Biliary disease is relatively uncommon in dogs and includes cholecystitis, cholelithiasis, biliary tract trauma, and gallbladder mucocele (GBM), a relatively recently described disease.\textsuperscript{1–5} GBM is characterized by accumulation of thick, mucinous bile within the gallbladder.\textsuperscript{1,2,6,7} Gallbladder dysmotility, altered bile composition, and bile stasis have been found to be associated with GBM.\textsuperscript{8–10} Excessive secretion and defective unpackaging of mucin within the gallbladder epithelium is present in dogs with GBM, but the cause of these mucin defects is unknown.\textsuperscript{11} Additionally, many affected dogs have concurrent endocrinopathies such as hypoadrenocorticism or hypothyroidism, which may alter metabolic pathways associated with bile or mucus production.\textsuperscript{12,14} Shetland Sheepdogs, Cocker Spaniels, and Miniature Schnauzers appear to be predisposed to develop GBMs, which supports genetic dysfunction in bile metabolism as a possible cause.\textsuperscript{1–3,14,15}

Dogs with GBM may not have any clinical signs, however, in up to 70% of cases, the unchecked production of thickened bile sludge distends the biliary tree and gallbladder, leading to clinical signs associated with extrahepatic biliary obstruction.\textsuperscript{3,5,14,15} Focal necrosis develops in the gallbladder wall as GBM progresses as a result of pressure necrosis secondary to gallbladder overdistension, necrotizing cholecystitis, or the cytotoxic effects of static bile salts.\textsuperscript{2,5,6,7} Necrotic foci can develop into transmural defects, leading to gallbladder rupture (GBR) and bile peritonitis.\textsuperscript{2,6,13,14}

Most veterinary surgeons agree that exploratory surgery and cholecystectomy should be performed on an emergency basis in patients with GBM that...
have a rapidly declining physical status, icterus, or bile ascites.3,5,7,14 There is a relatively high (7% to 50%) mortality rate associated with cholecystectomy for treatment of GBM in dogs with concurrent bile peritonitis, cholestasis-associated systemic toxicity, or gallbladder necrosis–related systemic inflammatory response syndrome,4,15 and GBR prior to cholecystectomy can worsen the surgical prognosis. A recent study15 showed that dogs with GBR at the time of cholecystectomy had a mortality rate 2.7 times that for dogs with an intact gallbladder undergoing cholecystectomy.

Mucinous bile appears as gravity-independent, immobile material with a striated or stellate pattern when visualized by ultrasonographic imaging.2,6,15–17 Ultrasonographic imaging of the abdomen, besides providing a noninvasive diagnosis of GBM, is valuable for assessing the preoperative status of affected dogs. Ultrasonographic imaging may provide evidence of gallbladder wall necrosis, bile duct obstruction, or GBR prior to the onset of severe clinical signs. In the case of GBR, it is important to obtain an accurate diagnosis as early as possible, with the goal of performing emergency cholecystectomy prior to the onset of severe systemic compromise associated with prolonged biliary obstruction or diffuse bile peritonitis.4,15 However, GBR can be difficult to diagnose accurately with abdominal ultrasonography, and the reported diagnostic sensitivity of preoperative abdominal ultrasonography to identify GBR is 56% to 86%.15–19 Contrast-enhanced ultrasonography has been shown to have enhanced diagnostic accuracy for GBR,20 but this technique is not commonly available. CT cholangiography and CT angiography have been used to delineate the biliary tree and diagnose GBM but have not been reported for use in detecting GBR.21,22 We sought to determine whether an additional test could enhance or replace the use of abdominal ultrasonography for preoperative diagnosis of GBR. To this end, we evaluated serum concentration of C-reactive protein (CRP), a biomarker of cellular inflammation, as a preoperative indicator of GBR in dogs with GBM.

CRP is an acute phase protein released into the bloodstream during systemic inflammatory conditions23–28 and is synthesized primarily by hepatocytes stimulated by proinflammatory cytokines such as tumor necrosis factor, interleukin-1, and interleukin-6. CRP is also synthesized by adipocytes and leukocytes under inflammatory stimulation.28,29 The serum concentration of CRP can increase 1,000-fold within 24 to 72 hours after severe tissue damage but decreases rapidly when the inflammatory stimulus resolves.23,26,29 However, the CRP response is nonspecific, and CRP concentration is elevated during numerous inflammatory conditions, including cardiovascular disease, neoplasia, trauma, immune-mediated disease, and microbial infection.23,26–29 CRP promotes opsonization of pathogens and clearance of necrotic and apoptotic cells.30 Additionally, CRP promotes generalized leukocyte chemotaxis to sites of inflammation. CRP binds to surface receptors on leukocytes, activating phagocytosis and release of inflammatory mediators.29,31

Because the serum concentration of CRP changes rapidly with the onset and resolution of inflammatory conditions, CRP concentration has found utility as a prognostic indicator for many disease conditions in humans, including atherosclerosis, myocarditis, numerous cancers, meningitis, appendicitis, pancreatitis, peritonitis, and numerous hepatobiliary abnormalities.29,32–38 The Japanese Society of Hepato-Biliary-Pancreatic Surgery added CRP concentration to the standard diagnostic screening criteria for human patients suspected to have acute cholecystitis.39–42 Similarly in dogs, CRP concentration has been used as a biomarker and prognostic indicator for multiple inflammatory diseases, including pyometra, autoimmune hemolytic anemia, bronchopneumonia, systemic inflammatory response syndrome, surgical trauma and infection, cardiac disease, steroid-responsive meningitis-arthritis, idiopathic immune-mediated polyarthritis, necroinflammatory liver disease, hepatoencephalopathy, response to cancer treatment, and pancreatitis.43–65

The objective of the retrospective clinical study reported here was to determine whether serum CRP concentration could be used to detect GBR prior to surgery in dogs undergoing cholecystectomy for treatment of GBM. We hypothesized that preoperative CRP concentration would be more accurate than preoperative abdominal ultrasonography and other common clinicopathologic tests for detection of GBR in dogs with GBM.

Materials and Methods

Animals

Electronic medical records of the Veterinary Specialists Emergency Center were searched to identify all dogs that underwent cholecystectomy between April 2017 and March 2020. Dogs were eligible for inclusion in the study if the medical record was complete and contained a detailed, preoperative abdominal ultrasonography report; complete surgical report; a histologic report confirming the diagnosis of GBM; and a serum CRP concentration measured within 24 hours prior to surgery. All dogs included in the study were reexamined at our hospital within 2 weeks after the initial surgery.

Data collection

Information retrieved from medical records of dogs included in the study consisted of signalment, physical examination findings, medical history, results of clinicopathologic testing (eg, CBC and results of serum biochemical testing, including measurement of serum CRP concentration), results of preoperative abdominal ultrasonography, American Society of Anesthesiologists physical status score, durations of anesthesia and surgery, duration of in-
traoperative hypotension, surgical findings, bacterial culture results, and results of histologic evaluation of biopsied tissues.

All serum CRP concentrations were measured within 24 hours prior to surgery; all CBCs and other serum biochemical testing were performed within 48 hours prior to surgery. During the study period, serum CRP concentration was routinely measured in all dogs undergoing nonelective surgery in our hospital. Serum CRP concentration was measured with a spectrophotometric canine-specific CRP assay according to the manufacturer’s instructions (canine-specific vc-CRP-P assay; Fujifilm Corp). The assay has been independently validated for use on canine serum.64

All dogs received antimicrobials IV prior to surgery, including 1 of the following: ampicillin (22 mg/kg), cefazolin (22 mg/kg), ceftriaxone (25 mg/kg), sulbactam-ampicillin (20 mg/kg), or enrofloxacin (10 mg/kg).

Procedures

The initial diagnosis of GBM was based on ultrasonographic visualization of gravity-independent, granular, echogenic material in a stellate or striated pattern within the gallbladder. GBR was suspected when a defect in the gallbladder wall was seen along with adjacent hypechoic peritoneal fat or peritoneal effusion.20 All dogs were evaluated prior to surgery and assigned an American Society of Anesthesiologists physical status score.65 The anesthetic protocol was at the discretion of the attending anesthetist.

A complete exploratory celiotomy was performed on each dog, and the gallbladder was examined carefully for evidence of bile leakage. Any full-thickness defect in the gallbladder wall or any bile leakage from the gallbladder was considered evidence of GBR. The liver and surrounding tissues were examined for signs of inflammation, and the biliary tract was inspected for inspissated bile and choleliths. Cholecystectomy was performed in all dogs. Common bile duct patency was determined by retrograde passage of a catheter through the major duodenal papilla or by normograde passage of a catheter through the cholecystectomy incision. After cholecystectomy, wedge biopsy of the liver and placement of a nasogastric tube were performed in all dogs. Gallbladder and liver biopsy samples were submitted for histopathologic examination and were reviewed by a single board-certified veterinary pathologist. Bile, abdominal fluid, choleliths, and urine samples were submitted for cytologic examination. Bacterial culture and antimicrobial susceptibility testing of abdominal fluid samples was done at the attending surgeon’s discretion.

Dogs that were alive at a 2-week postoperative recheck examination were considered to be survivors. Dogs that died during the perioperative period or at any time during the 2-week period following surgery were classified as nonsurvivors.

Statistical analysis

Results of preoperative clinicopathologic testing (CBC, serum biochemical panel, and serum CRP concentration), histologic findings (gallbladder wall necrosis, cholecystitis, and sclerosing cholangitis), signalment (age, sex, and weight), clinical signs (anorexia, vomiting, and lethargy), and physical examination findings (rectal temperature, heart rate, and respiratory rate) were compared between groups, with dogs grouped on the basis of whether GBR was or was not present at the time of surgery. Continuous data were assessed for normality with the D’Agostino-Pearson omnibus normality test. Normally distributed continuous variables were compared between groups with a 2-tailed, unpaired Student’s t test; nonnormally distributed continuous variables were compared between groups with a two-tailed Mann-Whitney test. Categorical data were tested for an association with group with a 2-tailed Fisher exact test. Results were recorded as mean and SD or as median and interquartile (25th to 75th percentile) range (IQR). After initial exploratory statistical evaluations were completed, results were corrected for the large number of comparisons with the Benjamini-Hochberg method to control the false discovery rate.66,67 Clinicopathologic test results that were significantly (P < 0.05) different between groups after correction for the false discovery rate were analyzed further for associations with physical status score; the presence of cholangiohepatitis, cholecystitis, or gallbladder wall necrosis; and outcome (survivor vs nonsurvivor) with Mann-Whitney or 2-tailed, unpaired Student’s t tests. A Bonferroni correction was applied to results of these secondary analyses to adjust for multiple comparisons.

Abdominal ultrasonography findings and results of preoperative clinicopathologic tests that were found to be significantly different between groups (GBR vs intact gallbladder) after correction for false discovery rate were analyzed by means of receiver operating characteristic curve analysis, and the maximum Youden index (sensitivity + specificity – 1) was used to determine the optimal cutoff for detecting GBR. The resulting dichotomous data (ie, above or below the cutoff value) were evaluated with a Fisher exact test to derive sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio (LR), OR, and accuracy for detection of GBR. Accuracy was calculated as the percentage of true positive plus true negative results divided by the number of dogs. The area under the receiver operating characteristic curve was derived by computer software to further evaluate and confirm test accuracy.

For all analyses, a value of P < 0.05 was considered significant. Statistical analyses were performed with commercially available software (Prism 8 statistical software; GraphPad Software; R version 4.2; R Foundation for Statistical Computing).
Results

Animals

Eighty-five dogs underwent gallbladder surgery during study period, and of these, 45 met the criteria for inclusion in the study. Thirty of the dogs included in the study had an intact gallbladder at the time of surgery, 15 had GBR. Breeds included Chihuahua (n = 10), Toy Poodle (8), Shiba Inu (6), Pomeranian (4), Miniature Schnauzer (3), Miniature Dachshund (3), Yorkshire Terrier (3), mixed-breed (3), Cocker Spaniel (2), Pug (2), and Shih-Tzu (1). Mean ± SD age at the time of surgery was 11.2 ± 2.9 years for dogs with an intact gallbladder and 10.2 ± 2.9 years for dogs with GBR. Median body weight was 6 kg (IQR, 4.2 to 10 kg) for dogs with an intact gallbladder and 4.7 kg (IQR, 2.5 to 10.4 kg) for dogs with GBR. Of the 30 dogs with an intact gallbladder, 14 (47%) were spayed females, 12 (40%) were castrated males, 3 (10%) were sexually intact females, and 1 (3%) was a sexually intact male. Of the 15 dogs with GBR, 10 (67%) were castrated males, 3 (20%) were spayed females, 1 (6.5%) was a sexually intact male, and 1 (6.5%) was a sexually intact female. There was no significant difference between groups with regard to age, body weight, or sex distribution.

Clinical signs

Mean ± SD rectal temperature at the time of initial examination was 38.1 ± 0.65 °C for dogs with an intact gallbladder and 38.4 ± 0.72 °C for dogs with GBR. Median heart rate was 120 beats/min (IQR, 103 to 141 beats/min) for dogs with an intact gallbladder and 140 beats/min (IQR, 126 to 150 beats/min) for dogs with GBR. Median respiratory rate was 34 breaths/min (IQR, 24 to 48 breaths/min) for dogs with an intact gallbladder and 42 breaths/min (IQR, 34.5 to 55.5 breaths/min) in dogs with GBR (dogs that were panting were assigned a respiratory rate of 60 breaths/min). There was no significant difference between groups in regard to rectal temperature, heart rate, or respiratory rate at the time of initial examination.

Common preoperative clinical signs included anorexia (23/30 [77%] dogs with an intact gallbladder and 12/15 [80%] dogs with GBR), vomiting (18/30 [60%] dogs with an intact gallbladder and 13/15 [87%] dogs with GBR), lethargy (18/30 [60%] dogs with an intact gallbladder and 13/15 [87%] dogs with GBR), tachypnea (4/30 [13%] dogs with an intact gallbladder), abdominal pain (4/30 [13%] dogs with an intact gallbladder and 2/15 [13%] dogs with GBR), polyuria and polydipsia (2/30 [7%] dogs with an intact gallbladder), diarrhea (1/30 [3%]) dogs with an intact gallbladder and 1/15 [7%] dogs with GBR), and respiratory distress (2/30 [7%] dogs with an intact gallbladder). The percentage of dogs with anorexia, vomiting, or lethargy did not differ significantly between groups.

Concurrent medical problems were present in 23 dogs and included 1 or more of the following conditions: mitral valve disease (n = 10), urinary incontinence (2, including 1 dog with bladder atony secondary to spinal disease that was medically managed), renal calculi (2), hyperadrenocorticicism (2; previously diagnosed in both dogs and medically managed), diabetes mellitus (2; previously diagnosed in both dogs and medically managed), abdominal distension (2), epilepsy (1; previously diagnosed and medically managed), pancreatitis (1), hypothyroidism (1; previously diagnosed and medically managed), intervertebral disk herniation (1), cystic calculi (1), hydrometra (1; diagnosed at presentation), decreased proprioception in a pelvic limb (1), rheumatoid arthritis (1), and tricuspid valve disease (1).

Abdominal ultrasonographic findings

All 45 dogs underwent abdominal ultrasonography prior to cholecystectomy, and GBM was diagnosed in all 45 on the basis of gravity-independent material with a stellate or finely striated pattern in the gallbladder. Other abdominal ultrasonographic findings included peritoneal effusion (n = 20 [44%]), cystic fluid around the gallbladder (13 [29%]), common bile duct enlargement (21 [47%]), gallbladder wall thickening and irregular mucosal surface (8 [18%]), pancreatitis (9 [20%]), common bile duct obstruction (7 [16%]), and cholelithiasis (4 [9%]). Preoperative abdominal ultrasonography provided imaging evidence suggestive of GBR in 17 dogs. Fourteen (82%) of these dogs had GBR confirmed at surgery and 3 did not. One dog considered to have an intact gallbladder on the basis of abdominal ultrasonography was found to have GBR during surgery. Results of abdominal ultrasonography were significantly (P < 0.001) associated with gallbladder status (intact vs ruptured). Sensitivity of abdominal ultrasonography for detection of GBR was 93%, and specificity was 90% (OR, 126; LR, 9.33).

Clinicopathologic test results

During initial statistical testing, serum CRP concentration, WBC count, neutrophil count, and total serum bilirubin concentration were significantly higher in dogs with GBR than in dogs with an intact gallbladder (Table 1). However, after adjustment for multiple (n = 46) comparisons, only serum CRP concentration remained significantly (P = 0.03) different between groups. The preoperative serum CRP concentration was higher than the upper reference limit (0.7 mg/dL) in all 15 dogs with GBR and in 26 of the 30 (87%) dogs with an intact gallbladder (Figure 1). Other CBC or serum biochemical test results were not significantly different between groups after correction for multiple tests (Supplementary Table S1).

Receiver operating characteristic curve analysis suggested that the optimal serum CRP concentration cutoff to detect GBR was 6.3 mg/dL (LR, 3.0). The area under the curve at this cutoff was 0.79 (95% CI, 0.42 to 0.83). Diagnostic performance of using this cutoff to identify GBR was calculated and compared...
with diagnostic performance of preoperative abdominal ultrasonography and with diagnostic performance of abdominal ultrasonography combined with serum CRP concentration (ie, ultrasonography positive for GBR and CRP > 6.3 mg/dL vs ultrasonography negative for GBR and CRP < 6.3 mg/dL). CRP concentration was > 6.3 mg/dL in all 15 dogs with surgically confirmed GBR. The CRP concentration was 7.0 mg/dL in the 1 dog with GBR that was falsely diagnosed as having an intact gallbladder on abdominal ultrasonography. The CRP concentration was < 6.3 mg/dL in 20 of the 30 dogs confirmed at surgery to have an intact gallbladder. Abdominal ultrasonography correctly diagnosed an intact gallbladder in 8 of the 10 dogs that had a CRP concentration greater than the cutoff (Table 2).

Median preoperative CRP concentration was significantly ($P < 0.001$) higher in dogs with physical status scores > 2 (8.2 mg/dL; IQR, 6 to 16.4 mg/dL) than in dogs with physical status scores of 2 (1 mg/dL; IQR, 0.4 to 1.63 mg/dL). There was no significant difference in preoperative serum CRP concentration in dogs with or without cholangiohepatitis, cholecystitis, or histologic evidence of gallbladder necrosis. There was also no significant difference in preoperative serum CRP concentration between dogs alive 2 weeks after surgery and dogs that died prior to the 2-week recheck examination.

### Surgical findings

Median time between diagnosis of GBM and surgery was 1 day (IQR, 1 to 1 day; range, 1 to 36 days). An intact gallbladder was documented during surgery in 30 (67%) dogs. External areas of necrosis of the gallbladder wall were noted in 1 (2%) dog with an intact gallbladder. Inspissated bile was noted in the abdominal cavity or within omental adhesions adjacent to the gallbladder in 15 (33%) dogs. The site of bile leakage appeared to be the gallbladder in all 15 dogs. Choleliths were present in the gallbladder of 2 (4%) dogs. Liver nodules were seen in 5 (11%) dogs, and splenic nodules were present in 3 (7%).

Patency of the common bile duct was confirmed in all 45 dogs. Normograde catheterization was performed in 31 (69%) dogs and retrograde catheterization via duodenotomy was performed in 14 (31%).

Three dogs required a second exploratory surgery because of deterioration of clinical signs. The first dog underwent a second surgery 4 days after initial surgery, leakage from the common bile duct was identified. The common bile duct was repaired, and a choledochal stent was placed, but the dog died 3 days later of respiratory distress. The second dog underwent a second surgery 6 days after initial surgery, and a small dehiscence of the duodenotomy incision was found. The dehiscence was repaired with a serosal patch, but the dog had cardiopulmonary arrest intraoperatively after treatment of medically nonresponsive hypotension. The third dog underwent a second surgery 15 days after initial surgery, and an

### Table 1 — Selected preoperative clinicopathologic test results for 45 dogs undergoing cholecystectomy for treatment of gallbladder mucocele that had gallbladder rupture (n = 15) or an intact gallbladder (30) at the time of surgery.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Reference interval</th>
<th>Intact gallbladder</th>
<th>Gallbladder rupture</th>
<th>$P$ value</th>
<th>Adjusted $P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (X $10^3$ cells/μL)</td>
<td>6.0–17.0</td>
<td>17.7 (6.5–23.9)</td>
<td>4.0–61.3</td>
<td>0.019</td>
<td>0.22</td>
</tr>
<tr>
<td>Neutrophils (X $10^3$ cells/μL)</td>
<td>3.0–11.5</td>
<td>15.4 (6.5–21.8)</td>
<td>1.1–50.9</td>
<td>0.012</td>
<td>0.18</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.1–0.5</td>
<td>1.6 (0.2–5)</td>
<td>0.1–20.1</td>
<td>0.036</td>
<td>0.27</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0–0.7</td>
<td>2.6 (0.97–13.4)</td>
<td>0–20.2</td>
<td>0.001</td>
<td>0.03</td>
</tr>
</tbody>
</table>

CRP = C-reactive protein. IQR = Interquartile (25th to 75th percentile) range. $P$ values were calculated with a Student $t$ (parametric values) or Mann-Whitney (nonparametric values) test. Adjusted $P$ values were calculated with the Benjamini-Hochberg method to control for multiple comparisons.

Figure 1 — Dot plots of preoperative serum C-reactive protein (CRP) concentration (reference interval, 0 to 0.7 mg/dL) in 45 dogs undergoing cholecystectomy for treatment of gallbladder mucocele that had gallbladder rupture (GBR; n = 15) or an intact gallbladder (GBI; 30) at the time of surgery. Preoperative CRP concentration was significantly ($P = 0.03$) different between the 2 groups.
abscess between the liver and the lesser curvature of the stomach was excised without further complications. The dog was alive when examined 2 weeks after the second surgery.

Mean ± SD anesthesia and surgical times for all dogs were 163.4 ± 35.6 minutes and 118.0 ± 35.2 minutes, respectively. Mean ± SD duration of hypotension was 44 ± 32.8 minutes. There was no significant difference in duration of surgery, anesthesia, or hypotension between dogs with and without GBR. Additional procedures were performed in some dogs, including cystotomy, splenectomy, and gastrotomy. The physical status score was > 2 in all 15 dogs with GBR and in 16 of the 30 (53%) dogs with an intact gallbladder, and the physical status score was significantly different between groups during initial statistical screening (P = 0.001) and after adjustment for multiple comparisons (P = 0.03).

All dogs were treated after surgery with IV administration of crystalloid fluids, pain medications (intermittent or constant rate infusion of opioids), and antiemetics, as needed. One dog with GBR received a packed RBC transfusion postoperatively to treat anemia. All dogs received antimicrobials every 90 minutes during surgery. Injectable antibacterials used in the postoperative period were the same as those used intraoperatively. Oral antibacterials administered postoperatively included amoxicillin, amoxicillin–clavulanic acid, cephalaxin, enrofloxacin, and metronidazole. Nasogastric tubes were aspirated intermittently to check for fluid accumulation in the stomach. In 16 (36%) dogs, metoclopramide was administered as a constant rate or intermittent infusion at the discretion of the attending clinician. Ursodeoxycholic acid (10 to 15 mg/kg/d, PO) was administered to 19 (42%) dogs.

There was no significant difference in median duration of hospitalization between dogs with GBR (5 days; IQR, 4 to 9 days) and dogs with an intact gallbladder (5 days; IQR, 3 to 6 days). There was also no significant difference in median duration of hospitalization between dogs that underwent retrograde catheterization of the common bile duct (5 days; IQR, 5 to 10.5 days) and dogs that underwent normograde catheterization (5 days; IQR, 3 to 6 days).

**Microbiologic and histologic findings**

Bacterial culture and susceptibility testing were performed on gallbladder contents of 41 dogs, and results of bacterial culture were negative for all 41 dogs. Histologic evaluation of the gallbladder and liver was done in all dogs, and histologic findings compatible with GMB were present in all 45. Thirty-four (76%) dogs, including 21 of 30 (70%) dogs with an intact gallbladder and 13 of 15 (87%) dogs with GBR, had histologic evidence of focal or diffuse gallbladder wall necrosis. Histologic evidence of cholecystitis was present in 6 of 30 (20%) dogs with an intact gallbladder and 5 of 15 (33%) dogs with GBR. There was no significant difference in the incidence of gallbladder wall necrosis or cholecystitis between dogs with and without GBR. Cholangiohepatitis was diagnosed in 22 of 30 (73%) dogs with an intact gallbladder and in 10 of 15 (67%) dogs with GBR; these proportions were not significantly different. Suppurative serositis was found in 3 of 30 (10%) dogs with an intact gallbladder and 4 of 15 (27%) dogs with GBR. Fibrosis was present in the liver of 17 of the 45 (37.8%) dogs, and hepatocellular vacuolation without inflammation was noted in 12 (27%). Lobular atrophy was diagnosed in the liver of 2 (4%) dogs. Liver nodules were present in 5 dogs and were identified as well-differentiated hepatocellular carcinoma (2 dogs), nodular hyperplasia (2 dogs), and regenerative nodules (1 dog). The splenic nodules present in 3 dogs were diagnosed as lymphoid hyperplasia.

**Outcome**

Thirty-eight of the 45 (84%) dogs were alive at a recheck examination 2 weeks after surgery. No dog died during the initial intraoperative period; however, 6 dogs died prior to discharge between 5 and 12 days postoperatively. One dog died at home 9 days after surgery. Three of the 7 dogs that died had an intact gallbladder and 4 had GBR. There was no significant difference in death rate between dogs with GBR (4/15 [27%]) and dogs with an intact gallbladder (3/30 [10%]). All dogs that died underwent surgery within 24 hours after the diagnosis of GMB.

**Discussion**

In the present study, we found that for dogs with GMB undergoing cholecystectomy, preoperative serum CRP concentration was significantly higher in dogs with GBR than in dogs with an intact gallbladder. The 33% incidence of GBR in our study was within the range (20% to 61%) reported in other stud-

### Table 2—Diagnostic performance of abdominal ultrasonography (AUS), preoperative CRP concentration, and the combination of AUS and CRP concentration (AUS + CRP) for prediction of gallbladder rupture in 45 dogs undergoing cholecystectomy for treatment of gallbladder mucocele.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUS</td>
<td>93%</td>
<td>90%</td>
<td>82%</td>
<td>96%</td>
<td>91%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CRP &gt; 6.3 mg/dL</td>
<td>100%</td>
<td>67%</td>
<td>60%</td>
<td>100%</td>
<td>78%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AUS + CRP</td>
<td>100%</td>
<td>93%</td>
<td>88%</td>
<td>100%</td>
<td>96%</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

NPV = Negative predictive value. PPV = Positive predictive value.
Abdominal ultrasonography was able to better identify true negative cases (ie, dogs with an intact gallbladder) because it focused directly on imaging the gallbladder wall. The greatest discriminatory power was achieved when ultrasonographic detection of GBR was combined with a CRP concentration > 6.3 mg/dL. This combination increased the accuracy of detecting GBR to 96% and increased sensitivity and specificity to 100% and 93%, respectively. Although CRP is a stronger predictor than other inflammatory biomarkers (eg, WBC or neutrophil count) for the diagnosis of multiple forms of acute cholecystitis in humans, it has comparable discriminatory power in diagnosing pericholecystic abscesses and GBR with biliary peritonitis.

The overall perioperative mortality rate among the dogs in the present study (7/45 [16%]) was similar to that (7% to 40%) reported previously. The mortality rate among dogs with GBR (4/15 [27%]) was not significantly higher than that among dogs with an intact gallbladder (3/30 [10%]). However, the statistical power to differentiate the 2 groups was low owing to the low overall mortality rate and small population size. GBR was not associated with an increased mortality rate in several previous studies. However, a recent multi-institutional study found that the risk of death with GBR was 2.7 times the risk after cholecystectomy when the gallbladder was intact. Another recent investigation involving 121 dogs reported a 33% mortality rate among dogs with GBR and a 16% mortality rate among dogs with an intact gallbladder.

Two of the 3 dogs in our study that underwent a second surgery to address complications associated with the initial surgery died during the perioperative period. One of these dogs had leakage from the initial cystic duct ligation, and the other had dehiscence of the duodenotomy performed to allow for retrograde and normograde catheterization. The overall complication rate during the study period was low, with a decreased incidence of biliary peritonitis. There is considerable controversy about the usefulness of bile duct catheterization in dogs undergoing cholecystectomy for treatment of GBM, with some experts questioning the need for common bile duct catheterization at all. One study reported an increased incidence of postoperative pancreatitis and a slight increase in the duration of hospitalization after common bile duct catheterization, regardless of the technique used. Two other studies reported a benefit of normograde over retrograde catheterization, with a decreased incidence of hyperthermia and a more rapid decrease in serum bilirubin concentration after normograde catheterization. We did not find a difference in complication rate, mortality rate, or hospitalization time between retrograde and normograde catheterization.

The breed distribution of dogs in the present study was slightly different from that of previous reports. Along with breeds commonly reported in previous studies of GBM in dogs, such as Shetland Sheepdog, Miniature Schnauzer, Cocker Spaniel, and Chihuahua, the third most common breed in the
present study was the Shiba Inu. This breed has not been reported previously as being predisposed to GBM, and the high number of Shiba Inus in the present report may simply have been due to the great popularity of the breed in Japan.

Limitations of the present study included the retrospective nature of the investigation. Inclusion criteria required a complete medical record, histopathologic confirmation of GBM, and measurement of CRP concentration within 24 hours prior to cholecystectomy, which limited the number of cases available for analysis. Dogs included in the present study may have been skewed toward more severe cases, because only dogs that were suspected to have GBM on the basis of abdominal ultrasonographic findings underwent cholecystectomy. Dogs with equivocal abdominal ultrasonographic findings were treated medically or had surgery delayed and were therefore excluded. Another possible limitation of our study was that we based our analysis on a single preoperative measurement of CRP concentration. Serial measurement of CRP concentration has been used as a prognostic indicator for several medical conditions. For example, in a study comparing dogs with septic or nonseptic systemic inflammatory response syndrome, although there was no difference between groups for a single measurement of CRP concentration, there was a significant difference in the 3-day decrease in CRP concentration between survivors and non-survivors. Serial measurement of serum CRP concentration in the present study may have provided novel prognostic information.

In conclusion, we found in our study population that preoperative serum CRP concentration was a biomarker for GBR rupture in dogs with GBM undergoing cholecystectomy, with CRP concentration significantly higher in dogs with GBR than in dogs with an intact gallbladder. We also found that a preoperative serum CRP concentration > 6.3 mg/dL could be used to identify GBR with a sensitivity of 100%, specificity of 87%, and overall accuracy of 88% in dogs with GBM. We did not find that measuring CRP concentration was superior to performing abdominal ultrasonography for detection of GBR in our study population. However, the accuracy of preoperative CRP concentration for detection of GBR was superior to that reported for abdominal ultrasonography in several previous publications. We propose using preoperative serum CRP concentration as an adjunct to abdominal ultrasonography for evaluation of dogs with GBM and for surgical decision-making. The greatest discriminatory power for preoperative diagnosis of GBR in our study was achieved when ultrasonographic detection of GBR was combined with a serum CRP concentration > 6.3 mg/dL. The combination increased the sensitivity and specificity of GBR detection to 100% and 93%, respectively, and the accuracy to 96%. Future studies are planned to further investigate the efficacy of combined abdominal ultrasonography and CRP concentration for detecting GBR. The combination of abdominal ultrasonography and CRP concentration may also prove to be useful for staging and establishing a prognosis for GBM early in the disease course, prior to the need for emergency cholecystectomy.

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**Supplementary Materials**

Supplementary materials are posted online at the journal website: avmajournals.avma.org