Association of periodontal disease with systemic health indices in dogs and the systemic response to treatment of periodontal disease

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Objective—To determine whether severity of periodontal disease (PD) was associated with systemic health indices in dogs and whether treatment of PD altered systemic health indices.

Design—Prospective cohort study.

Animals—38 dogs.

Procedures—Healthy dogs with clinical signs of PD were included in the study. Physical examination, serum biochemical analysis, a CBC, urine evaluation, measurement of serum C-reactive protein (CRP) concentration, and a microalbuminuria test were performed prior to treatment of PD. All tooth roots were scored for gingivitis and attachment loss, and appropriate treatment of PD was performed. Laboratory data were obtained 4 weeks after treatment. The Spearman rank correlation and Wilcoxon signed rank test were used for statistical analysis.

Results—Analyses of the correlation of several variables with attachment loss or gingivitis or of differences before and after treatment revealed significant results for several variables. After applying Bonferroni corrections for family-wise error rate, significant rank correlations were found between attachment loss and platelet number (r = 0.54), creatinine concentration (r = –0.49), and the within-dog difference in CRP concentrations before and after treatment (r = 0.40). The BUN concentration was significantly higher after treatment than before treatment.

Conclusions and Clinical Relevance—Increasing severity of attachment loss was associated with changes in systemic inflammatory variables and renal indices. A decrease in CRP concentration after treatment was correlated with the severity of PD. The BUN concentration increased significantly after treatment of PD. There is a need for continued research into the systemic impact of PD. (*J Am Vet Med Assoc* 2011;238:601–609)

Oral disease is the most common physical examination finding in all age categories of dogs, with PD being the most commonly diagnosed oral disease.[1,2] Periodontal disease is inflammation and infection of the periodontium compromising the gingiva, periodontal ligament, cementum, and alveolar bone. The first sign of PD is gingivitis, which is evident clinically as hyperemia, edema, ulceration, or spontaneous bleeding of the gingiva. Gingivitis is the tissue's inflammatory response to periodontopathogenic bacteria in dental plaque accumulated on the tooth surface. As plaque continues to accumulate in the gingival sulcus, early lesions (which are characterized by inflammatory mediators and infiltration of polymorphonuclear neutrophilic leukocytes) progress to chronic infection in which plasma cells dominate the cellular population.[3] Connective tissue and gingival epithelium are destroyed by the cellular inflammatory infiltrate and bacterial by-products, such as collagenase, hyaluronidase, protease, chondroitin sulfatase, and endotoxin. These elements lead to increased tissue vulnerability and infection of deeper tissues.[1,3,4]

Untreated gingivitis can progress to periodontitis, which is the infection of the nongingival components of the periodontium.[5] The transition from gingivitis to periodontitis is the result of changes in the pathogenic potential of dental plaque, inappropriate or inadequate host response to gingival infection, and various risk factors (eg, systemic disease, stress, age, medications,

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CRP	C-reactive protein
GGT	γ-Glutamyltranspeptidase
IL	Interleukin
PD	Periodontal disease
TMPS	Total mouth periodontal score
UPC	Urine protein-to-urine creatinine ratio
USG	Urine specific gravity

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lack of oral hygiene maintenance, poor diet, and size of the affected animal).[6] Although periodontitis can progress slowly (with periods of quiescence) or rapidly, it always results in some degree of regional destruction of the periodontium that is evident as attachment loss. Immunocompetent cells produce proinflammatory cytokines (IL-1β, IL-6, IL-8, and tumor necrosis factor-α) in addition to prostaglandin E_2, matrix metalloproteinases, and tissue inhibitors of matrix metalloproteinases; these mediators lead to destruction of collagen, connective tissue matrix, and bone.[6] Both gingivitis and periodontitis increase porosity of regional blood vessels via cytokine signaling and destruction of endothelial cells.[6]

In humans, associations have been detected between PD and increased circulating concentrations of inflammatory mediators and acute-phase proteins, systemic endothelial dysfunction, atherosclerosis, acute myocardial infarction, multiple complications of pregnancy, and diabetes mellitus.[7–13] Few studies have been performed on this topic in dogs. Two studies[14,15] that involved canine cadavers revealed that dogs with moderate to severe PD had increased microscopic changes in the myocardium, mitral valve, renal glomeruli and interstitium, and hepatic parenchyma, compared with results for dogs with less severe PD. In humans and dogs, transient bacteremia is associated with disruption of the gingival surface, which includes processes such as chewing and tooth brushing, and 30% to 100% of dogs undergoing ultrasonic dental scaling or tooth extraction procedures have transient bacteremia.[16–19] It is suspected that frequent transient bacteremia combined with chronic stimulation of the immune system plays a role in the development of systemic disease,[20] but we are not aware of any studies in which PD has been directly linked to pathological organ changes in dogs.

If porosity of periodontal vasculature increases as a result of tissue destruction and increases in concentrations of bacterial by-products, inflammatory mediators, cytokines, T cells, and B cells because of active periodontal infection (as has been determined in humans), then it is expected that these products will enter the systemic circulation in dogs. Therefore, the purpose of the study reported here was to analyze the relationship between PD and systemic effects in dogs. This relationship was quantified by scoring periodontal health indices and evaluating hematologic and urine indicators of organ health and systemic inflammation. The 2 hypotheses were that increasing severity of PD would be associated with increasing concentrations of systemic inflammatory markers and changes in enzyme activities or functional indicators of organ health and that appropriate treatment for PD would cause a change in these values.

Materials and Methods

Animals—Client-owned dogs examined for PD by the Dentistry and Oral Surgery Service at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania were used in the study. A written protocol for the study was reviewed and approved by a university institutional animal care and use committee, and written consent was obtained from each client prior to enrollment of their dog in the study.

Dogs included were > 1 year old and of various body weights and breeds and either sex. Dogs were evaluated via a serum biochemical panel, CBC, urinalysis, microbial culture of a urine sample, UPC, microalbuminuria test, and serum CRP test performed on the day prior to scheduled periodontal treatment. Exclusion criteria included dogs with major systemic disease (renal failure, severe hepatopathy, endocrine disease, pancreatitis, autoimmune-based pathological conditions, neoplasia, overt dermatitis, allergic disease, or draining wounds), oral disease other than PD, recent medical or surgical treatment (within 120 days of evaluation), positive results for microbial culture of a urine sample, or systolic blood pressure > 160 mm Hg.

Blood collection and analysis—A blood sample was collected from each dog into a tube that contained EDTA as an anticoagulant, and CBCs were performed by use of the appropriate cytology analyzers.[a,b] Another blood sample was collected from each dog into a serum tube; serum tubes were centrifuged and the serum harvested, and standard biochemical analyses were performed by use of a standard serum analyzer.[c] Remaining serum was placed in freezer-safe plastic vials and stored at –20°C until tested to detect CRP concentrations.

Serum CRP concentration was determined by use of a solid-phase sandwich immunoassay.[d] Prior to testing, all samples were warmed to 22°C and mixed by inverting the vials several times. Samples were diluted to a concentration of 1:500 before assay by the addition of 10 µL of sample to 5.0 mL of diluent buffer. Diluted sample was added to each well on a plate, and plates were incubated for 15 minutes at 37°C. After incubation, liquid was decanted from the wells, and wells were washed 4 times with diluted wash buffer. After the last wash, the wells were tapped on absorbent paper until dry. One hundred microliters of anti-canine CRP antibody labeled with horseradish peroxidase was added to each well, and plates then were incubated for 15 minutes at 37°C. The liquid was again decanted from the wells, wells were washed 4 times with diluted wash buffer, and wells were tapped on absorbent paper until dry. Tetramethylbenzidine substrate solution was added to each well and allowed to incubate for 15 minutes at 22°C. Stop solution was added to each well, and absorbance of each well was measured at 450 nm in a spectrophotometer, with absorbance at 630 nm used as a reference. Positive and negative control samples were included on each plate, and all samples were processed in duplicate. A standard calibration curve was constructed for each plate.

Urine collection and analysis—Urine was collected via ultrasonography-guided cystocentesis. Urinalysis was performed with a standard urine analyzer,[e] and the UPC was determined with measurements obtained via a serum analyzer.[c] The remainder of each urine sample was divided into 2 aliquots; 1 aliquot was submitted for microbial culture, and the other was placed in a freezer-safe plastic tube and stored at –20°C until further testing to evaluate microalbuminuria.

A rapid immunoassay[f] was used to measure concentrations of albumin in the urine samples. Samples were warmed to 22°C and mixed by inverting the tubes several times. A refractometer was used to determine USG. When the USG was < 1.020, the sample was added directly to the sample dilution tube. When the

USG was between 1.020 and 1.060, 1 mL of sample was added to the sample dilution tube, and the sample was diluted with distilled water to the line on the dilution tube that matched the specific gravity of the urine sample. When the USG was greater than the upper limit of the refractometer, the sample was diluted 1:1 with distilled water and the specific gravity of the diluted sample was measured again. The diluted USG was used for the test. Once a sample dilution tube was filled, the sample was mixed by inverting the tube 3 times. A test device that contained the immunoassay was placed in the sample tube and used to agitate the solution; the device was allowed to remain in the sample tube for 3 minutes. The test device then was removed and evaluated by comparing relative intensities of the color bands in the window of the test device. Results were reported as negative (0) or low, medium, or high positive (1, 2, or 3, respectively). To minimize variation for the semiquantitative scoring system, all microalbuminuria tests were completed by 1 research technician who was unaware of the sample source.

Scoring of PD—On the day after admission, dogs were anesthetized for evaluation and treatment of PD. Prior to any oral manipulation, dental and periodontal scoring was performed. The height of each of the 4 canine teeth was measured from the crown tip to the gingival margin on the buccal aspect, and each mouth was scored for gingivitis and attachment loss by use of a TMPS system, as described elsewhere.[21] Scoring was performed by 1 veterinary dental hygienist who was not aware of the history or results of physical examination and diagnostic testing for the dog being evaluated. A plastic mouth gag was gently placed between the maxillary and mandibular canine teeth to assist visual examination of the oral cavity. A standard periodontal probe with 1-mm black markings was used to determine gingivitis and attachment loss of all 120 root sites. Gingivitis was determined first and scored on a scale[l] of 0 to 3 (0 = normal gingiva, 1 = mild inflammation [slight change in color, slight edema, and no bleeding on probing], 2 = moderate inflammation [redness, edema, and glazing with bleeding on probing], and 3 = severe inflammation [bright red, edema or ulceration, and spontaneous bleeding]). Attachment loss was measured by use of the periodontal probe, and the greatest distance between the cementoenamel junction and the bottom of the gingival sulcus or periodontal pocket at each crown-root segment was recorded. Regions with substantial amounts of calculus deposition were carefully scaled (care was taken to avoid touching the gingiva) prior to measurement to aid visual identification of the cementoenamel junction. Missing teeth were not assigned a gingivitis or attachment loss score. Gingivitis scores and attachment loss depths were entered into a TMPS spreadsheet to generate a TMPS, taking into account missing teeth and differences in size of individual teeth.

Periodontal treatments—After scoring was completed, a thorough oral examination was performed, and intraoral dental radiographs were obtained when necessary as indicated by the severity of oral disease. Ultrasonic dental scaling with polishing was performed in all dogs, and additional treatment of PD or tooth extractions were performed as necessary. Nerve blocks were used to provide regional analgesia during and immediately after oral surgery.[22–24] Treatments of PD provided were considered standard of care and were not modified for the study. No fluoride treatment was applied. All dogs were closely monitored during recovery from anesthesia.

Depending on the extent of periodontal treatment and tooth extractions, some dogs were discharged to their owners with instructions for administration of antimicrobials, such as amoxicillin-clavulanic acid[g] (13.75 mg/kg [6.25 mg/lb], PO, q 12 h for 10 days) or clindamycin[h] (5 to 10 mg/kg [2.3 to 4.5 mg/lb], PO, q 12 h for 10 days). Some dogs received pain medication, such as deracoxib[i] (2 to 4 mg/kg [0.9 to 1.8 mg/lb], PO, q 24 h for 3 days), butorphanol tartrate[j] (0.25 mg/kg [0.11 mg/lb], PO, q 8 h for 3 days), or buprenorphine[k] (0.01 mg/kg [0.0045 mg/lb], sublingually, q 8 h for 3 days). Some dogs required no medication postoperatively.

Follow-up evaluation—Approximately 30 days after treatment of PD, follow-up evaluations were performed. These evaluations included a medical history, physical examination, serum biochemical analysis, CBC, urinalysis, microbial culture of a urine sample, determination of UPC, microalbuminuria test, and serum CRP test.

Statistical analysis—Data were analyzed by use of a statistical software package.[l] Nonparametric statistical analyses were performed because several variables did not have a Gaussian distribution as determined by use of the Shapiro-Wilk test. Significance was set at $P \leq 0.05$ within type of lesion (gingivitis or attachment loss) and within logical subsets of variables. Bonferroni corrections for family-wise error rate were made to lower the threshold for the *P* value for significance within each subset of variables based on multiple comparisons. The Spearman rank correlation was used to evaluate associations between gingivitis and attachment loss and other variables. All hematologic and urine variables obtained before treatment were tested separately to determine a correlation with gingivitis or attachment loss scores. Hematologic and urine values were assigned into 4 subsets of variables to decrease the impact of multiple variables; critical *P* values within each subset were established prior to review of statistical analysis. The 4 subsets focused on inflammation, renal function, liver function, and blood glucose–electrolytes. The inflammation subset included values for serum CRP, globulin, and albumin concentrations and counts for WBCs, neutrophils, monocytes, lymphocytes, and platelets. The renal subset included values for microalbuminuria, BUN concentration, creatinine concentration, USG, UPC, and phosphorous concentration. The liver function subset included ALP, ALT, AST, and GGT activities and total bilirubin concentration. The blood glucose–electrolytes subset included blood glucose, calcium, and magnesium concentrations. The Spearman rank correlation was used to evaluate whether the severity of gingivitis and attachment loss was associated with results in each subset. A 2-tailed Wilcoxon signed rank test was used to compare the difference in hematologic and urine values obtained before and after treatment of PD.

Two possible confounding factors in this study, antimicrobial administration and duration of anesthesia, were also evaluated by use of rank-sum tests to detect relationships with differences (before treatment minus after treatment). The Spearman rank correlation was used to determine whether differences in values were related to severity of gingivitis and attachment loss, antimicrobial administration, and duration of anesthesia.

Results

Of 43 dogs enrolled in the study, 5 were excluded from analysis because of missed appointments for follow-up evaluation (n = 2), severe irresolvable lipemia (1), initial undetected lymphoma (1), and a purulent draining lesion detected on a paw during follow-up evaluation (1). Therefore, 38 dogs with PD of various severities were included in the study. A portion of the data from this study has been reported elsewhere.[m]

Dogs ranged from 312 to 186 months of age (median, 112 months), and body weight ranged from 2 to 39 kg (4.4 to 85.8 lb), with a median of 16 kg (35.2 lb). Twenty-one dogs were neutered males, and 17 were spayed females. Gingivitis scores ranged from 0.65 to 2.00 (median, 1.50), and attachment loss scores ranged from 0.98 to 6.54 mm (median, 1.66 mm). Duration of anesthesia ranged from 1.5 to 6.8 hours (median, 3.2 hours). Fourteen dogs received amoxicillin-clavulanic acid, and 4 dogs received clindamycin. Twenty-six dogs received medications for pain control in the form of buprenorphine or butorphanol, and 4 dogs received deracoxib. The number of days until follow-up evaluation ranged from 20 to 80 (median, 34 days).

Gingivitis and attachment loss were significantly correlated (r = 0.63; $P < 0.001$); however, the correlation was not strongly collinear. Correlation analyses were conducted for gingivitis and attachment loss scores with results for variables before treatment (**Table 1**). Variables with a significant correlation for individual analysis with gingivitis score included globulin concentration, platelet count, microalbuminuria, creatinine concentration (a negative correlation), phosphorus concentration, ALP activity, and ALT activity. Variables with a significant correlation for individual analysis with attachment loss score included globulin concentration, monocyte count, platelet count, creatinine concentration (a negative correlation), phosphorus concentration, ALP activity, ALT activity, and blood glucose concentration. With Bonferroni corrections for family-wise error rate applied to each variable on the basis of the aforementioned subsets, there were no significant correlations between the severity of gingivitis and any hematologic and urine values before treatment. In the inflammation subset, platelet count was moderately correlated (r = 0.54; $P < 0.001$) with attachment loss; the platelet count ranged from 92 × 10^3 cells/µL to 899 × 10^3 cells/µL (median, 318 × 10^3 cells/µL). No other variables in this subset were significantly correlated with attachment loss. In the renal function subset, creatinine concentration was moderately negatively correlated

Table 1—Results of correlation analysis for hematologic and urine values obtained before treatment for PD with gingivitis or with attachment loss in 38 dogs.

Variable	Reference interval	Before treatment		Correlation with gingivitis		Correlation with attachment loss	
		Median	Range	*r*	*P* value	*r*	*P* value
Inflammation							
Serum CRP (µg/mL)	< 20.0	4.1	0–34.6	0.21	0.22	0.26	0.12
Globulin (g/dL)	2.4–4.0	3.3	2.4–4.5	0.34	0.04	0.37	0.02
Albumin (g/dL)	2.5–3.7	3.3	2.6–4.1	–0.08	0.62	–0.15	0.36
WBCs (× 10^3 cells/µL)	5.30–19.80	7.19	4.63–24.50	0.21	0.19	0.12	0.48
Neutrophils (× 10^3 cells/µL)	3.10–14.40	4.87	2.73–19.85	0.11	0.50	0.02	0.92
Monocytes (× 10^3 cells/µL)	0.10–1.40	0.42	0–2.05	0.25	0.12	0.34	0.04
Lymphocytes (× 10^3 cells/µL)	0.90–4.70	1.09	0.16–3.92	–0.03	0.85	0.05	0.76
Platelets (× 10^3 cells/µL)	177–398	318	92–899	0.37	0.02	0.54	0.001*
Renal							
Microalbuminuria	0	0	0–3	0.35	0.03	0.29	0.08
BUN (mg/dL)	5–30	13	6–41	0.18	0.29	0.16	0.34
Creatinine (mg/dL)	0.7–1.8	1.0	0.6–1.9	–0.39	0.02	–0.49	0.002*
USG	1.013–1.070	1.030	1.009–1.055	0.15	0.36	0.09	0.60
UPC	0–0.50	0.06	0.01–0.83	0.26	0.12	0.24	0.14
Phosphorus (mg/dL)	2.8–6.10	3.8	2.1–5.7	0.39	0.02	0.42	0.01
Liver							
ALP (U/L)	20–155	102	28–648	0.38	0.02	0.40	0.01
ALT (U/L)	16–91	47	18–328	0.36	0.03	0.39	0.02
AST (U/L)	23–65	36	24–78	0.29	0.08	0.18	0.29
GGT (U/L)	7–24	12	5–70	0.22	0.19	0.18	0.30
Total bilirubin (mg/dL)	0.3–0.9	0.3	0.1–1.1	0.02	0.90	0.03	0.88
Blood glucose–electrolytes							
Blood glucose (mg/dL)	65–112	100	78–115	0.26	0.11	0.36	0.03
Calcium (mg/dL)	9.8–11.7	10.5	9.2–11.6	0.13	0.42	–0.07	0.69
Magnesium (mg/dL)	1.6–2.5	2.1	1.7–2.7	0.08	0.64	0.30	0.07

Microalbuminuria was measured qualitatively; results were reported as negative (0) or low, medium, or high positive (1, 2, or 3, respectively).
*Value considered significant ($P \leq 0.05$) after applying a Bonferroni correction for family-wise error rate.

Table 2—Results of a 2-tailed Wilcoxon signed rank test for the difference between values before and after treatment for PD in 38 dogs.

Variable	After treatment		Difference		*P* value for difference
	Median	Range	Median	Range	
Inflammation					
Serum CRP (μg/mL)	2.98	0–12.50	0.72	–5.32 to 31.00	0.03
Globulin (g/dL)	3.3	2.4–4.3	0	–0.8 to 1.0	0.80
Albumin (g/dL)	3.3	2.7–4.3	–0.1	–1.0 to 0.4	0.03
WBCs ($\times 10^3$ cells/μL)	7.52	4.68–23.40	0.08	–3.97 to 3.66	0.92
Neutrophils ($\times 10^3$ cells/μL)	5.44	2.90–19.89	0.11	–3.54 to 4.00	0.82
Monocytes ($\times 10^3$ cells/μL)	0.39	0–1.11	0.08	–0.42 to 1.34	0.06
Lymphocytes ($\times 10^3$ cells/μL)	1.24	0.11–2.70	0.03	–2.04 to 2.28	0.59
Platelets ($\times 10^3$ cells/μL)	330	128–1,570	–3	–671 to 187	0.77
Renal					
Microalbuminuria	0	0–3	0	–3 to 2	0.40
BUN (mg/dL)	17	8–68	–2	–44 to 17	< 0.001*
Creatinine (mg/dL)	1.1	0.6–2.1	0	–1.0 to 0.3	0.63
USG	1.035	1.008–1.057	–0.002	–0.04 to –0.02	0.05
UPC	0.05	0.01–1.31	0.02	–0.48 to 0.21	0.49
Phosphorus (mg/dL)	4.0	1.9–43.0	–0.3	–39.4 to 2.1	0.13
Liver					
ALP (U/L)	101	34–964	–1	–316 to 148	0.63
ALT (U/L)	43	14–241	2	–65 to 276	0.43
AST (U/L)	38	22–72	–3	–22 to 33	0.17
GGT (U/L)	11	5–24	1	–7 to 52	0.18
Total bilirubin (mg/dL)	0.3	0.1–2.0	0	–1.8 to 0.8	0.31
Blood glucose–electrolytes					
Blood glucose (mg/dL)	96	70–129	3	–18 to 19	0.04
Calcium (mg/dL)	10.4	9.0–11.3	0.1	–0.6 to 1.2	0.28
Magnesium (mg/dL)	2.0	1.7–2.8	0	–0.3 to 0.4	0.69

*Difference (after treatment > before treatment) considered significant ($P \leq 0.05$) after applying a Bonferroni correction for family-wise error rate.

($r = –0.49$; $P = 0.002$) with attachment loss; the creatinine concentration ranged from 0.6 to 1.9 mg/dL (median, 1 mg/dL). No other variable in the renal subset was significantly correlated with attachment loss. No variable in the liver function subset was significantly correlated with attachment loss, and blood glucose, calcium, and magnesium concentrations were not significantly correlated with attachment loss.

A 2-tailed Wilcoxon signed-rank test was conducted for differences in variables before and after treatment (**Table 2**). Variables for which there was a significant reduction after treatment included serum CRP concentration, albumin concentration, BUN concentration (a negative correlation), USG, and blood glucose concentration. When a Bonferroni correction for family-wise error rate was applied, only BUN concentration had a significant change from before to after treatment; the BUN concentration before treatment ranged from 6 to 41 mg/dL (median, 13 mg/dL), whereas the BUN concentration after treatment ranged from 8 to 68 mg/dL (median, 17 mg/dL). The median within-dog increase in BUN concentration was 2 mg/dL, but the maximum increase was 44 mg/dL. Although there was no significant change after Bonferroni correction for serum CRP concentration when comparing values obtained before and after treatment, the within-dog change in serum CRP concentration was significantly correlated ($r = 0.40$; $P = 0.01$) with increasing severity of attachment loss (**Table 3**). No differences between before and after treatment were associated with antimicrobial administration or duration of anesthesia.

Table 3—Results of correlation analysis for hematologic and urine variable difference before and after treatment for PD with gingivitis or with attachment loss in 38 dogs.

Variable	Correlation with gingivitis		Correlation with attachment loss	
	r	*P* value	*r*	*P* value
Inflammation				
Serum CRP	0.21	0.20	0.40	0.01*
Globulin	0.07	0.67	0.13	0.42
Albumin	–0.17	0.29	–0.13	0.42
WBCs	0.14	0.38	–0.06	0.68
Neutrophils	0.10	0.52	–0.12	0.46
Monocytes	0.25	0.12	0.28	0.09
Lymphocytes	0.06	0.73	0.11	0.50
Platelets	0.18	0.27	0.19	0.25
Renal				
Microalbuminuria	–0.01	0.99	0.01	0.99
BUN	0.02	0.91	–0.08	0.63
Creatinine	–0.06	0.70	–0.27	0.11
USG	0.15	0.34	0.16	0.33
UPC	0.03	0.87	0.07	0.70
Phosphorus	–0.04	0.83	–0.01	0.99
Liver				
ALP	0.07	0.68	0.10	0.55
ALT	0.23	0.16	0.17	0.30
AST	0.06	0.71	0.06	0.70
GGT	0.19	0.26	0.09	0.60
Total bilirubin	0.22	0.18	0.15	0.38
Blood glucose–electrolytes				
Blood glucose	–0.05	0.76	0.03	0.87
Calcium	0.13	0.43	0.03	0.87
Magnesium	0.14	0.40	0.13	0.44

*Correlation considered significant ($P \leq 0.05$) after applying a Bonferroni correction for family-wise error rate.

Discussion

In the past, the heterogeneity of methods used for the assessment of oral disease has made it difficult to directly compare study results. Studies have varied with regard to age of dogs, breed, size of dogs, teeth evaluated, indices for gingivitis and attachment loss, scoring instruments, inclusion of missing teeth, and subject vitality. In the study reported here, every tooth was evaluated in vivo and the amount of systemic inflammation and organ health were assessed in each dog. The ranges for age and body weight indicated that this study included a variety of dogs. Because increasing age and decreasing body size negatively affect oral health in dogs,[25] the variety in the study population should enhance our ability to generalize the results. In comparison, the 2 previous studies[14,15] that linked PD to pathological changes in organs of dogs were retrospective cadaveric evaluations.

The TMPS system allows for a complete evaluation of canine periodontal health and provides a standardized method for use in future studies.[21] Because PD is influenced by body size, the weighted attachment loss score generated by use of the TMPS system takes into consideration the effect that body size has on total surface area of the tooth root and its influence on encompassing periodontal tissue. The weighted attachment loss score normalizes the proportion of tooth root surface per dog and results in comparable attachment loss scores among dogs of different sizes but similar periodontal health. Gingivitis was not weighted because the scoring system does not result in different scores for disease of equal severity in a large dog or a small dog. All dogs were considered to be their own control animal because healthy periodontal states were achieved by the time of the 30-day follow-up evaluation.

Use of the 2 scores, gingivitis and attachment loss, for comparison with systemic health indicators was essential because of the nature of PD. Periodontal disease is a dynamic pathological condition that progresses in some cases to loss of the tooth; there sometimes are periods of quiescence during which the amount of active inflammation around each tooth subsides. The gingivitis score represents the patient's active-inflammation burden at the time of examination. Attachment loss, on the other hand, measures the amount of destruction resulting from PD in the past as well as the present. Teeth in the quiescent phase can have a low gingivitis score but high attachment loss score, and teeth recently affected by PD can have a high gingivitis score with little to no attachment loss (normal gingival sulcus depth of 1 to 3 mm). It may be surprising that the median attachment loss was relatively low (1.66 mm), but the TMPSs used in the analyses were a weighted average of all teeth. Typically, not all teeth are equally affected by PD at any one time.

The 4 subsets (inflammation, renal function, liver function, and blood glucose–electrolytes) were chosen for evaluation on the basis of studies[10,14,15] in which investigators detected an association between PD, pathological conditions of organs, or changes in systemic hematologic values. The basic immune response underlying the pathogenesis of PD has been described.[3,6] The variables chosen in the present study were selected because of their accurate representation of organ and systemic functioning. Although correlation analyses of several individual variables had values of $P \leq 0.05$ (Tables 1 and 2), significant correlations were limited when the Bonferroni corrections for variable subsets was applied.

Periodontal disease can be considered an acute or chronic inflammatory state, depending on the stage of disease involving specific teeth. Inflammatory variables were chosen to reflect the dynamic nature of PD, their historically accurate representation of stimulation of the immune system (ie, WBCs and globulin concentration), and their recognized importance in the inflammatory pathway (ie, serum CRP concentration, albumin concentration, and platelet counts). Chronic inflammatory states may or may not be associated with an increase in the number of WBCs. It has been reported[26,27] that the number of WBCs is higher in humans with periodontitis, although this has not been determined in dogs.

Globulin concentration was chosen as an indirect measure of circulating antibodies. Immunoglobulins specific for plaque bacteria can be found in the systemic circulation of humans and dogs.[28–30] In addition, the serum globulin fraction is also composed of nonimmunoglobulins synthesized by the liver, some of which are acute-phase reactants whose production is increased in response to systemic inflammatory disease.[31] Therefore, globulin concentration was used as a nonspecific protein marker of cell-mediated and humoral immune system stimulation. An association of increasing globulin concentration with increasing severity of PD was anticipated because studies[29,30] have revealed the development of antibodies against a wide variety of periodontal pathogens. In addition, an increase in globulin concentration may be a reflection of hepatic stimulation attributable to a systemic inflammatory response triggered by PD.

Although acute-phase protein profiles currently are advocated as a more accurate representation of inflammation, at the time of the study, laboratory tests were readily available for only CRP (a strong acute-phase protein) and albumin. Hepatic production of CRP is induced by proinflammatory cytokines in response to tissue injury and infection, both of which are involved in periodontitis.[26] Concentrations of CRP correlate with some acute and chronic inflammatory conditions in dogs.[32–36] Increases in CRP concentrations have been strongly associated with PD in humans, and CRP concentrations significantly decrease following treatment for PD.[37] Research into acute-phase protein reactions in dogs has included investigation of the role of albumin (a negative acute-phase protein) in the inflammatory response.[38] It was surprising that serum CRP and albumin concentrations were not correlated with increasing severity of PD, but the chronic nature of inflammation associated with long-standing PD may explain the lack of an association. The significant association for decreasing CRP concentrations after treatment with increasing attachment loss hints at support for the hypothesis that alleviation of chronic periodontal inflammation can result in a systemic decrease of inflammatory burden.

Although it has been traditionally thought that platelets mainly function in homeostasis, they also play a critical role in inflammatory processes, with platelet

numbers increasing during chronic inflammation.[39,40] The reason for the significant correlation of increased platelet count with gingivitis and with attachment loss may be twofold. First, PD leads to the breakdown of the gingival sulcular epithelium, including the endothelium of the vessels associated with this tissue. As a result, continuous spontaneous or mechanically induced hemorrhage of this tissue is common. Therefore, the coagulation pathway is repeatedly stimulated in more severe cases of PD. Second, the influence and intimate relationship of platelets with the inflammatory response and wound healing might play a part in repair of damaged sulcular epithelium leading to stimulation of the megakaryocytic pathway.[40] The apparent lack of a response after treatment may be a result of long-term stimulation of the bone marrow that requires > 30 days to mitigate.

Histologic lesions of the renal glomeruli, interstitium, and tubules have been associated with PD in dogs.[14,15] The lesions observed were inflammatory or suspected to be from immune complex–mediated damage. There have been multiple human studies[41–43] in which PD was associated with decreasing renal function and chronic renal failure. The major markers for renal health in the study reported here were UPC, USG, and concentrations of creatinine, BUN, and phosphorus.

A test to measure low concentrations of albumin in the urine was added to the major markers of renal health to aid in identifying pathological changes from renal injury earlier than would be detectable by the other tests. Albumin is typically excluded from glomerular filtrate because of its size, but even when there are small amounts of albumin in healthy renal tubules, it is reabsorbed or degraded.[44] Pathological changes in glomerular permeability and failure in tubular cellular processing can lead to microalbuminuria without changes in the major markers of renal function. Various inflammatory disease processes have been linked to microalbuminuria without overt signs of chronic renal failure.[n,o] There was no significant increase in microalbuminuria when a Bonferroni correction was applied; however, the pattern for an increase in microalbuminuria with increasing severity of gingivitis was interesting. Systemic inflammation and infection can cause changes in renal filtration and cellular processing, without substantial pathological changes. Lack of a significant correlation may be explained by the semiquantitative nature of the test or the unexplained high rate of positive results (19%) in healthy dogs.[p]

A significant moderate negative correlation was detected between creatinine concentration and increasing severity of attachment loss. A direct association between severe PD and healthier kidneys is counterintuitive; therefore, we are suggesting another explanation for this relation. Dogs with increasing severity of PD may be more prone to decreases in muscle mass as a result of a decreased ability to masticate appropriately; consequently, subclinical chronic malnutrition may result. Decreased muscle mass leads to lower overall circulating creatinine concentrations.[45] Therefore, the decreasing creatinine concentration may be a reflection of the decreasing muscle mass as the severity of PD increases. A study conducted to carefully examine muscle mass of patients with PD before and after treatment is warranted. The increase in phosphorus concentration associated with increases for gingivitis and for attachment loss was not suspected to be of renal origin because of the decreasing creatinine concentration; creatinine and phosphorus are similarly dependent on glomerular filtration rates for excretion. Increasing phosphorus concentrations are most likely a component of increased metabolism and production attributable to bone loss associated with PD.

Treatment of PD significantly affected only the BUN concentration in the present study, with higher concentrations after treatment. Anesthesia was investigated as a possible cause, but no correlation was found between increasing duration of anesthesia and BUN values. The USG after treatment of PD did not indicate decreased renal function; therefore, another reason for the increase in BUN concentration was needed. Because BUN concentrations can be affected by protein consumption, we theorize that the increase in BUN concentration could have been attributable to dogs having less discomfort in their mouths after treatment and eating better, which resulted in increased protein consumption and a higher BUN concentration.

Hepatic histologic examination in dogs with PD has revealed mild to moderate, multifocal regions of inflammation.[14,15] In the study reported here, the hepatobiliary enzymes used to evaluate liver health included ALP, ALT, AST, and GGT; we also evaluated total bilirubin concentration. In addition, evaluation of globulin and albumin concentrations can be used in detection of hepatic disease; chronic inflammatory liver disease can be accompanied by hyperglobulinemia and hypoalbuminemia.[46] Although not significant after a Bonferroni correction, the increase in ALP and ALT activities and globulin concentration with increasing severity of PD may be worth exploring. Increases in ALT activity are seen with hepatocellular necrosis and inflammation, whereas AST activity is a more sensitive marker for hepatobiliary disease; hepatic injuries of greater severity are required for release of AST from hepatocellular mitochondria.[46] Increased ALP activity can also result from chronic inflammation of the liver.[46] It is possible that only the more sensitive indicators of hepatocellular inflammation (ie, ALT and ALP activities) would increase because the degree of inflammatory change in the liver parenchyma is relatively mild and multifocal. In this scenario, the severity of hepatic inflammation may not have reached the threshold for release of AST from hepatic mitochondria. In addition, enzyme histochemical analyses revealed that chronically inflamed gingiva has elevated ALP activity within gingival crevicular fluid, most likely as a result of the increased bone metabolism and remodeling caused by PD.[47] The lack of a response in all hepatic indices to treatment of PD warrants further investigation.

The relationship between PD and diabetes mellitus has clearly been established in humans.[13,48–50] Such a relationship has been suspected in veterinary medicine,[51,52] but to our knowledge, it has not yet been established. Although evaluating only blood glucose concentrations in a population of apparently healthy dogs does not substantiate a relationship between PD and

diabetes mellitus in dogs, the increase in blood glucose concentrations in relation to attachment loss and the difference in blood glucose concentrations before and after treatment of PD were interesting. Increased circulating concentrations of blood glucose during active PD may be a result of low-level insulin resistance triggered by inflammation. The same proinflammatory cytokines (IL-6, IL-1, interferon-γ, and tumor necrosis factor-α) that signal the production of acute-phase proteins are also capable of inducing insulin resistance.[53] Further research into the relationship between PD and diabetes mellitus in dogs is justified.

The sample size, broad scope, and abbreviated follow-up period were limiting factors of the study reported here. A similar study with a larger number of participants and that involves monitoring of dogs with advanced PD or systemic disease for a longer period may result in more conclusive findings. The 30-day reevaluation time point was chosen to allow for values to return to within the respective reference intervals and to ensure proper oral healing after treatment. Further follow-up monitoring was not pursued because of client expectations and limitations. The effects of long-standing inflammation, infection, and prolonged systemic response may explain the lack of change in values before and after treatment in such a short period, although only samples obtained during a longer follow-up period would confirm this suspicion.

We concluded that analysis of results of this prospective in vivo study reveals associations between the severity of PD and systemic inflammation. Some findings are consistent with results from more extensive studies that have been conducted in humans. Although no causal relationship can be proven by the present study, the association of PD to overall systemic inflammatory burden and organ health highlights the need for continued research.

a. Cell-Dyn 3700, Abbott Laboratories, Abbott Park, Ill.
b. Scil ABC Veterinary Hematology Analyzer, Scil Animal Care Co, Viernheim, Germany.
c. Vitros 350 Chemistry System, Ortho Clinical Diagnostics, Rochester, NY.
d. Tridelta phase range CRP—canine assay, Tridelta Development Ltd, Maynooth, Ireland.
e. IDEXX VetLab UA analyzer, IDEXX Laboratories Inc, Westbrook, Me.
f. E.R.D. HealthScreen canine urine test, Heska Corp, Loveland, Colo.
g. Clavamox, SmithKline Beecham, Exton, Pa.
h. Antirobe, Upjohn Co, Kalamazoo, Mich.
i. Deramaxx, Novartis Animal Health U8 Inc, Greensboro, NC.
j. Torbugesic, Fort Dodge Laboratories Inc, Fort Dodge, Iowa.
k. Buprenex, Reckitt Benckiser Pharmaceuticals Inc, Slough, Berkshire, England.
l. Statistix 8.0, Analytical Software, Tallahassee, Fla.
m. Rawlinson JE, Goldstein RE, Erb HN, et al. Tracking inflammatory and renal parameters in dogs pre- and post-treatment for periodontal disease (abstr), in *Proceedings*. Am Coll Vet Intern Med Forum 2005;871.
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