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Serum C-Reactive Protein as a Diagnostic Biomarker in Dogs with Bacterial Respiratory Diseases

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Background: C-reactive protein (CRP) is a major acute-phase protein in dogs. Serum concentrations are low in healthy animals, but increase rapidly after inflammatory stimuli.

Objective: The aim of the study was to investigate CRP concentrations in various respiratory diseases of dogs and to determine if CRP can be used as a biomarker in the diagnosis of bacterial respiratory diseases.

Animals: A total of 106 privately owned dogs with respiratory diseases (17 with bacterial tracheobronchitis [BTB], 20 with chronic bronchitis [CB], 20 with eosinophilic bronchopneumopathy [EBP], 12 with canine idiopathic pulmonary fibrosis [CIPF], 15 with cardiogenic pulmonary edema [CPE], and 22 with bacterial pneumonia [BP]) and 72 healthy controls.

Methods: The study was conducted as a prospective cross-sectional observational study. CRP was measured in serum samples. Diagnosis was confirmed by clinical and laboratory findings, diagnostic imaging, and selected diagnostic methods such as cytological and microbiological analysis of respiratory samples, echocardiography, and histopathology.

Results: Dogs with BP had significantly higher CRP concentrations (median, 121 mg/L; interquartile range, 68–178 mg/L) than dogs with BTB (23, 15–38, $P = .0003$), CB (13, 8–14, $P < .0001$), EBP (5, 5–15, $P < .0001$), CIPF (17, 10–20, $P < .0001$), or CPE (19, 13–32, $P < .0001$) and healthy controls (14, 8–20, $P < .0001$). Dogs with BTB had significantly higher CRP concentrations than dogs with CB ($P = .001$) or EBP ($P < .0001$) and healthy controls ($P = .029$).

Conclusion and Clinical Importance: These results indicate that CRP has potential for use as an additional biomarker, especially in the diagnostics of BP.

Key words: Acute-phase protein; Canine; Pneumonia.

Acute-phase response (APR) refers to a complex systemic reaction occurring shortly after tissue injury. APR, as part of the innate host defense system, is a nonspecific response to various possible causes, including infectious, immunologic, neoplastic, or traumatic processes.[1] APR is mainly mediated by pro-inflammatory cytokines, including interleukin-6, interleukin-1, and tumor necrosis factor-α secreted by local inflammatory cells such as monocytes and macrophages. This induces changes in plasma proteins, produced mainly in the liver, called acute-phase proteins (APPs).[2] Major and minor positive APPs (eg, C-reactive protein [CRP], serum amyloid A, haptoglobin, alpha-1-acid glycoprotein, ceruloplasmin, fibrinogen) increase in the APR, and negative APPs (eg, albumin, transferrin) decrease.[3] In dogs, CRP is a major APP. Its serum concentration is very low in healthy animals, but increases rapidly after stimuli, with a lag-phase of ~4 h, reaching peak concentrations ~24 h after the stimulus and then normalizing quickly during recovery.[4] These characteristics make CRP a useful marker of ongoing inflammatory activity.

C-reactive protein increases in various infectious disease processes as well as in immune-mediated and neoplastic diseases in dogs.[5–13] CRP has been shown to be useful in differentiating disease processes such as pyometra from endometrial hyperplasia as well as steroid-responsive meningitis and arteritis from other neurologic diseases and in identifying early postoperative complications.[9,14,15]

Canine lower respiratory diseases and cardiac diseases are common clinical entities in small animal practice. Many of these diseases present with similar signs and a definite diagnosis often requires advanced methods, including bronchoscopic sampling and echocardiography. Because these methods are not always readily available and airway sampling requires anesthesia, a need exists for new noninvasive markers, especially for the detection of bacterial infections. Early precise diagnosis decreases unnecessary antimicrobial use and lowers the risk of development of antimicrobial resistance. Increases in CRP previously have been identified in studies describing a variety of infectious diseases, including dogs with pneumonia and laboratory dogs with experimentally induced bacterial

Abbreviations:

APP	acute-phase protein
APR	acute-phase response
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BP	bacterial pneumonia
BTB	bacterial tracheobronchitis
CAP	community-acquired pneumonia
CB	chronic bronchitis
CIPF	canine idiopathic pulmonary fibrosis
CPE	cardiogenic pulmonary edema
CRP	C-reactive protein
EBP	eosinophilic bronchopneumopathy
TTA	transthoracic aspirate
TTW	transtracheal wash

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bronchitis, as well as in dogs with chronic valvular disease and congestive heart failure.[16–20] These studies have applied different methods for CRP analysis, preventing comparison of CRP concentrations among diseases. Studies describing CRP concentrations in a cohort of dogs with clinically defined different lower airway diseases are lacking. In human respiratory medicine, CRP is widely studied and used especially for identifying community-acquired pneumonia (CAP) and for assessing the need for antimicrobial therapy.[21–23]

The purpose of this study was to investigate serum CRP concentrations in various canine respiratory diseases and to examine whether CRP can be used as a biomarker in the diagnosis of bacterial respiratory diseases.

Materials and Methods

Study Design

The study was conducted as a prospective cross-sectional observational study.

Study Population

Privately owned dogs with respiratory signs examined at the Veterinary Teaching Hospital of the University of Helsinki between May 2006 and January 2013 with one of the following diagnoses were eligible for inclusion in the study: bacterial tracheobronchitis (BTB), chronic bronchitis (CB), eosinophilic bronchopneumopathy (EBP), canine idiopathic pulmonary fibrosis (CIPF), cardiogenic pulmonary edema (CPE), or bacterial pneumonia (BP). In addition, banked frozen (−80°C) serum samples from 10 dogs participating in a previously published study describing EBP were included.[24,25] Healthy blood donor dogs with no signs of illness, with normal physical examination findings, and with normal hematology and biochemistry findings were eligible for inclusion as healthy controls.

All dogs with respiratory signs underwent a full clinical examination with special emphasis on the respiratory tract. Thoracic radiographs (lateral and ventrodorsal or dorsoventral views) as well as venous blood samples for hematology, serum biochemistry, and CRP analysis were obtained at the time of presentation before other procedures. Fecal analysis and arterial blood gas analysis for partial pressures of oxygen, carbon dioxide, and alveolar-arterial oxygen gradient were performed. Bronchoscopy was performed with a 4.9 mm flexible endoscope,[a] and airway samples for cytology and quantitative bacterial culture were obtained by weight-adjusted bronchoalveolar lavage (BAL).[26] Dogs with BP that were considered unsuitable candidates for anesthesia because of the severity of their disease underwent transtracheal wash (TTW) or transthoracic fine needle aspiration (TTA) to obtain respiratory samples for cytology and bacterial culture.[27,28] Blood cultures were aseptically collected from the jugular vein in BP dogs. All dogs with findings typical for CPE on clinical examination and thoracic radiography underwent cardiac ultrasound examination after stabilization to further confirm the presence of a cardiac disease, and airway sampling was not performed.

Dogs were considered to have BP when there were typical acute signs (at least 3 of the following: fever, lethargy, dyspnea, tachypnea, cough) and newly diagnosed alveolar lung consolidation on thoracic radiographs. Bacterial origin was proven either by cytologic confirmation of bacterial infection in respiratory samples (>2 intracellular bacteria/oil immersion field), positive bacterial culture ($\geq 10^3$ colony-forming units/mL in airway samples), positive blood culture, or rapid response to antibiotics and full clinical and radiographic normalization with antibiotic treatment.[29] All dogs with BP were followed until complete cure or death.

Diagnosis of BTB was made by the presence of acute or chronic cough and a positive bacterial culture in BAL fluid (BALF) in the absence of alveolar consolidation on thoracic radiographs as well as in the absence of other diagnosed concurrent respiratory disease.

Negative bacterial culture and absence of intracellular bacteria in BALF were inclusion criteria for dogs with CB, EBP, and CIPF. Diagnosis of CB was based on the presence of chronic cough (>2 months of duration), typical bronchoscopic findings such as increased mucus production and irregular bronchial mucosa as well as possible additional findings such as bronchomalacia or bronchiectasia, and exclusion of other causes capable of causing the signs.[30] Dogs with both inflammatory (increased total cell count and predominance of nondegenerate neutrophils in BALF) and noninflammatory cytologic findings were included in the CB group. EBP was diagnosed by the presence of respiratory signs, >20% eosinophil count in BALF, and exclusion of other causes such as pulmonary parasites. Diagnosis of CIPF was based on typical respiratory signs and clinical findings as well as BALF cytology, radiographic and high-resolution computed tomography findings or postmortem pathology confirmation.[31] CPE was diagnosed by typical clinical presentation, including respiratory signs (eg, cough, tachypnea, dyspnea), interstitial or alveolar infiltration on thoracic radiographs, and presence of confirmed heart disease by echocardiography.

Dogs with other known or suspected concurrent infectious, inflammatory, or neoplastic diseases other than the respiratory diseases included in the study as well as dogs in groups other than BTB and BP that had received antimicrobial treatment 7 days before the examination were excluded from the study. Pregnant or lactating bitches and puppies <3 months of age also were excluded.[32]

Sample Collection, Handling, and Analysis

Hematology,[b] serum biochemistry,[c] and arterial blood gas analysis[d] were performed immediately after sampling. Serum samples obtained for CRP analysis either were immediately assayed or were stored at −80°C until assayed.[25] CRP was analyzed using LifeAssays® magnetic permeability-based immunoassay[e] previously validated for CRP measurement in dogs.[33] CRP results below the detection limit for the assay (<10 mg/L) were set to equal 5 mg/L. Results beyond the upper detection limit for the assay (>210 mg/L) were set to equal 211 mg/L. Fecal samples were examined for parasites with $MgSO_4$ flotation and Baermann's sedimentation methods.

Semi-quantitative aerobic and anaerobic bacterial cultures were performed immediately after sampling. In addition, an enrichment culture was performed because some of the patients had received antimicrobials before sampling. A 10 μL volume of the specimen was streaked onto agars,[f,g,h] and placed in enrichment broth.[i] Blood and chocolate agars were incubated at +35°C in 5% CO_2 atmosphere for 7 days and were evaluated daily to detect growth. Anaerobe agars were incubated under anaerobic conditions at +35°C for 7 days and were evaluated every second day. Species identification was performed by conventional methods and antimicrobial susceptibility testing by disk diffusion or E-test following standardized methods.[34,35] Bacterial counts were quantified by calculating the colonies on a plate and multiplying the number of colonies by the dilution factor. The limit of detection was 100 colony-forming units/mL. Enrichment tubes were incubated at +35°C for 7 days and inspected daily. If cloudiness was detected, a swab from the tube was streaked onto the

above-mentioned agars and only a qualitative result was given. Airway samples for mycoplasma culture were transferred into a transport medium[j] and sent for analysis to a national reference laboratory.[k] Blood cultures were aseptically collected into a vacuum culture medium[l] and incubated in an automatic microbial detection system.[m] Gram stain and cytologic examination were performed on BALF and TTW samples after cytocentrifugation.,[n,26,29]

Statistical Analysis

The differences in CRP among disease groups (BTB, CB, EBP, CIPF, CPE, BP, and healthy controls) were evaluated by ANOVA models. Together with disease, the variables of age, sex, and weight were used as fixed effects in the model to control for the possible confounding effects of these demographic variables. Because the distribution of CRP did not follow a normal distribution in several disease groups (based on Shapiro–Wilk's test of normality and normal Q-Q plots), the analysis was performed for rank-transformed data.[36] Several predefined pair-wise comparisons were estimated from the model using contrasts. Sidak's correction was used in the pair-wise comparisons to control for the family-wise error rate.

The correlation between CRP and clinical variables (body temperature, respiratory rate, arterial oxygen and carbon dioxide partial pressures, arterial-alveolar oxygen gradient, blood leukocyte parameters, and BALF cytology parameters) was assessed with Spearman's correlation coefficients for all data and separately for each disease group. In addition, Spearman's correlation was calculated to assess correlations between CRP and duration of signs in dogs with BP, BTB, and CPE. The effect of antibiotic use on CRP before entering the study was evaluated using ANOVA models for dogs with bacterial respiratory disease (BTB and BP). The fitted model included fixed effects for disease and antibiotic use. $P \leq .05$ was considered statistically significant. All statistical analyses were performed using commercial statistic software.[o,p,q]

Ethical Approval and Owner Consent

This study was approved by the Ethics Committee of the University of Helsinki. Owner consent was obtained from owners of the dogs before participation.

Results

Dogs

Altogether 106 dogs with respiratory signs were accepted into the study, including 17 dogs diagnosed with BTB, 20 with EBP, 20 with CB, 12 with CIPF (4/12 confirmed by high resolution computed tomography and in 8/12 by histopathology), 22 with BP, and 15 with CPE. In addition, 72 blood donor dogs were chosen as healthy controls for CRP measurements. Age, body weight, and sex distribution of the dogs are presented in Table 1. Dogs in the different disease groups represented different breeds, except for CIPF dogs, which all were West Highland White Terriers.

Clinical Findings

Dogs with CB, EBP, BTB, and CIPF were clinically stable on presentation, with the exception of 1 dog with CIPF presenting with severe dyspnea that led to euthanasia. Dogs with CPE and BP were presented as acute emergency cases with dyspnea of varying severity. Body temperature, respiratory rate, results from arterial blood gas analysis and hematology are presented in Tables 2 and 3. Fecal analysis performed on 77/91 diseased dogs (BTB 12/17, EBP 20/20, CB 16/20, CIPF 12/12, BP 17/22) was negative for lung parasites and gastrointestinal parasites with migrating larvae in all dogs. Two fecal samples were positive for other gastrointestinal parasites (*Coccidia* sp. in 1 dog and *Ancylostoma* sp. infection in the other dog); both dogs were diagnosed with EBP.

Respiratory Sampling

Bronchoalveolar lavage fluid was retrieved from all dogs with BTB, CB, EBP, and CIPF as well as from 13/22 dogs with BP. In 9/22 dogs with BP, anesthesia was not considered safe because of severity of the

Table 1. Demographic data for healthy dogs and dogs with BTB, CB, EBP, CIPF, CPE, and BP.

	Age (years) Mean ± SD (range)	Body Weight (kg) Mean ± SD (range)	Sex (%)
Healthy controls (n = 72)	3.5 ± 1.3 (1.2–6.0)	42.2 ± 15.7 (25.0–62.5)	Male 34 (47%) Female 38 (53%)
BTB (n = 17)	1.1 ± 1.8 (0.3–7.9)	15.8 ± 12.8 (3.4–49.2)	Male 11 (65%) Female 6 (35%)
CB (n = 20)	9.27 ± 4.03 (2.17–14.5)	12.6 ± 7.85 (5.0–38.0)	Male 13 (65%) Female 7 (35%)
EBP (n = 20)	4.2 ± 2.8 (0.8–8.5)	22.0 ± 9.7 (6.5–37.4)	Male 5 (25%) Female 15 (75%)
CIPF (n = 12)	12.1 ± 1.8 (10.2–16.3)	10.8 ± 2.0 (7.2–15.2)	Male 8 (67%) Female 2 (33%)
CPE (n = 15)	10.7 ± 2.8 (4.4–14.6)	18.3 ± 18.8 (4.1–58.8)	Male 8 (53%) Female 7 (47%)
BP (n = 22)	3.9 ± 3.2 (0.6–10.8)	30.3 ± 20.1 (7.3–81.7)	Male 12 (55%) Female 10 (45%)

BTB, bacterial tracheobronchitis; CB, chronic bronchitis; EBP, eosinophilic bronchopneumopathy; CIPF, canine idiopathic pulmonary fibrosis; CPE, cardiogenic pulmonary edema; BP, bacterial pneumonia.

Table 2. Body temperature, respiratory rate, and arterial blood gas analysis results in dogs with BTB, CB, EBP, CIPF, CPE, and BP.

	BTB (n = 17)	CB (n = 20)	EBP (n = 20)	CIPF (n = 12)	CPE (n = 15)	BP (n = 22)
	Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)
Body temperature (°C)	38.9 ± 0.5 (38.1–39.6)	38.7 ± 0.7 (37.7–39.5) n = 18*	38.7 ± 0.5 (38.1–39.4) n = 15*	38.5 ± 0.4 (38.0–39.2) n = 7*	38.2 ± 0.6 (36.8–39.0) n = 11*	39.4 ± 0.9 (39.1–41.2)
Respiratory rate (breaths/min)	41.5 ± 9.4 (32–60) n = 12*	45.1 ± 23.7 (24–120) n = 14*	24.8 ± 6.9 (12–36) n = 12*	57.6 ± 35.1 (24–120) n = 11*	61.0 ± 29.9 (40–132) n = 12*	55.7 ± 20.7 (24–100) n = 21*
Arterial pO_2 (mmHg)	85.3 ± 1.9 (53.7–111) n = 15*	81.3 ± 12.8 (61.8–96.1)	91.7 ± 11.1 (74.4–112.6) n = 18*	63.0 ± 8.7 (57.0–81.4) n = 10*		76.9 ± 11.1 (54.2–91.6) n = 21*
Arterial pCO_2 (mmHg)	35.3 ± 6.6 (27.9–50.3) n = 15*	30.1 ± 4.7 (20.1–41.9)	31.8 ± 3.8 (23.2–35.2) n = 18*	30.5 ± 4.1 (25.7–35.7) n = 10*		29.9 ± 3.6 (22.7–37.9)
A-a O_2 (gradient)	25.4 ± 10.81 (5.0–36.3) n = 9*	34.9 ± 12.8 (15.1–54.5) n = 19*	26.8 ± 8.1 (16.7–45.6) n = 13*	51.3 ± 10.2 (45.3–70.0) n = 10*		37.8 ± 12.6 (17.9–56.5) n = 21*

*Number of patients for whom information concerning the variable was available.

BTB, bacterial tracheobronchitis; CB, chronic bronchitis; EBP, eosinophilic bronchopneumopathy; CIPF, canine idiopathic pulmonary fibrosis; CPE, cardiogenic pulmonary edema; BP, bacterial pneumonia.

Table 3. Hematology results in dogs with BTB, CB, EBP, CIPF, CPE, and BP.

	BTB (n = 17)	CB (n = 20)	EBP (n = 20)	CIPF (n = 12)	CPE (n = 15)	BP (n = 22)
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Blood leukocyte count (10^9/L)	13.0 (10.1–19.9)	9.0 (7.1–10.4)	10.0 (8.9–12.4)	9.7 (7.4–11.9)	10.1 (6.0–12.3) n = 11*	12.6 (9.23–18.0)
Blood segmented neutrophil count (10^9/L)	8.3 (6.6–14.0)	6.4 (4.9–9.7) n = 19*	6.3 (5.4–7.9)	7.5 (5.2–9.4)	4.0 (3.8–7.5) n = 7*	9.8 (6.6–14.4)
Blood band neutrophil count (10^9/L)	0 (0–0.1)	0 (0–0) n = 19*	0 (0–0)	0 (0–0)	0 (0–0) n = 7*	0.4 (0–1.3)
Blood lymphocyte count (10^9/L)	2.8 (2.1–4.2)	1.6 (1.2–3.3) n = 19*	1.9 (1.3–2.5)	1.4 (1.1–1.5)	1.5 (1.4–1.7) n = 7*	1.1 (0.38–1.7)
Blood eosinophil count (10^9/L)	0.4 (0.2–0.7)	0.3 (0.2–0.5) n = 19*	1.2 (0.8–2.1)	0.3 (0.1–0.6)	0.35 (0.2–0.7) n = 7*	0.2 (0–0.6)
Blood monocyte count (10^9/L)	0.9 (0.6–1.1)	0.7 (0.4–1.1) n = 19*	0.5 (0.4–0.7)	0.5 (0.3–1.0)	0.5 (0.2–1.0) n = 7*	0.7 (0.3–1.5)
Blood basophil count (10^9/L)	0 (0–0.04)	0.02 (0–0.05) n = 19*	0.15 (0–0.3)	0 (0–0.01)	0.01 (0–0.01) n = 7*	0 (0–0)

*Number of patients for whom information concerning the variable was available.

BTB, bacterial tracheobronchitis; CB, chronic bronchitis; EBP, eosinophilic bronchopneumopathy; CIPF, canine idiopathic pulmonary fibrosis; CPE, cardiogenic pulmonary edema; BP, bacterial pneumonia.

disease, and other sampling methods were used, including TTW in 7/22, TTA in 1/22, and fresh sputum sample in 1/22 dogs. Results from cytology analysis of BALF and TTW fluid are shown in Table 4. In 1 BALF sample in a dog with CB and in 2/7 TTW samples in dogs with BP, cellularity of the sample was low and cytologic analysis was not performed. Cytology in TTA and fresh sputum samples disclosed markedly increased numbers of neutrophils and intracellular bacteria in both cases.

A single bacteria species was isolated in 15/17 dogs with BTB and included *Bordetella bronchiseptica* (11/15), *Pasteurella* sp. (2/15), *Escherichia coli* (1/15), and *Haemophilus* sp. (1/15). Multiple species of bacteria were identified in 2/17 dogs, including *B. bronchiseptica* and *Mycoplasma* sp. in 1 dog and *Klebsiella pneumoniae* and *Streptococcus* spp. in the other dog.

Bacterial culture of respiratory samples was performed on all BP dogs. Twelve of 22 dogs had received antimicrobial therapy before sampling. Bacterial

Table 4. Cytology analysis of BALF and TTW fluid in dogs with BTB, CB), EBP, CIPF, CPE, and BP.

	BTB (n = 17) Median (IQR)	CB (n = 20) Median (IQR)	EBP (n = 20) Median (IQR)	CIPF (n = 12) Median (IQR)	BP (n = 22) Median (IQR)	
	n = 19*				BALF (n = 13*)	TTW (n = 5*)
Total cell count (10^9/L)	0.60 (0.2–1.0)	0.23 (0.1–0.4)	0.99 (0.4–1.7)	1.09 (0.7–1.6)	1.8 (0.2–18.6)	
Neutrophils (%)	12.7 (3.6–55.2)	5.7 (2.7–14.4)	5.2 (2.4–10.1)	6.5 (4.2–13.0)	45.4 (5.9–88.6)	67.5 (16.9–97.7)
Eosinophils (%)	2.7 (0.4–4.0)	1.7(0.4–6.0)	50.7 (41.2–65.5)	0.2 (0–0.6)	0.7 (0–5.4)	1.4 (0–17.6)
Mast cells (%)	0.0 (0–0.4)	1.4 (0.7–3.0)	0.9 (0–1.9)	0.7 (0.2–1.0)	0 (0–0.35)	0 (0–1.2)
Lymphocytes (%)	13.4–(4.2–25.2)	12.0 (6.4–17.4)	7.9 (3.7–14.2)	6.0 (4.4–6.7)	7.0 (0.7–22.9)	3.0 (0–14.5)
Macrophages (%)	57.4 (34.4–68.7)	75.0 (54.0–85.0)	29.7 (16.2–37.2)	85.1 (77.6–89.4)	27.4 (10.6–63.7)	12.5 (1.2–43.2)
Epithelial cells (%)	0 (0–2.7)	0 (0–0.4)	0.4 (0–1.2)	0 (0–0)	0 (0–0)	0.7 (0–15.2)
Basophils (%)	0 (0–0–)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Plasma cells (%)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)

*Number of patients for whom information concerning the variable was available.

BTB, bacterial tracheobronchitis; CB, chronic bronchitis; EBP, eosinophilic bronchopneumopathy; CIPF, canine idiopathic pulmonary fibrosis; CPE, cardiogenic pulmonary edema; BP, bacterial pneumonia; BALF, bronchoalveolar lavage fluid; TTW, transtracheal wash.

growth ($\geq 10^3$ colony-forming units/mL in BALF, TTW, TTA and sputum samples) was isolated in primary culture from 13/22 samples: a single species of bacteria was detected in 11/13 including *E. coli* (4/11), *Pasteurella* sp. (2/11), *Streptococcus* sp. (1/11), *Mycoplasma* sp. (1/11), *Haemophilus* sp. (1/11), *Actinomyces* sp. (1/11), and *Nocardiopsis* sp. (1/11), and 2 species of bacteria in 2/13, including *Pasteurella* sp. and *Mycoplasma* sp.; 6/13 of these dogs had received prior antimicrobial treatment. In 1 dog with negative primary bacterial culture, >2 intracellular bacteria/oil immersion field were demonstrated, and *Actinomyces* sp. was cultured after enrichment. Positive bacterial growth was detected only after enrichment in 5/22 samples (single species of bacteria, including *Streptococcus* sp. [2/5], *Pasteurella* sp. [1/5], *Haemophilus* sp. [1/5], and *Actinomyces* sp. [1/5]). Three of 22 dogs with negative cultures in airway samples and blood cultures showed at least 3 of the following signs: fever, lethargy, dyspnea, tachypnea, or cough, and had new alveolar densities on thoracic radiographs as well as neutrophilia in BALF cytology. These dogs showed a rapid response to antibiotics, and full clinical and radiographic recovery was achieved. Gram staining was performed on 20/22 dogs with BP and showed intracellular bacteria in 7/22 samples. Blood culture was performed on 11/22 BP dogs and was positive in 4/11 samples, including dogs with TTA and fresh sputum respiratory samples (*E. coli* [1/4], *Haemophilus* sp. [1/4], *Arcanobacterium* sp. [1/4] and *Staphylococcus* sp. [1/4]). Blood culture findings were consistent with bacteria isolated in respiratory samples in 2/4 dogs.

CRP Measurements

C-reactive protein concentrations are presented in Figure 1. Age, sex, or body weight did not affect CRP concentrations. Effect of prior antibiotic treatment on serum CRP was found to be insignificant in dogs with bacterial diseases (BTB and BP; $P = .58$). Duration of clinical signs did not affect CRP concentration in dogs with BP ($P = .17$), BTB ($P = .61$), or CPE ($P = .15$).

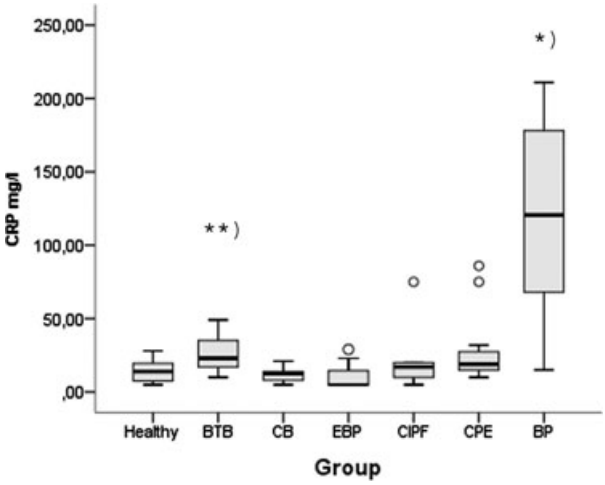

Fig 1. Box and whiskers plot showing serum C-reactive protein (CRP) concentrations in dogs with bacterial tracheobronchitis (BTB, n = 17), chronic bronchitis (CB, n = 20), eosinophilic bronchopneumopathy (EBP, n = 20), idiopathic pulmonary fibrosis (CIPF, n = 12), cardiogenic pulmonary edema (CPE, n = 15), or bacterial pneumonia (BP, n = 22) and in healthy controls (n = 72). (*) Dogs with BP had significantly higher CRP concentrations (median 121 mg/l, interquartile range 68–178 mg/l) than dogs with BTB (23, 15–38, $P = .0003$), CB (13, 8–14, $P < .0001$), EBP (5, 5–15, $P < .0001$), CIPF (17, 10–20, $P < .0001$), or CPE (19, 13–32, $P < .0001$), or healthy controls (14, 8–20, $P < .0001$). (**) Dogs with BTB had significantly higher CRP–concentrations than dogs with CB ($P = .0010$) or EBP ($P < .0001$) or healthy controls ($P = .029$).

In the entire patient group, CRP was positively correlated with body temperature ($r = 0.25$, $P = .020$), respiratory rate ($r = 0.33$, $P = .0029$), blood leukocyte count ($r = 0.23$, $P = .018$), blood segmented neutrophil count ($r = 0.34$, $P = .0007$), blood band neutrophil count ($r = 0.64$, $P < .0001$), and BALF neutrophil percentage ($r = 0.44$, $P < .0001$). In the entire group, CRP was negatively correlated with blood eosinophil count ($r = -0.48$, $P < .0001$), blood basophil count ($r = -0.36$, $P = .0002$), BALF eosinophil percentage

($r = -0.48$, $P < .0001$), and BALF mast cell percentage ($r = -0.27$, $P = .012$).

When analyzed for individual disease groups, CRP was positively correlated with blood band neutrophil count in dogs with BTB (0.59, $P = .012$), with BALF neutrophil percentage in dogs with BTB ($r = 0.58$, $P = .015$), and with BALF macrophage percentage in dogs with CB ($r = 0.67$; $P = .0016$). CRP was negatively correlated with arterial pCO_2 in dogs with BP ($r = -0.50$, $P = .022$), with blood eosinophil count in dogs with CB ($r = -0.58$, $P = .0091$) and CIPF ($r = -0.71$, $P = .0058$), with blood lymphocyte count in dogs with CB ($r = -0.49$, $P = .033$), and with BALF eosinophil percentage in dogs with CB ($r = -0.58$, $P = .0091$) and CIPF ($r = -0.74$, $P = .0058$).

Discussion

Dogs with bacterial lower airway diseases had increased CRP concentrations compared with dogs that had noninfectious diseases. The highest CRP concentrations were noted in dogs with BP, which had significantly higher CRP concentrations than healthy dogs or dogs from the BTB, CB, EBP, CIPF, or CPE disease groups. Our findings are in accordance with earlier studies by Nakamura et al and Yamamoto et al who reported increased CRP concentrations in dogs with bronchopneumonia, severe pneumonia, or "wild pneumonia" (a term used and not further defined by authors of the original article).[16,17]

We noted that BP was identified with 100% specificity when CRP was >100 mg/L and ruled out with 100% specificity when CRP was <20 mg/L when signs had lasted >24 h. This finding is important because CRP concentration increases within 24 h after an inciting stimulus, and in the very early phase of BP development CRP production may not have developed to the full extent.[4] According to the findings in this study, CRP concentrations between 20 and 100 mg/L may be found in dogs with several different respiratory diseases, and therefore are limited in their diagnostic value. In human medicine, CRP is widely used as an important biomarker in the diagnosis of CAP, and current guidelines recommend measuring CRP in patients suspected with CAP.[37,38] According to these guidelines, CAP is considered very likely when CRP is >100 mg/L in a patient with compatible clinical presentation and unlikely when CRP is <20 mg/L and clinical signs have lasted >24 h.[37] Similar to BP, CPE often presents with acute dyspnea or tachypnea. Thus, distinguishing between these conditions is sometimes difficult, especially before or in the absence of thoracic radiographs or echocardiography. Therefore, our results indicate that CRP has potential for use in dogs, similar to that in human medicine, contributing markedly to the diagnosis of BP.

In dogs with BTB, CRP was significantly higher than in dogs with other lower airway diseases presenting with cough (CB, EBP) or in healthy controls. Increases in CRP were mild (35–49 mg/L), and although significantly higher, there was marked overlap among groups. These findings indicate that increased CRP in a dog presenting with cough may arouse suspicion of bacterial bronchitis, but normal CRP does not rule out bacterial bronchitis. Yamamoto et al studied induced *B. bronchiseptica* bronchitis in laboratory dogs and found marked increases in CRP in the first 5 days after inoculation. CRP concentration returned nearly to normal within 10 days of inoculation.[18] It would be interesting to compare these findings with those of naturally occurring BTB.

Unexpectedly, prior antimicrobial use did not affect CRP concentration in dogs with either BTB or BP; a similar finding has been reported in humans.[22] This finding may be attributable to inadequate tissue penetration, inappropriate antimicrobial dosage, recent initiation of therapy, or bacterial resistance. The majority of patients with BP had received previous antimicrobial treatment, and bacteria were found in only 59% of respiratory samples in primary culture. This observation is in accordance with findings in human medicine, where identification of the causative bacterial organism is challenging, and negative results (up to 60% of samples) are common.[38,39] Therefore, respiratory tract sampling is not a part of the routine diagnostic evaluation in humans with suspected CAP.[37–39] We believe that 3 of the dogs in the BP group that had neutrophilia in BALF or TTW samples, but a negative culture result represented such cases because they showed a rapid response and complete clinical and radiographic recovery with antibiotic treatment. Based on these findings, it may be reasonable to follow similar recommendations in veterinary medicine concerning diagnosis of uncomplicated cases of pneumonia in dogs.

The highest CRP concentrations in dogs with noninfectious lower respiratory tract diseases were observed in dogs with severe dyspnea leading to death or euthanasia (1 dog with CIPF [CRP 75 mg/L] and acute respiratory distress syndrome and 1 dog with CPE [CRP 86 mg/L]). However, in most dogs with CIPF and CPE, CRP concentrations were low (mean 16.8 mg/L; range, 5–32 mg/L). Cunningham et al described mild-to-moderate increases in CRP in dogs with congestive heart failure, which is in accordance with findings in our study.[20] In people, CRP has been shown to increase with increasing severity of congestive heart failure.[40,41]

To further understand the interrelationships of CRP, correlations with other clinical parameters were evaluated. In all groups combined, CRP was positively correlated with other markers of inflammation, including body temperature, blood leukocyte count, blood segmented and band neutrophil counts, and BALF neutrophil percentage. This finding was not unexpected because the same pro-inflammatory cytokines responsible for stimulating APR are also important mediators of fever and neutrophil production in bone marrow.[42,43] However, these correlations were not found in the BP group. This can be partially explained by different timing of physiologic events in relation to CRP measurement in acute BP such as a delay in neutrophil production in early BP and a rapid decrease

in body temperature after initiation of antimicrobial therapy. Interestingly, duration of signs did not correlate negatively with CRP in dogs with BTB, CPE, or BP. This finding is in contrast to previously published results and warrants further research before drawing any conclusions.[18]

This study was intentionally designed to exclude dogs with other concurrent conditions capable of increasing CRP concentrations. For this reason, we cannot assess the applicability of CRP measurements in patients with respiratory disease and several other concurrent infectious, inflammatory, or neoplastic conditions. Certain neoplastic diseases have been demonstrated to result in significant increases in CRP potentially linked to tissue inflammation and necrosis induced by the tumor.[13] However, it remains to be investigated whether patients with pulmonary neoplasia or pleural diseases can be differentiated from patients with BP using CRP measurement. Other potential implications of CRP in canine respiratory medicine include the role of CRP as an indicator of treatment response and as a possible prognostic marker in patients with severe pneumonia.

In conclusion, CRP is significantly increased in dogs with BP relative to healthy dogs and dogs with BTB, CB, EBP, CIPF, or CPE, enabling its use as an additional diagnostic biomarker in BP. Moreover, increases in CRP are not typical in patients with CB, EBP, or CIPF, and if encountered in such patients, presence of a secondary infectious process should be suspected.

Footnotes

[a] Olympus GIF N180, Olympus Medical Systems Europa GMBH, Hamburg, Germany
[b] Advia 2120i, Siemens AG, Erlangen, Germany
[c] Konelab 30i Clinical Chemistry Analyzer, Thermo Scientific, Fischer Scientific Oy, Vantaa, Finland
[d] ABL 800 Flex analyzer, Radiometer Medical ApS, Brønshøj, Denmark
[e] LifeAssays canine CRP point-of-care system, LifeAssays AB, Ideon Science Park, Scheelevägen 19 F, Lund 22370, Sweden
[f] Tryptone Soya Agar with Sheep Blood, Oxoid Limited, Wade Road, Basingstoke, Hampshire, UK
[g] Chocolate Agar with Vitox, Oxoid Limited
[h] Fastidious Anaerobe Agar with Horse Blood, Oxoid Limited
[i] Fastidious Anaerobe Broth, Oxoid Limited
[j] Copan Universal Transport Medium UTM-RT System, Copan Italia, Brescia, Italy
[k] Evira, Neulaniementie 4, Kuopio 70210, Finland
[l] Bact/Alert®FA, Bact/Alert®SN Culture medium, Biomerieux Inc, Durham, NC
[m] Bact Alert 3D60, Biomerieux Inc
[n] Cytospin 4, Thermo Scientific, Fischer Scientific Oy, Vantaa, Finland
[o] SAS® System for Windows, version 9.3, SAS Institute Inc, Cary, NC
[p] R for Windows®, version 2.15.2, R Foundation for Statistical Computing, Vienna, Austria
[q] PASW Statistics 18, SPSS Inc, 233 South Wacker Drive, Chicago, IL

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