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STANDARD ARTICLE

Association of chronic enteropathy activity index, blood urea concentration, and risk of death in dogs with protein-losing enteropathy

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Background: Malnutrition is associated with increased risk of premature death in humans with inflammatory bowel disease.

Hypothesis/Objective: To determine if historical, clinical, and laboratory markers of malnutrition in dogs at the time of histologic diagnosis of protein-losing enteropathy (PLE) caused by chronic enteropathy (CE) or lymphangiectasia are associated with increased risk of death.

Animals: Seventy-one client-owned dogs diagnosed with PLE.

Methods: The medical records were retrospectively searched for cases of PLE, diagnosed with CE or lymphangiectasia on the basis of histopathology of intestinal biopsies at a referral hospital. For each case, various variables at the time of diagnostic investigation were recorded and follow-up obtained by telephone contact with the referring veterinarian.

Results: A multivariable cox model indicated that canine chronic enteropathy activity index (CCEAI) and blood urea concentration were significantly associated with death (*P* values <.01). For each unit increase in CCEAI, the hazard of death increased by 22.9% (confidence interval [CI]: 6.9%-41.2%). Dogs with a CCEAI of ≤8 and dogs with urea ≤7 mmol/L survived 256 days longer (*P* = .001, CI: 106.7-405.4 days) and 279 days longer (*P* = .009, CI: 70.0-488.7 days) than those with a CCEAI of >8 and urea >7 mmol/L on average, respectively, when followed up for 647 days.

Conclusions and Clinical Importance: Increased CCEAI and blood urea concentration at the time of diagnosis might be predictive of death in dogs with PLE caused by CE or lymphangiectasia.

KEYWORDS

bowel, canine, lymphangiectasia, malnutrition, survival

1 | INTRODUCTION

Dogs with protein-losing enteropathy (PLE) caused by chronic enteropathy (CE) or lymphangiectasia have poor prognosis after failure with immunomodulatory treatment.[1] Although, various studies have aimed to identify prognostic markers in dogs with PLE caused by CE or lymphangiectasia, these have led to variable and inconsistent results.[1–5] To the authors' knowledge, body condition score (BCS) has been assessed in only 1 study in dogs with PLE caused by CE, which documented that the median BCS at the time of diagnosis was not different between dogs with PLE that had a good or bad outcome.[4] However, this study used a relatively short follow-up period (ie, 4 months) to define outcome and, although not its main intention, it did not assess the effect of BCS adjusting for other variables in a multivariable model.

Abbreviations: AIC, Akaike information criterion; BCS, body condition score; BMI, body mass index; CCEAI, canine chronic enteropathy activity index; CE, chronic enteropathy; CI, confidence interval; GI, gastrointestinal; IBD, inflammatory bowel disease; LRT, likelihood ratio test; PH, proportional hazard; PLE, protein-losing enteropathy; RMST, restricted mean survival time.

Body condition score in dogs is one of the measures used to assess nutritional status.[6,7] Nutritional status in dogs with PLE caused by CE or lymphangiectasia might be important prognostically, as malnutrition is associated with increased in-hospital death and duration of stay in humans with inflammatory bowel disease (IBD).[8] This study hypothesized that, as malnutrition is associated with a weakened immune system,[9,10] the use of concurrent immunomodulatory treatment in humans with IBD could lead to increased death. As immunomodulatory treatment is commonly used in dogs with PLE caused by CE or lymphangiectasia, the investigation of malnutrition as a predictor for death might not only help prognostication, but might help to determine whether assisted dietary intervention at an earlier stage optimizes response to immunomodulatory treatment and therefore prognosis. Also, if markers of malnutrition are associated with death, then incorporation into index measures of clinical disease activity should be considered.

Although there is no complete agreement on the elements that define malnutrition in human or veterinary medicine, The European Society for Clinical Nutrition and Metabolism states that the diagnosis of malnutrition in humans should be based on either a body mass index (BMI) of <18.5 or a combined finding of unintentional weight loss with either reduced BMI or a low fat free mass.[11] Single micronutrient deficiencies such as cobalamin and folate can also help to define malnutrition.[12] Serum albumin and BMI are the most predictive of all variables of malnutrition in human IBD patients with active disease.[13] However, BMI and unintentional weight loss together with recent nutrient intake and severity of disease might be more appropriate for the assessment of malnutrition in human IBD patients.[14]

Therefore, the aim of our study was to assess whether BCS and other indicators of malnutrition used in human IBD patients, such as body weight, percentage weight loss, appetite, serum albumin, cholesterol, urea, creatinine, cobalamin and folate concentrations, as well as severity of disease and duration of clinical signs at the time of histologic diagnosis can be used as predictors for death after treatment failure in dogs with PLE caused by CE or lymphangiectasia. In our study, severity of disease was estimated using the canine chronic enteropathy activity index (CCEAI), which is based on the presence and severity of 9 factors including attitude/activity, appetite, vomiting, consistency of feces, frequency of defecation, weight loss, serum albumin concentrations, ascites and peripheral edema, and pruritus.[15]

2 | MATERIALS AND METHODS

2.1 | Data collection

The medical records at the University of Bristol Small Animal Referral Hospital were retrospectively searched for dogs presented between January 2010 and October 2017 with a diagnosis of PLE from either a CE or lymphangiectasia diagnosed on histopathology of endoscopically collected intestinal biopsies performed at our hospital. Inclusion criteria included those dogs diagnosed with PLE based on biochemical profile and consistent signs of gastrointestinal (GI) disease that had, to rule out other causes, appropriate diagnostic investigations, including at least an upper GI endoscopy with collection of intestinal biopsies, and which documented CE or lymphangiectasia. A minimum follow-up period of 4 months since discharge from the hospital was required for inclusion into the study if the dog was still alive at the end of the study. This minimum time period has been used in previous studies assessing outcome in dogs with PLE.[3,4] Those dogs that had died or were euthanized after histopathology of intestinal biopsies but before hospital discharge were also included. Exclusion criteria consisted of those cases that did not have collection of intestinal biopsies performed or were diagnosed with alimentary lymphoma. In addition, dogs diagnosed with CE or intestinal lymphangiectasia and still alive that were followed up <4 months since discharge from the hospital or had incomplete medical records including an absent BCS were also excluded. Seventy-one dogs meeting the inclusion criteria were included in the study.

The diagnostic evaluation for each of the 71 dogs included a complete blood count, serum biochemistry, serum cobalamin and folate concentrations, transabdominal ultrasound examination and upper GI endoscopy and collection of biopsy specimens. Of these dogs, a number had additional diagnostic procedures performed as indicated by the history, physical examination, and ultrasound examination findings: collection of intestinal biopsy specimens by lower GI endoscopy in 29 dogs (41%), pancreatic function testing (canine pancreatic lipase immunoreactivity in 26 dogs [37%] and trypsin-like immunoreactivity in 37 [52%]), basal cortisol concentration or ACTH stimulation test in 47 dogs (66%), preprandial or pre- and postprandial bile acid concentrations in 52 dogs (73%), fecal parasitology using zinc sulfate flotation with centrifugation in 53 dogs (75%), fecal culture (for *Salmonella*, *Campylobacter*, and *Clostridium difficile*) in 45 dogs (63%), empirical deworming in 64 dogs (90%), and urine protein:creatinine ratio in 59 dogs (83%). All medical records were reviewed by 1 of the authors (Aarti Kathrani) and included the following information for each dog: signalment (including breed, sex, neutering status, and age); clinical history including duration of clinical signs, appetite, lethargy, body weight, percentage weight loss, presence of ascites and peripheral edema, vomiting, and diarrhea; BCS (numerical score assessed on a 9-point[6] or 5-point scale[16]); results of diagnostic tests including laboratory findings, transabdominal ultrasound examination findings, endoscopic findings, and histopathology report; and treatment prescribed. For each dog, neutering status, age, BCS, and other historical, clinical, and laboratory markers of malnutrition used for analysis were recorded at the time of histologic diagnosis of PLE caused by CE or lymphangiectasia. Outcomes for each dog were obtained from medical records as well as follow-up phone contact with the referring veterinary practice.

2.2 | Data management

Many breeds were present in the dataset, most represented by only a few individuals, limiting further breed analysis. Thus, for the purposes of this study, only the animal's breed, classified as purebred or crossbred, was further assessed. The percentage weight loss was calculated as the difference between the last recorded stable body weight based on body weight histories obtained from the medical record from the referring veterinarian and the body weight at admission to our hospital. The CCEAI was retrospectively calculated for each dog based on

the history collected at admission and the referring veterinarian's medical records.[15] Appetite at diagnosis was categorized as anorexic (appetite 1), hyporexic (appetite 2), unchanged (appetite 3), or increased (appetite 4). Eleven out of 71 dogs (15%) had BCS assessed on a 5-point scale with the remainder of the dogs having their BCS assessed on a 9-point scale. Therefore, the 11 dogs that had their BCS assessed on a 5-point scale were converted to a 9-point scale by dividing their score by 5 and multiplying by 9 and rounding to the closest half-integer.

2.3 | Statistical analysis

Survival analysis was conducted on all dogs diagnosed with PLE caused by CE or lymphangiectasia. The study endpoint was determined to be death because of or euthanasia because of PLE. The survival time interval from the date of PLE histologic diagnosis caused by CE or lymphangiectasia to death was recorded. For those cases in which survival time and cause of death were not recorded in the medical records, follow-up information was obtained by telephone interview with the referring veterinary practice. Dogs that were still alive when they were lost to follow-up or still alive at the end of the study, as well as dogs dying from causes other than PLE caused by CE or lymphangiectasia were all right censored.

A multivariable Cox proportional hazards (PH) regression analysis was conducted to assess the association between several markers of malnutrition and death after treatment failure. Tied survival times were handled using the Efron method. Initial univariable screening was undertaken for 4 explanatory categorical variables: breed, sex, neutering status, and appetite; and 9 quantitative variables: age, duration of clinical signs, body weight, percentage weight loss, CCEAI, urea (mmol/L), creatinine (umol/L), BCS, and serum albumin concentrations (g/L). These univariable analyses were carried out using the same dataset, which contained information for each variable. Explanatory variables were retained for multivariable analysis if a likelihood ratio test (LRT) indicated $P \leq .20$ against a null model on univariable analysis. Polynomial terms were included for quantitative exploratory variables if an LRT indicated significantly improved fit. Multivariable models underwent step-wise backward elimination to minimize Akaike Information Criterion (AIC). The assumption of PH was tested by post-examining the relationship between scaled Schoenfeld residuals and time. If the PH assumption is not satisfied for a covariate of the model, the pattern of Schoenfeld residuals was used to help identify an appropriate time-dependent function, and then a covariate with a time-varying effect using the "time transfer" function "tt" in the R package "survival" was created.[17] Explanatory variables included in the final multivariable model were further evaluated individually by using the Kaplan-Meier survival analysis. Quantitative variables were categorized as dichotomous variables for the analysis only when a clinically relevant cut point was possible. A log-rank test was used to test whether the overall survival functions across groups were equal. The difference in restricted mean survival time (RMST) between groups was tested for 647 days of follow-up (ie, the minimum follow-up time during which all deaths after treatment failure occurred).

Detailed information about the continuous variables cholesterol concentrations (mmol/L), cobalamin concentrations (pmol/L), and folate concentrations (nmol/L) was only available for 72% of the dogs in the multivariable analysis. Therefore, to avoid reducing the number of dogs included in the multivariable model, the association between these variables and death was only evaluated by univariable Cox PH regression models.

All analyses were carried out using R language version 3.4.1.[17] Cox PH regression analysis and Kaplan-Meier survival analysis were conducted using the R package "survival"[17] and RMST analysis was carried out using the "rmst2" function from the R package "survRM2."[18] Statistical significance was defined as $P < .05$.

3 | RESULTS

3.1 | Study population

Seventy-one dogs with PLE were included in the study: 12 intact males, 33 neutered males, 1 intact female, and 25 neutered females. The age of the dogs ranged from 1.2 to 13.5 years, with a median age of 7.7 years. Breeds included Staffordshire Bull Terrier (7), Border Collie (6), cross breed (5), German Shepherd (4), Labrador Retriever (4), Cocker spaniel (4), Cavalier King Charles Spaniel (4), Jack Russell Terrier (3), English Bulldog (3), Rottweiler (3), Yorkshire Terrier (2), Border Terrier (2), Lurcher (2), Shetland Sheepdog (2), Hungarian Vizsla (2), Greyhound (2), Weimaraner (2), Springer Spaniel (2), Cockerpoo (2), and 1 each of the following breeds: Japanese Akita, Great Dane, Newfoundland, Sloughi, Miniature Schnauzer, Bichon Frise, Fox Terrier, Toy Poodle, Chihuahua, and Dachshund.

3.2 | Descriptive statistics

3.2.1 | Duration of clinical signs, appetite, BCS, body weight, percentage weight loss, and CCEAI

Duration of clinical signs for all dogs ranged between 1 day and 1095 days (median, 60 days). Eight dogs (11%) were anorexic, 26 dogs (37%) were hyporexic, 25 dogs (35%) had an unchanged appetite, 8 dogs (11%) were reported to be polyphagic, and appetite was unreported in 4 dogs (6%) at the time of presentation. Forty-seven dogs (66%) were under-conditioned (median, 3; range, 1 to 4), 18 dogs (25%) were ideal-conditioned (median, 4; range, 4 to 5), and 6 dogs (8%) were over-conditioned (median: 6 to 6-7; range: 5-6 to 8). Body weight ranged from 2.6 to 54.9 kg with a median of 17 kg. Sixty-one dogs (88%) had documented weight loss at the time of diagnosis; 30 (43%) had severe (≥10%; median, 17.2%; range, 10%-32%), 22 (32%) had moderate (5%-9.9%; median, 7.5%; range, 5%-9.6%), and 9 (13%) had mild (0.1%-4.9%; median 3.1%; range, 2.1%-4.7%) loss; the remaining 10 dogs had documented 0% body weight loss and for 2 dogs, weight loss could not be objectively quantified based on the medical records provided by the referring veterinarian. The CCEAI ranged from 3 to 16 with a median of 8.

3.2.2 | Histologic diagnosis

All dogs had histopathology of intestinal biopsies that were collected via upper GI endoscopy. Sixty-eight dogs (96%) were diagnosed with a chronic inflammatory enteropathy; a total of 21 (31%) had

lymphoplasmacytic and eosinophilic enteritis; 15 (22%) had lymphoplasmacytic enteritis; 12 (18%) had eosinophilic enteritis; 7 (10%) had lymphoplasmacytic, eosinophilic, and neutrophilic enteritis; 7 (10%) had lymphoplasmacytic and neutrophilic enteritis; 3 (4%) had plasmacytic and eosinophilic enteritis; and 1 had each of plasmacytic enteritis, lymphoplasmacytic and granulocytic enteritis, and plasmacytic and neutrophilic enteritis. Thirteen of the 68 dogs (19%) with chronic inflammatory enteropathy had concurrent lacteal dilatation on histopathology and 9 (13%) had crypt abscesses. Three dogs were diagnosed with primary lymphangiectasia.

Colon biopsies were collected in 29 dogs (41%), 25 of which also had ileal biopsies performed; 10 (15%) had lymphoplasmacytic colitis, 8 (12%) were within normal limits, 3 (4%) had lymphoplasmacytic, neutrophilic and eosinophilic colitis, 2 (3%) had lymphoplasmacytic and eosinophilic colitis, 2 (3%) had lymphoplasmacytic and neutrophilic colitis, 2 (3%) had eosinophilic colitis, and 1 had each of the following (3%): plasmacytic and eosinophilic colitis and plasmacytic and neutrophilic colitis.

3.2.3 | Serum albumin, cholesterol, cobalamin, folate, urea, and creatinine concentrations

All dogs had hypoalbuminemia (median, 17.1 g/L; range, 12-29.3 g/L; reference range, 32-38 g/L). Forty dogs (56%) had hypocholesterolemia, 17 dogs (24%) had cholesterol within the reference range, and cholesterol was not measured in 14 dogs (20%) (median, 2.6 mmol/L; range, 1.1-7.1 mmol/L; reference range, 3.5-7.0 mmol/L). Thirty-eight dogs (54%) had cobalamin concentrations below the reference range, 23 dogs (32%) had concentrations within or above the reference range, and 10 dogs (14%) had received supplementation before measurement and therefore were not included (median, 165 pmol/L; range, 107-748 g/L; reference range, 200-408 g/L). Thirty-three dogs (46%) had folate concentrations below the reference range, 19 dogs (27%) had concentrations within the reference range, 9 (13%) were above the reference range, and 10 dogs (14%) had concentrations that were not available for review (median, 11.4 nmol/L; range, 2.8-49.4 g/L; reference range, 12.0-30.0 nmol/L). Ten dogs (14%) had urea concentrations above the reference range and 1 dog (1%) had concentrations below the reference range (median, 5.0 mmol/L; range, 1.8-15.1 mmol/L/L; reference range, 2-7 mmol/L). Fifty-three dogs (75%) had creatinine concentrations below the reference range and no dogs (0%) had concentrations above the reference range (median, 72 umol/L; range, 35-132 umol/L; reference range, 100-133 umol/L).

3.2.4 | Treatment

Seven dogs (10%) received dietary treatment alone with either a commercial therapeutic hydrolyzed or limited-ingredient novel protein or GI low-fat diet. Six dogs (8%) received dietary treatment and antimicrobial treatment. Fifteen dogs (21%) received a combination of dietary treatment and corticosteroid treatment and 17 dogs (24%) received this combination with antimicrobial treatment. Nine dogs (13%) received combined dietary treatment, corticosteroid and cyclosporine and 12 dogs (17%) received this same combination with antimicrobial treatment. Two dogs (3%) received a combination of dietary treatment, corticosteroid, and azathioprine, and 3 dogs (4%) received this same combination with antimicrobial treatment.

Sixty-four dogs (90%) received empirical deworming, 43 dogs (61%) received parenteral cobalamin supplementation, and 12 dogs (17%) received either low-dose aspirin or clopidogrel at standard doses.

3.2.5 | Outcome

Twenty-six out of 71 dogs (37%) were alive at the end of the study (n = 24 dogs) or were lost to follow-up (2), and 45 of 71 dogs (63%) were dead. Of the 45 dogs that were dead at the end of the study, 15 died for reasons other than PLE, 3 died, and 27 were euthanized after treatment failure. Of these 30 dead dogs, 9 failed to respond to a combination of diet, antimicrobial, corticosteroid, and cyclosporine treatment; 6 to a combination of diet, antimicrobial, and corticosteroid treatment; 5 to diet and corticosteroid treatment; 6 to diet, corticosteroid, and cyclosporine treatment; 3 to diet, antimicrobial, corticosteroid, and azathioprine treatment; and 1 to diet, corticosteroid, and azathioprine treatment. The median follow-up time (from the time of PLE histologic diagnosis to death or end of the study, or to the last observation recorded before the animal was lost to follow-up) was 240 days (minimum value - maximum value: 0-2250 days; interquartile range: 1035 days). Median time to death or euthanasia after treatment failure was 41 days (0-647; 121).

3.3 | Survival analysis

Results from all the univariable analyses including information on the total number of dogs and events in each model can be found in Table 1. After univariable screening, variables retained for multivariable modeling were CCEAI, urea (mmol/L), breed, serum albumin concentrations (g/L), and appetite. The most parsimonious model using the AIC criterion was a multivariable model including CCEAI and urea (mmol/L) as explanatory variables. The assumption of PH was met for CCEAI (Z:ph test, $P = .65$), but it was not satisfied for urea (Z:ph test, $P = .006$). Thus, urea was modeled as a covariate with a time-varying effect, such that its regression coefficient varied as a linear function of time t ($f(t) = \log(t + 1)$) after adjusting for CCEAI. Results from the final multivariable Cox PH regression model are shown in Table 2. At any point in time after PLE histologic diagnosis caused by CE or lymphangiectasia, for each unit increase in CCEAI the dogs had experienced, the hazard of death increased by 22.9% (95% confidence interval [CI]: 6.9%-41.2%; Table 2), given that urea is held constant. The time-dependent coefficient for urea is estimated to be $ß(t) = 0.528-0.120 * \log(t + 1)$ (P values <.01) (Table 2) indicating that urea has a positive effect on death which decreases over time and drops off by approximately day 80, given that CCEAI is held constant.

To further explore the association between CCEAI and survival in dogs with PLE, we compared the Kaplan-Meier survival function of 2 groups of dogs formed based on a clinically relevant cutoff for CCEAI of 8, as a CCEAI of ≤8 indicates a CE ranging from insignificant to moderate, whereas a CCEAI of >8 denotes a CE ranging from severe to very severe. The group of dogs with a CCEAI of ≤8 had significantly longer survival time than the group with a CCEAI of >8 throughout the entire follow-up time period ($P = .002$; Figure 1).

TABLE 1 Parameter estimates from a series of 16 univariable Cox proportional hazards regression models assessing the association between a number of proposed markers of malnutrition and death after treatment failure in dogs with protein-losing enteropathy caused by chronic enteropathy or lymphangiectasia

							LRT[e]		
Variable	N[a]	n[b]	β	SE[c]	Hazard ratio (95% CI)[d]	*P* value	Value	DF[f]	*P* value
Urea	53	23	0.341	0.093	1.407 (1.173-1.687)	<.001	11.33	1	<.001
CCEAI[g]	53	23	0.220	0.068	1.246 (1.091-1.424)	.001	10.16	1	.001
Appetite1	53	23	0.936	0.547	2.549 (0.873-7.444)	.09	9.6	3	.02
Appetite3	53	23	−0.796	0.509	0.451 (0.166-1.223)	.12			
Appetite4	53	23	−1.435	1.045	0.238 (0.031-1.848)	.17			
Albumin (g/L)	53	23	−0.100	0.055	0.904 (0.812-1.007)	.07	3.87	1	.049
Purebred (Yes)	53	23	1.115	1.023	3.051 (0.411-22.65)	.28	1.7	1	.19
BCS[h]	53	23	−0.168	0.153	0.846 (0.626-1.142)	.28	1.23	1	.27
Creatinine	53	23	0.010	0.009	1.011 (0.99-1.029)	.26	1.22	1	.27
Age	53	23	0.060	0.073	1.062 (0.921-1.226)	.41	0.7	1	.40
Signs duration	53	23	−0.001	0.001	0.999 (0.997-1.001)	.48	0.56	1	.45
Sex (male)	53	23	0.180	0.427	1.198 (0.518-2.768)	.67	0.18	1	.67
Neutered (Yes)	53	23	−0.146	0.506	0.864 (0.321-2.329)	.77	0.08	1	.78
Percentage weight loss	53	23	−0.007	0.026	0.993 (0.943-1.046)	.79	0.07	1	.79
Body weight	53	23	−0.001	0.017	0.999 (0.967-1.032)	.95	0	1	.95
Cholesterol (mmol/L)	57	26	−0.351	0.187	0.704 (0.488-1.015)	.06	4.04	1	.04
Cobalamin (pmol/L)	61	26	−0.003	0.002	0.997 (0.993-1.001)	.12	3.28	1	.07
Folate (nmol/L)	61	24	−0.025	0.023	0.975 (0.932-1.02)	.27	1.37	1	.24

[a]Total number of dogs in the model.
[b]Number of dogs dead or euthanized because of protein-losing enteropathy caused by chronic enteropathy or lymphangiectasia.
[c]Standard error.
[d]95% Confidence interval.
[e]Likelihood ratio test.
[f]Degrees of freedom.
[g]Canine chronic enteropathy activity index.
[h]Body condition score.

Median survival time of the dogs with a CCEAI of ≤8 and with a CCEAI of >8 was undefined (95% CI: 647 - undefined; n = 32) and 109 days (95% CI: 6 - undefined; n = 21), respectively (Figure 1). After following up the dogs for 647 days, the RMST of the dogs with a CCEAI of ≤8 and with a CCEAI of >8 was 504.7 days (95% CI: 418.5-590.9; n = 32) and 248.6 days (95% CI: 126.6-370.5; n = 21) respectively. Dogs with a CCEAI of ≤8 survived 256.1 days longer (*P* = .001, 95% CI: 106.7–405.4) than those with a CCEAI of >8 on average, when followed up for 649 days.

The association between urea and survival was further assessed through comparing the Kaplan-Meier survival function of 2 groups of dogs formed based on a cutoff of 7 mmol/L, as this was the upper end of the laboratory reference range for urea. A value for urea of ≤7 mmol/L is considered normal based on our laboratory reference range, whereas a urea value of >7 mmol/L indicates an increased

TABLE 2 Parameter estimates from a final multivariable Cox proportional hazards regression model assessing the association between proposed markers of malnutrition and death after treatment failure in dogs with protein-losing enteropathy caused by chronic enteropathy or lymphangiectasia

Variable	β	SE[a]	Hazard ratio (95% CI)[b]	*P* value
CCEAI[c]	0.206	0.071	1.229 (1.069-1.412)	.004
Urea	0.528	0.126	1.695 (1.323-2.171)	<.001
Time-transform term (urea)	−0.120	0.046	0.887 (0.810-0.970)	.01

[a] Standard error.
[b] 95% Confidence interval.
[c] Canine chronic enteropathy activity index.

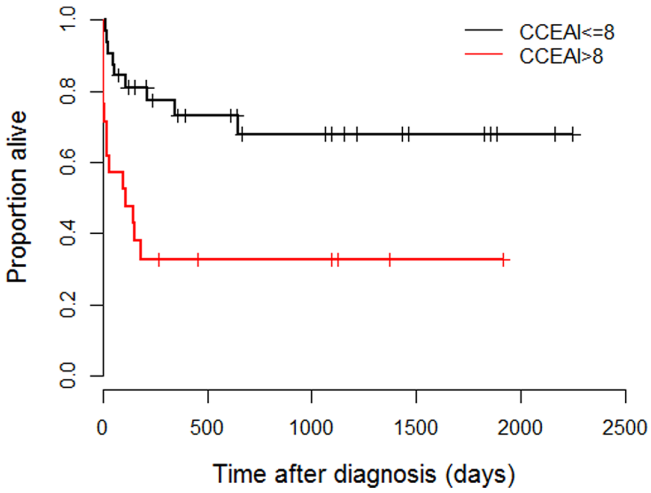

FIGURE 1 Kaplan-Meier estimates of survival based on death after treatment failure in 2 groups of dogs with protein-losing enteropathy caused by chronic enteropathy or lymphangiectasia divided based on the study population's median value for canine chronic enteropathy activity index (CCEAI) of ≤8 (black line, n = 34) or >8 (red line, n = 26). Marks in the lines indicate each censoring time

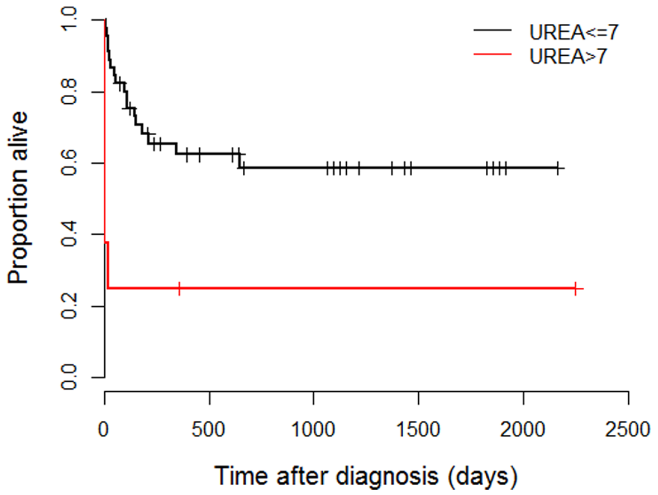

FIGURE 2 Kaplan-Meier estimates of survival based on death after treatment failure in 2 groups of dogs with protein-losing enteropathy caused by chronic enteropathy or lymphangiectasia divided based on the laboratory reference range cutoff for urea of ≤7 mmol/L (black line, n = 45) or >7 mmol/L (red line, n = 8). Marks in the lines indicate each censoring time

concentration. The group of dogs with urea ≤7 mmol/L had significantly longer survival time than the group with urea >7 mmol/L throughout the entire follow-up time period (P = .0006; Figure 2). Median survival time of the dogs with urea ≤7 and with urea >7 was undefined (95% CI: 215 - undefined; n = 45) and 0.5 days (95% CI: 0 - undefined; n = 8), respectively (Figure 2). After following up the dogs for 647 days, the RMST of the dogs with urea ≤7 and with urea >7 was 443.4 days (95% CI: 362.9-523.8; n = 45) and 164.0 days (95% CI: 29.38-357.3; n = 8) respectively. Dogs with urea ≤7 survived 279.4 days longer (P = .009, 95% CI: 70.0–488.7) than those with urea >7 on average, when followed up for 649 days.

4 | DISCUSSION

The role of malnutrition in the prognosis of dogs with PLE caused by CE or lymphangiectasia has not been extensively studied. Malnutrition is an important comorbidity in human IBD patients with a prevalence as high as 40%.[19] Malnutrition is linked to adverse outcomes[20,21] and is associated with in-hospital death and increased hospital stay in human IBD patients.[8] Therefore, our study aimed to assess whether markers associated with malnutrition in human IBD patients can be used as predictors for death after treatment failure in dogs with PLE caused by CE or lymphangiectasia. Our study showed that BCS, percentage weight loss, appetite and serum albumin, creatinine, cobalamin, and folate concentrations were not significantly associated with death in dogs with PLE caused by CE or lymphangiectasia after treatment failure.

Our study did show a significant association between the CCEAI and urea concentrations and death in dogs with PLE caused by CE or lymphangiectasia. The CCEAI is an index for clinical severity, which is associated with outcome in dogs with IBD and PLE.[2,15] The CCEAI was included as a marker of malnutrition in our study as severity of disease is 1 of the variables used for nutrition risk screening in humans as well as for the assessment of malnutrition in human IBD.[14,22] The CCEAI incorporates serum albumin concentrations, which is a known laboratory marker for malnutrition. However, serum albumin concentration changes caused by malnutrition are confounded by the PLE because of loss into the intestinal tract and the effect of inflammation. This makes serum albumin concentrations difficult to use as a definitive indicator of malnutrition in dogs with PLE. Similarly, loss of cholesterol into the GI tract and malabsorption of cobalamin and folate might also limit their usefulness in the assessment of malnutrition in dogs with PLE. In our study, an increase in serum albumin concentration was significantly associated with a reduction in the hazard of death in dogs with PLE in the univariable Cox PH regression model. However, this variable did not reach significance in the multivariable model. This may have been because of decreased power of the study and therefore a larger sample population might have resulted in a significant finding. If serum albumin concentrations are significantly associated with death, this is possibly an indicator of increased loss from the GI tract, and increased inflammation, or a combination of this, together with malnutrition. The concurrent measurement of fecal alpha 1 proteinase inhibitor and fecal calprotectin in these cases might help to determine the contribution to the hypoalbuminemia of enteric loss and inflammation, respectively, versus malnutrition. Such discrimination might then help to determine if active dietary interventions in those cases with malnutrition help to improve the response to treatment in dogs with PLE caused by CE or lymphangiectasia. An increase in cholesterol concentration was significantly associated with a reduction in the hazard of death in dogs with PLE in the univariable Cox PH regression model. However, in order to maximize the number of dogs included in the multivariable model, this variable was excluded. Therefore, the effect of serum cholesterol concentration on death in dogs with PLE cannot be definitively confirmed.

The results of our study corroborate previous studies, in which increased blood urea concentration is a negative prognostic indicator in dogs with PLE.[1,2] Possible causes of high urea include consumption of a high protein diet, GI hemorrhage, increased protein catabolism from starvation, prerenal causes because of severe diarrhea, kidney disease, and postrenal causes. Renal and postrenal causes were unlikely in our study; however, further prospective studies determining the hydration status and muscle condition score as well as diet history and presence of melena and hematochezia are needed to definitively ascertain the cause of high urea in dogs with PLE caused by CE or lymphangiectasia. Interestingly, dogs that were euthanized or died before discharge from the hospital (n = 4) had the highest urea concentrations (Figure 2). Therefore, our study suggests that increased urea concentrations might be predictive of in-hospital death in dogs with PLE caused by CE or lymphangiectasia rather than death after discharge from the hospital. However, because of the small number of in-hospital deaths in our study, additional statistical analysis to determine which factors are predictive of death in-hospital versus after discharge from the hospital could not be performed. Additional studies are needed to determine a cause or effect relationship of high urea (>7 mmol/L) and death as well as determining if specific intervention in those dogs with high urea (>7 mmol/L) might help to improve response to treatment and therefore prognosis.

In our study, body weight, percentage weight loss, and BCS were some of the markers used to assess for malnutrition. However, given

the variability in size of different dog breeds, body weight is not as informative of nutritional status as it is in humans. The reason for inclusion of this variable was to determine if larger breed dogs had a decreased response to immunomodulatory treatment compared to smaller breed dogs. Percentage weight loss might have underestimated the extent of malnutrition as nearly half of our cases had some degree of abdominal effusion, peripheral edema, or both. The use of BCS is informative of peripheral fat status in dogs; however, this score does not take into account intra-abdominal fat, muscle condition, or bone density, all of which can be reduced by malnutrition and are therefore likely important in its assessment.[23] Unfortunately, a major limitation of our study was the absence of muscle condition score in our cases and the effect of this on death after treatment failure. Although, serum creatinine was used in our study as an attempt to act as a surrogate for muscle condition, our study found that this was not a prognostic marker. However, 75% of the dogs in our study had serum creatinine concentrations below the reference range, which indicates that muscle atrophy is likely prevalent in this disease.

Other limitations of our study include the retrospective study design, which could have made the interpretation of the clinical history and assignment of the CCEAI score inconsistent because of multiple clinician involvement in the cases, especially because 3 of the variables used for assessment of the activity index (lethargy, appetite, and ascites and peripheral edema) involve a subjective scale.[15] Also, BCS is a subjective variable and therefore can differ between clinicians. Some variables, such as serum cholesterol concentrations or appetite, were not assessed or reported in all cases. This limited the number of dogs that were included in the statistical analysis, as only dogs that had complete information for all covariates were included (Table 1). Also, although all dogs in our study were normobilirubinemic and did not have significant liver or biliary tree abnormalities on abdominal imaging, pre- and postprandial bile acid concentrations to eliminate hepatic dysfunction definitively were not measured for all dogs. Similarly, renal function via the assessment of urinalysis and early biochemical markers for glomerular filtration rate was not assessed for all dogs. Therefore, the presence of possible concurrent hepatic and renal dysfunction, although considered unlikely, could not be definitively eliminated in all dogs. In our study, all but 1 of the deaths related to PLE occurred within the first year of diagnosis and also included 4 dogs that were euthanized or died before discharge from the hospital; therefore, assessing the effects of malnutrition during a specific time period might have allowed for tighter statistical comparisons. Furthermore, regarding dogs that were censored because they died of another cause, these were assumed to die of a competing cause independent of the event in question. Consequently, interpretation of the survival analysis in our study in the presence of competing risks is subject to at least some ambiguity because of uncertainty about the degree of dependence among the competing outcomes. In our study, the diagnosis of CE or lymphangiectasia for all cases was based on histopathology of endoscopic biopsies and not all cases had ileal biopsies performed; therefore, intestinal neoplasia might have been missed in some cases, which would have then impacted the prognosis.[1] Finally, the assessment of percentage weight loss in our cases was dependent on previously recorded values by the referring veterinarian and so any inaccuracies would have affected this measurement. Similarly, differences in weighing scales as well as the dog's water balance, especially for those cases of dehydration, hypovolemia, ascites, and peripheral edema could have impacted this measurement.

In conclusion, our study demonstrated that of the markers of malnutrition studied, only the CCEAI and blood urea concentrations were significantly associated with death in dogs with PLE caused by CE or lymphangiectasia. Body condition score, body weight, percentage weight loss, appetite, duration of signs and serum albumin, creatinine, cobalamin, and folate concentrations at the time of histologic diagnosis could not be used as predictors of death after treatment failure in dogs with PLE caused by CE or lymphangiectasia. Alternative measures of malnutrition such as muscle condition score and bone density might be required to definitively investigate the role of malnutrition in the prognosis of PLE in dogs.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The University of Bristol granted ethical approval for the study (VIN/17/026).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Nakashima K, Hiyoshi S, Ohno K, et al. Prognostic factors in dogs with protein-losing enteropathy. *Vet J*. 2015;205:28-32.
2. Gianella P, Lotti U, Bellino C, et al. Clinicopathologic and prognostic factors in short- and long-term surviving dogs with protein-losing enteropathy. *Schweiz Arch Tierheilkd*. 2017;159:163-169.
3. Simmerson SM, Armstrong PJ, Wunschmann A, et al. Clinical features, intestinal histopathology, and outcome in protein-losing enteropathy in Yorkshire Terrier dogs. *J Vet Intern Med*. 2014;28:331-337.
4. Allenspach K, Rizzo J, Jergens AE, Chang YM. Hypovitaminosis D is associated with negative outcome in dogs with protein losing enteropathy: a retrospective study of 43 cases. *BMC Vet Res*. 2017;13:96.
5. Equilino M, Theodoloz V, Gorgas D, et al. Evaluation of serum biochemical marker concentrations and survival time in dogs with protein-losing enteropathy. *J Am Vet Med Assoc*. 2015;246:91-99.
6. Laflamme D. Development and validation of a body condition score system for dogs. *Canine Practice*. 1997;22:10-15.
7. Mawby DI, Bartges JW, d'Avignon A, et al. Comparison of various methods for estimating body fat in dogs. *J Am Anim Hosp Assoc*. 2004;40:109-114.
8. Nguyen GC, Munsell M, Harris ML. Nationwide prevalence and prognostic significance of clinically diagnosable protein-calorie malnutrition

in hospitalized inflammatory bowel disease patients. *Inflamm Bowel Dis*. 2008;14:1105-1111.
9. Detsky AS, Smalley PS, Chang J. The rational clinical examination. Is this patient malnourished? *JAMA*. 1994;271:54-58.
10. O'Sullivan MA, O'Morain CA. Nutritional therapy in Crohn's disease. *Inflamm Bowel Dis*. 1998;4:45-53.
11. Cederholm T, Bosaeus I, Barazzoni R, et al. Diagnostic criteria for malnutrition - an ESPEN Consensus Statement. *Clin Nutr*. 2015;34:335-340.
12. Yakut M, Ustun Y, Kabacam G, et al. Serum vitamin B12 and folate status in patients with inflammatory bowel diseases. *Eur J Intern Med*. 2010;21:320-323.
13. Mijac DD, Jankovic GL, Jorga J, et al. Nutritional status in patients with active inflammatory bowel disease: prevalence of malnutrition and methods for routine nutritional assessment. *Eur J Intern Med*. 2010;21:315-319.
14. Valentini L, Schulzke JD. Mundane, yet challenging: the assessment of malnutrition in inflammatory bowel disease. *Eur J Intern Med*. 2011;22:13-15.
15. Allenspach K, Wieland B, Grone A, et al. Chronic enteropathies in dogs: evaluation of risk factors for negative outcome. *J Vet Intern Med*. 2007;21:700-708.
16. Hand MS, Thatcher CD, Remillard RL, Roundebush P, Novotny BJ. Small animal clinical nutrition: an iterative process. In: Hand MS, Thatcher CD, Remillard RL, Roundebush P, Novotny BJ, eds. *Small Animal Clinical Nutrition*. 5th ed. Topeka, KS: Mark Morris Institute; 2010, 2010:3-20.
17. Team RC. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. 2017; http://www.R-project.org/.
18. Therneau TM, Lumley T. Survival: survival analysis. R package version 2.41-3. 2017.
19. O'Sullivan M, O'Morain C. Nutritional therapy in inflammatory bowel disease. *Curr Treat Options Gastroenterol*. 2004;7:191-198.
20. Pirlich M, Schutz T, Kemps M, et al. Prevalence of malnutrition in hospitalized medical patients: impact of underlying disease. *Dig Dis*. 2003;21:245-251.
21. Gassull MA. Nutrition and inflammatory bowel disease: its relation to pathophysiology, outcome and therapy. *Dig Dis*. 2003;21:220-227.
22. Kondrup J, Allison SP, Elia M, Vellas B, Plauth M, Educational and Clinical Practice Committee, European Society of Parenteral and Enteral Nutrition (ESPEN). ESPEN guidelines for nutrition screening 2002. *Clin Nutr*. 2003;22:415-421.
23. Scaldaferri F, Pizzoferrato M, Lopetuso LR, et al. Nutrition and IBD: malnutrition and/or sarcopenia? a practical guide. *Gastroenterol Res Pract*. 2017;2017:8646495.

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