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Prognostic factors in dogs with protein-losing enteropathy

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ABSTRACT

Canine protein-losing enteropathy (PLE) is associated with severe gastrointestinal disorders and has a guarded to poor prognosis although little information is available regarding factors affecting prognosis. The purpose of this study was to identify the prognostic factors for survival of dogs with PLE. Ninety-two dogs diagnosed with PLE from 2006 to 2011 were included in a retrospective cohort study. Survival analysis was performed using the Kaplan–Meier method and log-rank test. Variables recorded at the time of diagnosis were statistically analysed for possible prognostic factors in a univariate and multivariate Cox proportional hazard model.

In the multivariate analysis, the predictors for mortality in dogs with PLE were more highly scored in terms of canine inflammatory bowel disease activity index (CIBDAI) ($P = 0.0003$), clonal rearrangement of lymphocyte antigen receptor genes ($P = 0.003$), and elevation of blood urea nitrogen (BUN) ($P = 0.03$). Using histopathological diagnosis, both small- and large-cell lymphomas were associated with significantly shorter survival times than chronic enteritis (CE) and intestinal lymphangiectasia (IL). Normalization of CIBDAI and plasma albumin concentration within 50 days of initial treatment was associated with a longer survival time. In conclusion, CIBDAI, clonal rearrangement of lymphocyte antigen receptor genes, histopathological diagnosis, and response to initial treatments would be valuable in separating the underlying causes and could be important in predicting prognosis in dogs with PLE.

Introduction

Protein-losing enteropathy (PLE) in dogs is characterized by hypoalbuminaemia due to excessive loss of plasma proteins through the gastrointestinal mucosa. PLE can be associated with various diseases, and chronic enteritis (CE), intestinal lymphangiectasia (IL), and gastrointestinal (GI) lymphoma are the common underlying diseases in canine PLE (Dossin and Lavoue, 2011; Dandrieux et al., 2013). Because PLE results from severe GI disorders, the prognosis is usually considered guarded to poor (Dossin and Lavoue, 2011).

In dogs with CE, hypoalbuminaemia has previously been reported as a poor prognostic factor (Craven et al., 2004; Allenspach et al., 2007) although little information is available on survival and prognostic factors in canine PLE. One small data set indicated that the 3-year survival rate was 70% in CE dogs with hypoalbuminaemia (Allenspach et al., 2007). Another report estimated that PLE dogs with hypercoagulability had a death rate of 66% within 5 months (Goodwin et al., 2011). With canine IL, there are no data about long-term survival rate and prognosis. On the other hand, the prognosis is poor for dogs with GI lymphoma, even for those undergoing multidrug chemotherapy (Rassnick et al., 2009).

The purpose of this retrospective study was to identify the prognostic factors for survival of dogs with GI disorders associated with hypoalbuminaemia.

Materials and methods

Case selection

Dogs diagnosed with PLE from 1 April 2006 to 31 January 2011 were identified from the medical records of the Veterinary Medical Center of the University of Tokyo (VMC-UTokyo). Inclusion criteria for the PLE dogs included hypoalbuminaemia (<2.7 g/dL), histological or cytological observations of gastrointestinal diseases known to be associated with PLE, and absence of other causes of hypoalbuminaemia (e.g. hepatic insufficiency, protein-losing nephropathy, severe cutaneous lesions, exocrine pancreatic insufficiency, and previous history of severe bleeding episodes).

The minimum diagnostic evaluation performed included complete blood cell count, plasma biochemistry, plasma C-reactive protein (CRP) concentration, serum bile acid concentration (fasted and postprandial), serum trypsin-like immunoreactivity, urinalysis, direct smear and flotation examination of faeces for nematode and protozoan parasites, abdominal radiography, and ultrasonography. For histological examination, mucosal biopsy specimens were obtained from dogs by gastroduodenoscopy or ileocolonoscopy or both. In some dogs, fine-needle aspiration of thickened intestinal wall or mesenteric lymph node was performed for

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cytological examination. Cases were excluded if underlying or concurrent disorders other than PLE were confirmed.

Data collection

Information collected included breed, sex, neuter status, age, and bodyweight. The severity of the clinical signs was assessed using two previously described activity indexes, namely, the canine inflammatory bowel disease activity index (CIBDAI) (Jergens et al., 2003) and the canine chronic enteropathy clinical activity index (CCECAI) (Allenspach et al., 2007). The CIBDAI is the sum of the scores for six different clinical signs including attitude/activity, appetite, vomiting, stool consistency, stool frequency, and weight loss. The CCECAI is based on the CIBDAI and further includes scores for albumin (ALB) concentration, peripheral oedema or ascites, and severity of pruritus.

Laboratory parameters for the analysis of PLE included packed cell volume (PCV), total white blood cell count (WBC), plasma chemistry profiles of ALB, globulin (GLB), blood urea nitrogen (BUN), creatinine (CRE), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total cholesterol (TCHO), magnesium (Mg), calcium (Ca), sodium (Na), potassium (K), chloride (Cl), and CRP.

Histopathology

At the time of diagnosis, at least six mucosal samples per location (stomach, duodenum, ileum, and colon) were obtained from each dog by endoscopic biopsy. Biopsy samples were fixed in 10% neutral buffered formalin, and haematoxylin and eosin stained sections were prepared. Histopathological assessment was retrospectively performed by one pathologist, who was blinded to the previous diagnosis, and classified objectively into four groups (CE, IL, large-cell lymphoma, and small-cell lymphoma; see Appendix: Supplementary Figs. S1–S4, respectively).

CE and IL were diagnosed according to the histopathological standards of the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group (Day et al., 2008). In addition, we identified IL by marked lacteal dilation of villous lamina propria (Day et al., 2008). Large- and small-cell lymphomas were diagnosed according to the World Health Organization lymphoma diagnostic criteria (Valli et al., 2002). Regarding small-cell lymphoma, we also referred to a past report on feline small-cell GI lymphoma (Kiupel et al., 2011) in which severe infiltration of intraepithelial lymphocytes, termed nests and plaques, was statistically significant in determining a diagnosis of lymphoma versus IBD.

PCR for antigen receptor gene rearrangement (PARR)

Endoscopic biopsy samples were subjected to PARR, following previously described methods (Fukushima et al., 2009). The determination of clonality of a sample required the presence of appropriate and same-sized single sharp bands in duplicate samples with heteroduplex analysis; all other samples were interpreted as not clonal.

Response to treatments

Regarding the response to treatments, the dogs were classified as responders or non-responders by plasma ALB concentration, CIBDAI, and CCECAI within 50 days of starting the initial treatments. Dogs with normalized ALB (≥2.7 g/dL), CIBDAI (≤3), or CCECAI (≤3) were classified as responders, whereas dogs with abnormal ALB (<2.7 g/dL), CIBDAI (≥4), or CCECAI (≥4) throughout the period were classified as non-responders.

Follow-up

The dates that dogs were last known to be alive before being lost to follow-up or the dates of death or euthanasia were confirmed from the medical records at the participating institutions or by phone contact. Telephone interviews with the owners or the referring veterinarians were performed during November 2011.

Statistical analysis

The baseline characteristics were compared among histological diagnoses with one-way ANOVA. For comparison of the survival time from the day of diagnosis among the groups, we used Kaplan–Meier curves and two-sided log-rank tests. We conducted univariate Cox proportional hazard analysis for each variable that was a potential prognostic factor in dogs with PLE. With a stepwise model selection method using multivariate Cox proportional hazard analysis, we developed a model for significant covariates identified in the previous univariate model. We adopted the following criteria for allowing and keeping a variable in the model: slentry = 0.25 and slstay = 0.15. All statistical analyses were performed using SAS 9.3 software (SAS Institute). Statistical significance was defined as $P < 0.05$.

Results

Cases

Ninety-two dogs with PLE were included in this study. The breeds were as follows: Shiba inu (11), miniature Dachshund (10), Yorkshire terrier (10), Chihuahua (9), Maltese (6), Papillon (6), French bulldog (4), Labrador retriever (4), Pomeranian (4), mixed breed (4), Shetland sheepdog (3), Border collie (2), German shepherd dog (2), Italian greyhound (2), and toy Poodle (2). The remaining 13 dogs were of other breeds. Forty-four out of 92 dogs were male (30 intact, 14 castrated) and 48/92 were female (21 intact, 27 spayed). Median age at the time of diagnosis of PLE was 6.9 years (range, 1.7–14.0).

On the basis of the histological evaluation, 34 dogs were diagnosed with CE, 28 with IL, 19 with small-cell lymphoma, and eight with large-cell lymphoma. On the basis of cytological examination, three dogs were diagnosed with large-cell lymphoma. Baseline characteristics of the dogs with CE, IL, small-cell lymphoma, and large-cell lymphoma are summarized in Table 1. Values of all variables were statistically compared among groups. Age, PCV, ALP, Cl,

Table 1
Summary of signalment and laboratory results at diagnosis of protein-losing enteropathy dogs divided into four histological diagnoses.

	CE		IL		Small-cell lymphoma		Large-cell lymphoma		
	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	*P*
BW (kg)	7.2 ± 7.0	34	5.2 ± 3.4	28	8.9 ± 8.8	19	9.3 ± 6.8	11	0.24
Age (years)	7.1 ± 2.7	34	6.9 ± 2.5	28	7.8 ± 2.3	19	8.9 ± 2.5	11	0.04
ALB (g/dL)	1.6 ± 0.4	34	1.6 ± 0.3	28	1.6 ± 0.4	19	1.8 ± 0.4	11	0.29
GLB (g/dL)	2.0 ± 0.7	33	1.7 ± 0.6	28	2.1 ± 0.7	19	1.8 ± 0.6	11	0.97
PCV (%)	43.2 ± 9.4	33	40.3 ± 4.4	28	37.9 ± 6.6	19	36.0 ± 9.3	11	0.002
WBC (/μL)	17,803 ± 9965	33	19,475 ± 7490	28	15,779 ± 7182	19	26,027 ± 8996	11	0.13
BUN (mg/dL)	15.8 ± 4.7	33	14.9 ± 6.3	27	14.9 ± 7.2	19	12.1 ± 3.8	11	0.11
CRE (mg/dL)	0.7 ± 0.4	33	0.4 ± 0.2	27	0.6 ± 0.3	19	0.5 ± 0.3	11	0.13
ALP (U/L)	216 ± 308	32	242 ± 360	27	561 ± 863	19	924 ± 1260	11	0.001
ALT (U/L)	87 ± 99	32	109 ± 107	27	197 ± 239	19	128 ± 148	11	0.06
Na (mEq/L)	147 ± 2	29	144 ± 5	21	146 ± 4	19	144 ± 4	11	0.07
K (mEq/L)	4.0 ± 0.6	29	4.0 ± 0.7	21	3.9 ± 0.5	19	3.8 ± 0.7	11	0.34
Cl (mEq/L)	112 ± 6	29	107 ± 6	21	109 ± 7	19	107 ± 7	11	0.05
Ca (mg/dL)	8.17 ± 1.3	34	7.6 ± 1.4	27	8.1 ± 1.2	19	8.2 ± 0.9	11	0.97
TCHO (mg/dL)	104 ± 45	34	90 ± 38	27	106 ± 43	19	105 ± 60	11	0.86
Mg (mg/dL)	1.4 ± 0.4	34	1.3 ± 0.6	27	1.2 ± 0.3	19	1.5 ± 0.4	11	0.84
CRP (mg/dL)	1.7 ± 2.2	34	1.3 ± 1.4	28	2.0 ± 2.6	19	2.8 ± 3.5	10	0.22
CIBDAI	5.3 ± 4.2	33	6.9 ± 4.0	28	7.3 ± 3.5	19	11.0 ± 3.0	11	0.0001
CCECAI	7.7 ± 4.7	33	10.3 ± 4.5	28	9.9 ± 3.1	19	12.6 ± 4.0	11	0.002

CE, chronic enteritis; IL, intestinal lymphangiectasia; BW, bodyweight; ALB, albumin; GLB, globulin; PCV, packed cell volume; WBC, total white blood cell count; BUN, blood urea nitrogen; CRE, creatinine; ALP, alkaline phosphatase; ALT, alanine aminotransferase; TCHO, total cholesterol; CRP, C-reactive protein; CIBDAI, canine inflammatory bowel disease activity index; CCECAI, canine chronic enteropathy clinical activity index; SD, standard deviation.