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Increasing age and severe intraoperative hypotension associated with nonsurvival in dogs with gallbladder mucocele undergoing cholecystectomy

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OBJECTIVE
To identify prognostic indicators and inflammatory markers associated with nonsurvival in dogs with gallbladder mucoceles (GBMs) following cholecystectomy and to evaluate C-reactive protein (CRP) and haptoglobin concentrations in dogs with GBMs compared to healthy controls.

ANIMALS
25 dogs that underwent cholecystectomy for removal of GBM and 20 healthy control dogs.

METHODS
A prospective, multicenter cohort study. Survival outcomes to hospital discharge and 2 weeks postdischarge were recorded from medical records. Laboratory variables, inflammatory markers (CRP and haptoglobin), and 25-hydroxyvitamin(OH) D (25[OH]D) concentrations were measured preoperatively. Associations between signalment, clinicopathologic variables, acute patient physiologic and laboratory evaluation ($APPLE_{FAST}$) scores, inflammatory markers, 25(OH)D concentration, and survival were analyzed using logistic regression.

RESULTS
76% (19/25) and 68% (17/25) of dogs survived to hospital discharge and 2 weeks postdischarge, respectively. For each additional year of age, the odds of nonsurvival in hospital and 2 weeks postdischarge increased by 2.2 (P = .01; 95% CI, 1.2 to 5.0) and 1.7 (P = .04; 95% CI, 1.0 to 3.2), respectively. Intraoperative systolic blood pressure ≤ 65 mm Hg increased the probability of nonsurvival in hospital (P < .04). Gallbladder perforation, $APPLE_{FAST}$ scores, and preoperative serum concentrations of CRP, haptoglobin, and 25(OH)D were not associated with survival. Serum CRP and haptoglobin concentrations were greater in dogs with GBM compared to controls (P < .001).

CLINICAL RELEVANCE
Increasing age and intraoperative systolic blood pressure ≤ 65 mm Hg were associated with nonsurvival in dogs with GBM undergoing cholecystectomy. Serum CRP, haptoglobin, and 25(OH)D were not associated with nonsurvival postcholecystectomy in this sample population.

Keywords: biliary disease, C-reactive protein, haptoglobin, vitamin D, APPLE fast

Gallbladder mucocele (GBM) is an increasingly recognized biliary disease in dogs[1–8] and is characterized by an accumulation of thick amorphous mucus in the gallbladder, possibly due to biliary stasis and hypersecretion.[9,10] Risk factors associated with GBM include hyperlipidemia,[11] endocrinopathies such as hyperadrenocorticism and hypothyroidism,[6,12,13] breeds such as the Shetland Sheepdog[6,14,15]

and Border Terrier,[15,16] and hyperleptinemia.[17] Dogs with GBM can present with a spectrum of illness ranging from subclinical to critically ill because of extrahepatic biliary obstruction, bile peritonitis, and systemic inflammatory response syndrome.[1–5,7,8,14,18] Gallbladder mucocele and perforation can be diagnosed with imaging methods such as abdominal ultrasonography or CT and addressed surgically with a cholecystectomy. However, the average rate of postoperative mortality is approximately 20% (range, 7% to 45%).[1–5,7,8,14,18,19] Given the high risk of mortality and costs of surgery, it is important to identify prognostic indicators and predictors of postsurgical survival outcomes. Identification of such predictors could help triage patients, counsel pet owners, and strategize treatment approach.

Dogs that survive 2 weeks postcholecystectomy have a good prognosis for long-term survival,[2,3,19] and therefore identifying prognostic indicators of short-term survival is valuable. Attempts in various studies to identify such predictors have yielded conflicting results for clinical variables such as gallbladder rupture,[1,3,5,7,18,19] age,[1,4,18,20] and presence of clinical signs[20]; laboratory variables such as serum creatinine, phosphorous, ALP activity, hyperbilirubinemia, and postoperative lactate[3,4,19,20]; and intraoperative nadir systolic blood pressure.[4,21] These contradicting results justify further investigation of prognostic indicators of nonsurvival postcholecystectomy in dogs with GBM.

Because the sequelae of GBM include complications such as cholecystitis, gallbladder perforation, bile peritonitis, pancreatitis, acute kidney injury, extrahepatic biliary duct obstruction, and systemic inflammatory response syndrome,[1–5,7,8,14,18,19] identification of serum markers of inflammation might improve patient management and prediction of postsurgical complications and survival. Examples of such markers include C-reactive protein (CRP), haptoglobin, and 25-hydroxyvitamin(OH)D (25[OH]D).

CRP and haptoglobin are nonspecific positive acute phase proteins that increase in response to inflammatory stimuli[22,23] and diseases such as acute pancreatitis,[24,25] parvovirus,[26] immune-mediated diseases,[27] and neoplasia in dogs.[28,29] CRP is a highly sensitive and moderately specific marker for gallbladder rupture in dogs with GBM undergoing cholecystectomy.[30] Furthermore, in humans, higher CRP is an independent predictor of more advanced acute cholecystitis[31] and surgical complexity.[32] Thus, CRP and haptoglobin have the potential to be valuable markers of disease and surgical outcomes in dogs with GBM.

Vitamin D is a steroid hormone that regulates calcium homeostasis but is also an indirect marker of inflammation.[33,34] Decreased serum 25(OH)D concentrations have been found in dogs with critical illness,[35] sepsis,[35] and acute pancreatitis,[36] and serum 25(OH)D concentrations have been correlated with illness severity (measured using acute patient physiologic and laboratory evaluation [$APPLE_{FAST}$] scores)[35] and nonsurvival.[36] Decreased 25(OH)D concentrations have also been found in dogs with GBM,[37] but the association between 25(OH)D and survival postcholecystectomy has not yet been examined.

The primary objective of this study was to examine the association of several variables ($APPLE_{FAST}$ scores and serum concentrations of CRP, haptoglobin, and 25[OH]D) with survival to discharge and 2 weeks post–hospital discharge in dogs with GBM undergoing cholecystectomy. A secondary objective was to determine whether dogs with GBM have higher serum CRP and haptoglobin concentrations compared to healthy control dogs. We hypothesized that variables such as gallbladder perforation, higher $APPLE_{FAST}$ scores, higher CRP and haptoglobin concentrations, and lower 25(OH)D concentrations would be associated with nonsurvival in hospital and 2 weeks post–hospital discharge and that serum CRP and haptoglobin concentrations would be higher in dogs with GBM compared to control dogs.

Methods

Criteria for case selection

A subset of dogs with GBM enrolled in a previous study[37] evaluating 25(OH)D concentrations were analyzed because these dogs had sufficient serum samples available to measure CRP and haptoglobin. Cases from 4 different academic teaching hospitals (Midwestern University College of Veterinary Medicine, University of Missouri Veterinary Health Center, Colorado State University James L. Voss Veterinary Teaching Hospital, and Utrecht University) were enrolled prospectively, primarily between July 2018 and November 2019. Four dogs were enrolled outside of the primary enrollment period. One dog was enrolled in 2021, 2 dogs in 2014, and 1 in 2017. This study was conducted in accordance with guidelines for clinical studies and approved by each institution's animal care and use committee (Midwestern University College of Veterinary Medicine, No. 2925; University of Missouri Veterinary Health Center, No. 7334; and Colorado State University James L. Voss Veterinary Teaching Hospital, No. 2019-203). Approval from the Utrecht University Animal Care and Use Committee was not required because only dogs with serum remaining from other diagnostic testing were included. Dogs were included if they had been diagnosed with a GBM via ultrasonography, gross or histopathological examination, or both and underwent cholecystectomy. Ultrasound images were obtained by a board-certified veterinary radiologist, veterinary radiology resident, board-certified small animal internist, or diagnostic sonographer. An ultrasonographic diagnosis of GBM was achieved with the identification of gravity-independent, immobile material.[4,5] Two board-certified veterinary radiologists reviewed all ultrasound images to reach a diagnosis and assign a consensus GBM type between I and VI based on previously established criteria.[38] Gross or histopathologic features consistent with GBM included a distended gallbladder with an abnormal accumulation of inspissated, amorphous

mucus in combination with cystic mucosal hyperplasia or hypertrophy.[10]

Gallbladder rupture was diagnosed intraoperatively by a board-certified surgeon when a perforation in the gallbladder wall, bile leakage, or both were identified.[5] Exclusion criteria included clinically relevant comorbidities that could affect prognosis such as diabetic ketoacidosis, neoplasia, congestive heart failure, or immune-mediated diseases.

The following data were collected for each dog with GBM: academic hospital of admission (institution), age, sex, breed, clinical signs prior to and on presentation, duration of clinical signs in days, $APPLE_{FAST}$ score on presentation, preoperative WBC count (/µL), ALP (IU/L), ALT (IU/L), GGT (IU/L), bilirubin (mg/dL), cholesterol (mg/dL), ultrasonographic GBM type (I to VI), presence of intraoperative hypotension (systolic blood pressure < 90 mm Hg), nadir intraoperative systolic blood pressure (mm Hg), gallbladder perforation (present/absent), surgery duration (minutes), gallbladder histopathology results, anaerobic and aerobic bile culture results (positive/negative), survival in hospital (yes/no), and survival 2 weeks post–hospital discharge (yes/no). Nonsurvival was defined as death or euthanasia. $APPLE_{FAST}$ score was calculated as previously described using the 5 variables of glucose (mg/dL), albumin (g/dL), mentation score, platelet count (number of platelets/µL), and lactate (mmol/L).[39] Possible scores ranged from 0 to a maximum score of 50. Higher scores were indicative of more severe disease. Intraoperative blood pressure measurements were obtained via Doppler or oscillometric techniques according to previously established guidelines.[40] Dogs were considered clinical if they had any signs of lethargy, hyporexia to anorexia, abdominal pain, vomiting, diarrhea, adipsia, jaundice, or fever within 7 days of presentation to the hospital. Laboratory variables such as WBC count or ALP were interpreted as a fold change with respect to the upper limit of the reference interval (xULN) because of different analyzers and reference intervals used at different institutions.

A second cohort of healthy control dogs used in a previous study[37] was used as a comparison group. These dogs were selected on the basis of a review of clinical history, physical examination, CBC, serum biochemical profile, and urinalysis by a board-certified small animal internist (JAJ) to confirm good health. Control dogs could not have any known illnesses, vaccination, or medications, except monthly parasiticides within 60 days of enrollment. For each control dog, ultrasonography by a board-certified internist confirmed the absence of GBM formation on the basis of anechoic contents or small volume of gravity-dependent sludge.

Blood samples were collected via venipuncture for dogs with GBM and healthy controls. Samples were centrifuged, and a minimum of 0.5 mL of serum was stored for each dog. Serum was stored in freezer-resistant conical microcentrifuge tubes at –80 °C for batch analysis of CRP, haptoglobin, and serum 25(OH)D concentrations. Samples were packed with dry ice and shipped overnight to VDI Laboratory LLC. Serum 25(OH)D quantification was performed on thawed samples using a competitive chemiluminescence immunoassay as previously described.[41] The minimum detectable concentration for serum 25(OH)D was 4.0 ng/mL. Serum CRP and haptoglobin measurements were performed as previously described using canine-specific sandwich ELISAs.[27] Precision data for each assay have been previously reported.[41,42] Laboratory reference intervals for the CRP assay in dogs were normal (≤ 3.9 mg/L), mildly increased (4 to 9.9 mg/L), moderately increased (10 to 39.9 mg/L), and markedly increased (≥ 40 mg/L).[27] The reference interval for the haptoglobin assay in dogs was 30 to 250 mg/dL.[27] The minimum detectable concentration for the CRP assay was 0.5 mg/L and for the haptoglobin assay was 10 mg/dL. Laboratory technicians performing the assay were blinded to the identity of each sample.

Statistical analysis

Statistical analysis was performed with commercially available software (Stata version 17; Stata Corp). Data were assessed for normality using tests of skewness and kurtosis. Normal data were described by the mean and SD, while nonnormal data were described by the median and IQR, expressed as the first and third quartiles. Concentrations of CRP, haptoglobin, and 25(OH)D below the lower limit of detection were imputed as the lower limit of detection for statistical purposes. The association of age, sex, presence of clinical signs, CRP, haptoglobin, 25(OH)D, $APPLE_{FAST}$ score, perforation (present/absent), nadir intraoperative systolic blood pressure, xULN total bilirubin, xULN ALP, and xULN WBC with survival data in hospital and 2 weeks following hospital discharge were determined via exact logistic regression. After the initial statistical analyses, lower-than-expected nadir readings for systolic blood pressure prompted a more in-depth examination via probability tables and conditional density plots generated by marginal analysis following logistic regression. Multivariable models were built using a backward stepwise approach informed by prior literature and biological plausibility incorporating variables with a P value ≤ .2 in univariable regression. Models were validated using the Pearson goodness-of-fit test to check the model's assumptions and ensure that it fit the data appropriately. Proportions were compared using a 2-sided test of proportions. The Shapiro-Wilk test was used to assess for normality, after which a nonparametric Wilcoxon rank sum test was used to compare age, CRP, and haptoglobin concentrations between dogs with GBM and healthy controls. A Pearson χ^2 test was used to compare sex distribution between GBM and healthy control groups. The level of significance was set at $P < .05$. The Strengthening the Reporting of Observational Studies in Epidemiology checklist was used to ensure all criteria were met when reporting results. Formal power calculations were not

performed to estimate an ideal sample size because this was an exploratory study, but sample size was determined by enrolling cases prospectively that met the inclusion criteria during the designated study period.

Results

Demographics of the study cohort of dogs with GBM

Twenty-six GBM dogs were included, of which 1 dog was excluded because sufficient serum sample was not available within 24 hours of cholecystectomy to measure CRP, haptoglobin, and 25(OH)D concentrations. Ten cases were enrolled from Utrecht University, 7 from University of Missouri, 5 from Midwestern University, and 3 from Colorado State University. Median (IQR) age was 10.3 years (11.3 to 12.9 years). There were 13 castrated males, 10 spayed females, and 1 each of intact female and intact male dogs. Breed distribution included Chihuahua (n = 5), mixed breed (4), Beagle (3), Maltese (2), Shetland Sheepdog (2), Labrador Retriever (1), Cavalier King Charles Spaniel (1), Pomeranian (1), West Highland White Terrier (1), Chow Chow (1), Jack Russell Terrier (1), Soft Coated Wheaten Terrier (1), Miniature Poodle (1), and Dachshund (1).

Demographics of control cohort dogs

Twenty healthy control dogs were included. Median (IQR) age was 5.3 years (1.4 to 9.0 years), and 7 intact males, 9 spayed females, 3 intact females, and 1 intact male comprised the group. Breed information regarding control dogs can be found in a previously published study.[37] Age was significantly higher in GBM dogs compared to healthy controls ($P < .001$), but sex distribution was not significantly different ($P = .44$; $\chi^2 = 0.59$) between the 2 groups.

Clinical presentation, diagnostics, and survival outcomes of the study cohort of dogs with GBM

Three of the 25 (12%) dogs were subclinical on presentation and evaluated for increased liver enzyme activity (n = 2) or for a recheck ultrasound to monitor the appearance of a previously identified GBM (1). Of the cases that presented with clinical signs, hyporexia to anorexia or lethargy were the most common clinical signs found in 77% (17/22) of cases followed by vomiting in 64% (14/22). Other clinical signs commonly noted were icterus in 32% (7/22), abdominal pain in 23% (5/22), and polyuria and polydipsia in 18% (4/22). Median (IQR) number of days the dog was clinical prior to presentation was 5 (3 to 14).

Median and IQR for the following laboratory variables were WBC, 12,900/µL (10,580/µL to 22,610/µL); ALP, 2,198 IU/L (688 to 4,050 IU/L); ALT, 1,019 IU/L (328 to 1,506 IU/L); GGT, 67 IU/L (19 to 90 IU/L); bilirubin, 0.7 mg/dL (0.2 to 6.5 mg/dL); and cholesterol, 412 mg/dL (188 to 562 mg/dL). Of 20 dogs with available $APPLE_{FAST}$ scores, median (IQR) was 21 (19 to 23). There were 24 dogs with available ultrasonographic GBM type, of which there were type 1 (7/24 [29%]), type 2 (5/24 [21%]), type 3 (4/24 [17%]), and type 4 (8/24 [33%]). In 1 case, the ultrasound was performed at the referring veterinary practice and the ultrasonographic type could not be ascertained, but a GBM was later confirmed on gross and histopathological examination postoperatively.

Three dogs were reported to have hyperadrenocorticism. One dog was suspected on the basis of clinical signs and an enlarged left adrenal gland on ultrasound and the other 2 dogs were diagnosed with low-dose dexamethasone suppression testing. One dog was receiving budesonide for treatment of anorexia due to suspected, but not confirmed, inflammatory bowel disease. One dog was reported to have diabetes mellitus.

The median (IQR) duration of surgery was 110 minutes (70 to 150). During cholecystectomy, 24 of 25 underwent open laparotomy and 1 of 25 underwent laparoscopy. One dog also underwent splenectomy for a splenic mass later confirmed to be a benign hematoma. A different dog underwent a partial liver lobectomy because of suspicion for a hepatic mass, but histopathology revealed multifocal regions of hepatic necrosis and infarction with periportal neutrophilic infiltrates with no evidence of malignancy. Nadir intraoperative systolic blood pressure was available for 23 of 25 cases with a median (IQR) of 70 mm Hg (60 to 85 mm Hg). Four of 25 (16%) dogs had gallbladder perforation. Aerobic and anaerobic culture of bile (n = 9), swab of gallbladder content (9), swab of gallbladder wall (2), gallbladder tissue (1), or a combination of bile or swab of gallbladder content with tissue (3) was performed in 24 of 25 cases, and results were positive in 5 of 24 (21%) cases. Bacterial species cultured included *Micrococcus luteus* and *Staphylococcus* spp coagulase negative (n = 1), *Roseomonas mucosa* (1), *Lactococcus* sp (1), *Pseudomonas aeruginosa* (1), and *Escherichia coli* (1). Of 25 dogs, 24 had gallbladder tissue submitted for histopathological examination, of which all confirmed GBM and cystic mucinous hyperplasia. Accompanying findings included cholecystitis in 12 dogs (lymphoplasmacytic in 4, neutrophilic in 4, mixed cell in 3, and cell type not specified in the report in 1). Two of 12 (17%) cases had mucosal necrosis associated with the inflammatory changes.

Survival to hospital discharge and 2 weeks following discharge in dogs with GBM

Eight of 25 (32%) dogs died or were euthanized (in hospital, n = 6; 2 weeks postdischarge, 2). Causes of death prior to discharge were due to cardiac arrest (n = 2) or unknown cause (1), and causes of euthanasia were concern for sepsis (2) and disseminated intravascular coagulation (1). Two cases were euthanized following discharge due to worsening jaundice and clinical signs (n = 1) and a bile peritonitis (1).

Serum CRP and haptoglobin concentrations between dogs with GBM and controls

Of the 25 dogs with GBM, median (IQR) concentration for CRP was 34.2 mg/L (10.5 to 62.2 mg/L) and

for haptoglobin was 598 mg/dL (425 to 654 mg/dL). Of 20 healthy control dogs, median (IQR) concentration for CRP was 2.0 mg/L (1.3 to 5.4 mg/L) and for haptoglobin was 156 mg/dL (62 to 369 mg/L). Serum CRP concentrations in dogs with GBM were normal (5/25 [20%]), mildly increased (1/25 [4%]), moderately increased (8/25 [32%]), or markedly increased (11/25 [44%]). Serum CRP concentrations in healthy control dogs were normal (17/20 [85%]), mildly increased (2/20 [10%]), and markedly increased (1/20 [5%]). The control dog with markedly increased serum CRP was diagnosed with coccidiomycosis 4 months after CRP measurement. Serum haptoglobin concentrations in dogs with GBM were normal (3/25 [12%]), decreased (1/25 [4%]), or increased (21/25 [84%]) above the reference interval and in healthy control dogs were below the reference interval (3/20 [15%]), normal (8/20 [40%]), or increased above the reference interval (9/20 [45%]). Haptoglobin concentrations were increased above the reference interval in dogs with GBM that were administered exogenous corticosteroids (n = 1) or diagnosed with diabetes mellitus (1) or hyperadrenocorticism (3). In 7 of 25 (28%) dogs with GBM, concentrations of haptoglobin or CRP were within the normal reference interval, of which 5 either had subclinical (n = 3) or very minimal clinical signs of lethargy (2).

Dogs with GBM had significantly higher serum CRP ($P < .001$) and haptoglobin ($P < .001$) than controls **(Figures 1 and 2)**.

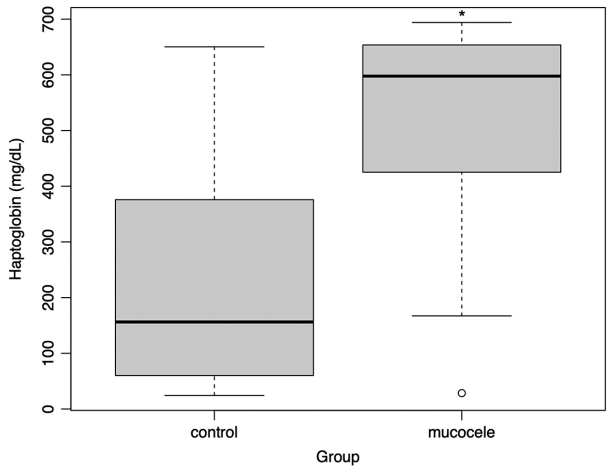

Figure 2—Comparison of serum haptoglobin concentrations (mg/dL) in dogs with gallbladder mucocele (n = 25) prior to cholecystectomy and healthy control dogs (20). Serum haptoglobin concentrations were higher in dogs with gallbladder mucoceles before cholecystectomy compared to healthy control dogs ($P < .001$). The bottom and top of the boxes represent the 25th and 75th quartiles, respectively, with the black horizontal line representing the median. The whiskers extend up to 1.5 X the IQR below and above the 25th and 75th quartiles, respectively. Open circles outside of the whiskers represent outlier values. Significant difference ($P < .05$) between groups is marked by the asterisk.

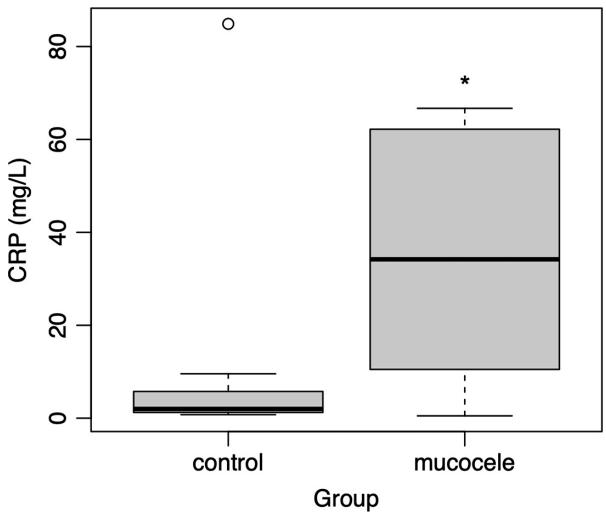

Figure 1—Comparison of serum C-reactive protein (CRP) concentrations (mg/L) in dogs with gallbladder mucocele (n = 25) prior to cholecystectomy and healthy control dogs (n = 20). Serum CRP concentrations were higher in gallbladder mucocele dogs before cholecystectomy compared to healthy control dogs ($P < .001$). The bottom and top of the boxes represent the 25th and 75th quartiles, respectively, with the black horizontal line representing the median. The whiskers extend up to 1.5 X the IQR below and above the 25th and 75th quartiles, respectively. Open circles outside of the whiskers represent outlier values. Significant difference ($P < .05$) between groups is marked by the asterisk.

Risk factors for nonsurvival in dogs with GBM undergoing cholecystectomy

Increasing age was associated with nonsurvival in hospital and within 2 weeks of discharge. The odds of nonsurvival in hospital and within 2 weeks of hospital discharge increased for each additional year by 2.2 ($P = .01$; 95% CI, 1.2 to 5.0) and 1.7 ($P = .04$; 95% CI, 1.0 to 3.2), respectively. A margins plot showed a sigmoid pattern, with the probability of nonsurvival before discharge and 2 weeks post-discharge low and insignificant **(Table 1)** for ages 8 to 11 and 8 to 10, respectively, then rising rapidly before leveling off at ages 15 to 16 years **(Supplementary Figure S1)**. No multivariable model was an improvement on the univariable model for age. None of the other examined clinical variables (clinical on presentation, gallbladder perforation, and $APPLE_{FAST}$ score), clinicopathologic variables (WBC count, ALP activity, or bilirubin,) nor inflammatory markers (CRP, haptoglobin, or 25[OH]D concentrations) were significantly associated with survival in hospital or 2 weeks post–hospital discharge **(Tables 2 and 3)**. While nadir intraoperative systolic blood pressure was also not associated with survival, examination of the margins showed that dogs in the lowest end of the range, with systolic blood pressure of ≤ 65 mm Hg, had a significantly increased probability of nonsurvival in hospital **(Table 4; Supplementary Figure S2)**.

Table 1—Predicted probability of nonsurvival in hospital and within 2 weeks postdischarge based on age of dogs with gallbladder mucocele undergoing cholecystectomy.

	Nonsurvival before discharge			Nonsurvival within 2 weeks postdischarge		
Age (y)	**Probability of nonsurvival (%)**	***P* value**	**95% CI**	**Probability of nonsurvival (%)**	***P* value**	**95% CI**
8	1%	.57	0%–4%	5%	.41	0%–17%
9	2%	.48	0%–9%	9%	.28	0%–24%
10	5%	.34	0%–15%	14%	.13	0%–32%
11	11%	.16	0%–26%	23%	**.02**	3%–42%
12	22%	**.03**	2%–42%	34%	**< .001**	13%–55%
13	39%	**.01**	12%–67%	48%	**< .001**	21%–75%
14	60%	**< .001**	22%–97%	62%	**< .001**	27%–96%
15	77%	**< .001**	40%–100%	74%	**< .001**	37%–100%
16	89%	**< .001**	61%–100%	83%	**< .001**	49%–100%

P values (*P* < .05 are bolded) and 95% CIs are provided.

Table 2—Descriptive statistics and univariable exact logistic regression results examining the association between variables and nonsurvival at discharge in 25 dogs with gallbladder mucocele following cholecystectomy.

	n	**No. of dogs that died or were euthanized or median (IQR)**	**No. of dogs that survived (n) or median (IQR)**	**OR (95% CI)**	***P* value**
Outcome	25	6	19		
Clinical on presentation	25	5	17	0.6 (0.0–41.6)	1.0
Gallbladder perforation present	25	2	2	3.7 (0.2–71.3)	.47
Age (y)	25	14 (14–14)	11 (10–12)	2.2 (1.2–5.0)	**.01**
Nadir intraoperative systolic blood pressure (mm Hg)	23	57 (45–70)	73 (60–85)	1.0 (0.9–1.0)	.17
Intraoperative hypotension (< 90 mm Hg)	23	4	14	1.1 (0.1–70.2)	1.00
xULN tbili	17	1 (1–26)	1 (1–11)	1.0 (0.9–1.1)	.57
xULN ALP	24	26 (14–57)	19 (4–30)	1.0 (1.0–1.0)	.57
xULN WBC	25	1.2 (0.8–1.8)	0.8 (0.6–1.4)	1.0 (1.0–1.0)	.46
$APPLE_{FAST}$ score	19	22 (19–24)	21 (18–23)	1.1 (0.7–1.7)	.75
CRP (mg/L)	25	42 (14–64)	34 (6–62)	1.0 (1.0–1.0)	.68
Haptoglobin (mg/dL)	25	560 (285–675)	598 (425–654)	1.0 (1.0–1.0)	.53
25(OH)D (ng/mL)	25	49 (16–101)	54 (36–65)	1.0 (1.0–1.0)	.86

CRP = C-reactive protein. tbili = Total bilirubin. xULN = Factor times upper limit of normal of reference interval. 25(OH)D = 25 hydroxy vitamin D. *P* values < .05 are bolded.

Table 3—Descriptive statistics and univariable exact logistic regression results examining the association between variables and nonsurvival 2 weeks postdischarge in 25 dogs with gallbladder mucocele following cholecystectomy.

	n	**No. of dogs that died or were euthanized (n) or median (IQR)**	**No. of dogs that survived (n) or median (IQR)**	**OR (95% CI)**	***P* value**
Outcome	25	8	17		
Clinical on presentation	25	6	16	4.9 (022–333.8)	.46
Gallbladder perforation present	25	2	2	2.4 (0.1–40.6)	.76
Age (y)	25	14 (11–14)	11 (10–12)	1.7 (1.0–3.2)	**.04**
Nadir intraoperative systolic blood pressure (mm Hg)	23	73 (60–83)	70 (45–90)	1.0 (0.9–1.0)	.43
Intraoperative hypotension (systolic < 90 mm Hg)	23	3	1	0.6 (0.0–9.1)	.98
xULN tbili	17	2 (1–26)	1 (1–6)	1.0 (1.0–1.1)	.56
xULN ALP	24	21 (15–46)	17 (4–35)	1.0 (1.0–1.1)	.87
xULN WBC	25	0.9 (0.6–1.7)	0.9 (0.6–1.4)	1.0 (1.0–1.0)	.60
$APPLE_{FAST}$ score	19	22 (20–23)	21 (18–22)	1.1 (0.8–1.7)	.58
CRP (mg/L)	25	28 (10–63)	38 (11–62)	1.0 (1.0–1.0)	.93
Haptoglobin (mg/dL)	25	560 (376–668)	598 (425–653)	1.0 (1.0–1.0)	.73
25(OH)D (ng/mL)	25	32 (14–84)	60 (44–65)	1.0 (1.0–1.0)	.39

See Table 2 for key.

Table 4—Predicted probability of nonsurvival in hospital based on nadir intraoperative systolic blood pressure in dogs with gallbladder mucoceles undergoing cholecystectomy.

Nadir intraoperative systolic blood pressure (mm Hg)	**Probability of nonsurvival (%)**	***P* value**	**95% CI**
45	47%	**.04**	3%–91%
55	35%	**.02**	6%–64%
65	24%	**.01**	5%–44%
75	16%	.07	0%–34%
85	11%	.23	0%–28%
95	7%	.39	0%–22%
105	4%	.52	0%–17%

P values < .05 are bolded and 95% CIs are provided.

Discussion

This study aimed to evaluate several factors to identify potential prognostic indicators of short-term survival in dogs with GBM postcholecystectomy. Results found that increasing age was significantly associated with nonsurvival, both postoperatively in hospital and within 2 weeks of hospital discharge. Additionally, nadir intraoperative systolic blood pressure ≤ 65 mm Hg carried an increased probability of nonsurvival in hospital. Preoperative $APPLE_{FAST}$ scores and serum concentrations of CRP, haptoglobin, and 25(OH)D

were not associated with survival. However, CRP and haptoglobin concentrations were significantly higher compared to control dogs, suggesting these concentrations could be used as biomarkers of disease.

Similar to other published studies,[4,18,20] the present study found a significant association between age and short-term survival. Odds of nonsurvival increased 2-fold with every additional year of age, implying that older dogs with GBM have a higher risk for worse prognoses postcholecystectomy. A potential explanation for this recurring finding could be age-associated declines in health, physical function, or accumulated comorbidities[43] that result in greater surgical complications and nonsurvival. Alternatively, pet owners might be more prone to elect euthanasia in such cases and thus older age should not necessarily be used as a stand-alone negative prognostic indicator. In contrast to other studies, the present study did not identify gallbladder perforation,[5,18] nadir intraoperative systolic blood pressure, or laboratory variables such as serum ALP[19] or bilirubin[18] as poor prognostic indicators. However, these predictors have not consistently been identified as risk factors in other studies.[1,3,4,7,19,21] Additionally, investigation of the margins of the systolic blood pressure data did find a higher probability of nonsurvival in hospital for dogs with a nadir intraoperative systolic blood pressure ≤ 65 mm Hg. Thus, severe intraoperative hypotension might be a poor prognostic indicator, which corroborates the findings of a previous study[4] evaluating survival in a cohort of dogs with GBM undergoing cholecystectomy in Asia.

Acute patient physiologic and laboratory evaluation scores did not correlate with survival in this study, contrary to other studies performed in dogs with critical illness.[39,44] This could be because the 5 variables used to calculate the $APPLE_{FAST}$ score do not account for surgical and anesthetic factors that could impact prognosis for GBM dogs undergoing cholecystectomy. Examples of such factors include whether the surgery was elective or emergent, whether the surgical approach was laparoscopic or open, and whether intraoperative hemorrhage or hypotension occurred. Furthermore, the median $APPLE_{FAST}$ score of the dogs with GBM in this study was 21 and no dog had a score higher than 25, which is the numerical cutoff that provides the highest specificity to predict nonsurvival.[39] Thus, this cohort of dogs might have lacked the number of critically ill dogs needed to appropriately assess $APPLE_{FAST}$ scores as a negative prognostic indicator. Small sample sizes could have compromised finding statistically significant associations between survival and serum CRP, haptoglobin, and 25(OH)D concentrations or any of the other variables examined. Nevertheless, a previous study[37] showed that serum 25(OH)D concentrations are lower in dogs with GBM and the present study found significantly higher serum CRP and haptoglobin concentrations in dogs with GBM compared to controls, suggesting that these markers of systemic inflammation could be useful biomarkers of GBM disease. In human patients, increased CRP or reduced 25(OH)D correlates with more advanced cholecystitis,[31] gallbladder perforation,[44] gallbladder stasis,[45] and a more complicated, painful cholecystectomy surgery.[31,32,46] Thus, these markers might be applicable to dogs with GBM in predicting gallbladder rupture[30] or serially monitoring disease progression or surgical recovery in individual patients.[24,47]

The statistical differences in serum CRP and haptoglobin concentrations between the 2 groups could be explained by age because the healthy control group in the present study was significantly younger than the GBM group. However, there are no data in dogs to suggest that concentrations of haptoglobin or CRP change with age.[23,48,49] Although there was a statistically significant difference in CRP and haptoglobin concentrations between GBM and healthy controls, there was overlap between the 2 groups. One healthy control dog had a markedly increased CRP concentration. This dog was diagnosed with coccidiomycosis 4 months after CRP measurement and therefore could have been subclinically infected at the time of enrollment. Nine healthy control dogs had haptoglobin concentrations above the reference interval, possibly because haptoglobin lacks specificity for GBM disease in dogs or because the upper limit of the reference interval is not the optimal cutoff to differentiate GBM from healthy control dogs. In 7 GBM dogs, concentrations of haptoglobin or CRP were within the normal reference interval. A possible explanation for this finding is that 5 of the 7 dogs had subclinical (n = 3) to very minimal clinical signs (2), and systemic inflammation was insufficient in these dogs to raise haptoglobin or CRP concentrations above the reference interval. These findings do highlight potential limitations in the sensitivity and specificity of these inflammatory markers, as has been previously observed.[27] Factors such as exogenous corticosteroid administration or endocrine diseases such as hyperadrenocorticism or diabetes mellitus might also have increased haptoglobin concentrations.[50]

Additional limitations to consider in interpretation of this study's findings are that although this was a prospective study, data such as bilirubin measurement and the information needed to calculate $APPLE_{FAST}$ scores were not available for every dog. Additionally, the surgical and treatment approach was not standardized across dogs, which could have impacted patient outcome. Furthermore, dogs from 4 different institutions were included in the study, which could have introduced some variability.

Despite these limitations, this study found age and severe intraoperative hypotension to be pertinent prognostic indicators of survival postcholecystectomy in dogs with GBM, which is valuable information considering the expenses, potential risks, and survival statistics of cholecystectomy surgery. Additionally, this was the first study to demonstrate that CRP and haptoglobin concentrations are significantly higher in dogs with GBM compared to healthy control dogs. Thus, although no significant associations were found between these inflammatory markers and short-term survival in this study, it is possible that larger sample sizes and serial measurements will elucidate the utility of these markers to help ascertain prognosis and monitor dogs with GBM.

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Disclosures

VDI Laboratory LLC (Randy Ringold) offers testing for C-reactive protein and haptoglobin in companion animals.

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Supplementary Materials

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