500/μl) (n=529, median=0.3 mg/dl), the CRP concentration was significantly higher in the group with high WBC counts (P<0.001). The correlations of CRP concentration with band neutrophil and segmented neutrophil counts were subsequently examined. No correlation was observed with the band neutrophil counts (r=0.24) and with the segmented neutrophil counts (r=0.38).

Plasma albumin and CRP concentrations: We analyzed the correlation between the CRP and plasma albumin concentrations in 536 dogs. Spearman's rank correlation coefficient was r=–0.30, indicating that no correlation was observed. However, when we examined the correlation in 444 dogs without chronic intestinal diseases (n=88) or nephrotic syndrome (n=4), which are the most common causes of protein loss, Spearman's rank correlation coefficient was r=–0.40, and a weak correlation was observed (P<0.001) (Fig. 2). Moreover, when we compared the CRP concentration of the group with plasma albumin concentrations higher than 2.6 mg/dl (n=408, median=0.8 mg/dl) to that of the group with low plasma albumin concentrations (≤2.6 mg/dl) (n=128, median=5.0 mg/dl), the CRP concentration was significantly higher in the group with low plasma albumin concentrations (P<0.001).

DISCUSSION

In this study, we evaluated the plasma CRP concentration in dogs with various diseases and compared its elevation in each disease. Although the CRP concentration was elevated in many diseases, it was most frequently observed in neoplastic diseases and immune-mediated diseases. Moreover, the extent of elevation of CRP levels in these diseases was also greater than that in other diseases. It has been reported that elevated CRP concentrations are also commonly observed in patients with neoplastic [10] and immune-mediated diseases in human medicine [7, 9]. However, we have to consider that the frequency of the diseases studied was biased since this study was conducted at a teaching hospital in a university.

The CRP concentration increases in canine tumor patients, particularly in those with hematopoietic tumors such as lymphoma and leukemia; moreover, the elevation of CRP concentration does not vary depending on the origin of solid tumors [18]. In mammary tumors, it is reported that the increase in CRP concentration is higher in patients with

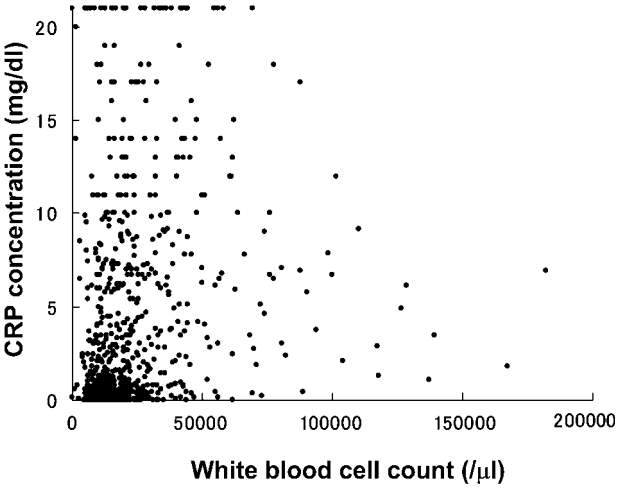

Fig. 1. The correlation of CRP concentration and WBC count in 914 canine patients. Spearman's rank correlation coefficient was r=0.44, P<0.001. CRP values higher than the measurement limit (>20 mg/d*l*) were statistically analyzed as 21 mg/d*l*.

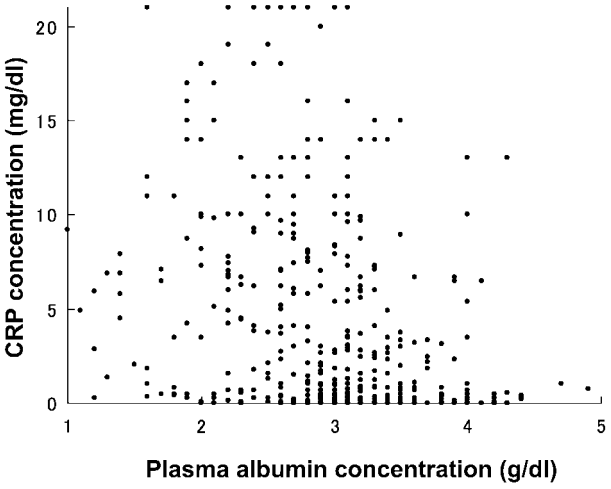

Fig. 2. The correlation of CRP and albumin concentrations in 444 canine patients. Patients with chronic intestinal diseases and nephrotic syndrome were excluded. Speaman's rank correlation coefficient was r=–0.40, P<0.001. CRP values higher than the measurement limit (>20 mg/d*l*) were statistically analyzed as 21 mg/d*l*.

disseminated lesions than in those with localized or benign lesions [4]. Further, we observed that patients with tumors affecting systemic organs, such as lymphoma and hemangiosarcoma demonstrated significantly elevated CRP concentrations in this study. On the other hand, most patients with localized tumors such as leiomyosarcoma did not demonstrate elevated CRP concentrations. The fact that the CRP concentration did not increase in most patients with upper respiratory diseases (e.g. rhinitis) but did increase in patients with lower respiratory diseases (e.g., pneumonia) indicates that localized lesions might not reflect on the CRP concentration even in inflammatory diseases.

Regarding neurological diseases, CRP concentration was not elevated in patients with necrotizing meningoencephalitis, indicating that we cannot determine intracranial inflammation based on the CRP concentration. Moreover, since the CRP concentration did not increase in patients with intervertebral disk protrusion, it might be useful in distinguishing arthritis from spinal/brain diseases in patients with lameness. Thus, although CRP is a nonspecific inflammatory marker, it could facilitate diagnosis by indicating the presence and the extent (e.g., localized vs. generalized, neurological vs. other) of inflammation.

In veterinary medicine, WBC counts and band neutrophil counts have been commonly used as inflammatory markers. The correlation of the CRP concentration with body temperature and WBC counts in dogs with experimentally induced inflammation has been reported [20]; however, statistical analysis in clinical patients has not been performed. Although we examined numerous clinical patients in this study, only a weak correlations of CRP concentration with WBC counts was observed and no correlation was observed with band neutrophil counts. Moreover, during inflammation, the CRP level and WBC count were dissociated in many cases. This can be attributed to several reasons. Firstly, as CRP concentration might increase prior to the WBC count since CRP levels change more rapidly than WBC counts. Further, myelosuppression and/or myeloproliferative diseases may have caused this result. The dissociation between the CRP concentration and band neutrophil counts indicates that not only WBC counts but also CRP concentration should be measured routinely as an inflammatory marker.

Although albumin is a traditional negative acute phase protein (APP) [5], there was no correlation between CRP and albumin concentrations in this study. However, when we excluded cases with chronic intestinal diseases (inflammatory bowel disease, lymphangiectasis, etc.), and nephrotic syndrome, which often cause protein loss, a weak negative correlation was observed. A previous study had also reported that albumin concentration decreases in dogs with experimentally induced inflammation [20]. However, as albumin concentration is affected by dehydration and malnutrition in canine patients, it would be difficult to be used it as an inflammatory marker in a clinical setting. Since inflammatory markers, including CRP and albumin concentrations have been used to score general conditions in human medicine recently [17, 19], and these might also be used to assess the severity and prognosis of chronic diseases in canine patients.

In conclusion, we observed elevated CRP concentrations in many diseases with inflammation and tissue damage, and this elevation was significant, particularly in neoplastic and immune-mediated diseases. Measuring the CRP concentration along with WBC counts is clinically valuable as a screening test for new patients to detect inflammation. In

human medicine, CRP has been reported to indicate the severity and prognosis of various diseases [9, 10, 16, 17]. In veterinary medicine, it has been reported to indicate the response to treatment and prognosis in idiopathic polyarthritis [14]. Further studies are needed to examine the variation in the CRP concentration during clinical courses and to evaluate its relationship with disease severity in order to determine its potential as a prognostic factor.

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