Measurement of feline-specific pancreatic lipase aids in the diagnosis of pancreatitis in cats

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OBJECTIVE
To establish a reference interval for a feline-specific pancreatic lipase assay (Spec fPL test; Idexx Laboratories Inc) in healthy cats and determine the sensitivity and specificity of the Spec fPL test in a large group of ill cats with and without pancreatitis.

ANIMALS
41 healthy cats, 141 cats with clinical signs consistent with pancreatitis, and 786 stored sera with known feline pancreatic lipase immunoreactivity (fPLI) concentrations.

METHODS
This was a prospective, cross-sectional, nonrandomized study. Based on a detailed review of the medical history and results of physical examination, CBC, serum biochemical profile, urinalysis, abdominal ultrasonography, and clinical outcome, each cat was categorized by 2 board-certified internists masked to the fPLI test results into 1 of 6 categories from definitely pancreatitis to definitely not pancreatitis.

RESULTS
The reference interval for the Spec fPL test, determined from the central 95th percentile of results from healthy cats, was fPLI of 0.7 to 3.5 µg/L. An fPLI concentration of $\geq 5.4$ µg/L was determined to be consistent with pancreatitis. With an fPLI of 5.4 µg/L as the diagnostic cutoff, the sensitivity of the Spec fPL test for feline pancreatitis (definitely pancreatitis and probably pancreatitis) was 79.4%, the specificity for cats characterized as probably not pancreatitis and definitively not pancreatitis was 79.7%, and positive and negative predictive values were 69% and 87%, respectively.

CLINICAL RELEVANCE
These findings support the use of the Spec fPL test as a valuable diagnostic test for feline pancreatitis.

Keywords: cats, ultrasound, cytology, histopathology, pancreas
measure of pancreatitis severity.\textsuperscript{31,32} The assay was shown to be both sensitive in cats with moderate to severe pancreatitis (100%) and specific in healthy cats (100%); however, a lower sensitivity (54%) was noted in cats with mild pancreatitis. Subsequently, a commercial assay for the measurement of serum fPLI concentration (Spec fPL test; Idexx Laboratories Inc) has replaced the original radioimmunoassay. This assay is species specific and utilizes monoclonal antibodies to bind a single specific binding site.

Transabdominal pancreatic ultrasonographic evaluation has been determined to be a moderately sensitive test (73%) for pancreatitis in cats; however, variable specificity (24% to 67%) has been reported.\textsuperscript{7,14,27,29} Unlike serology, this test is operator, equipment, and patient compliance dependent; however, it permits the detection of concurrent nonpancreatic disorders, screening for underlying causes of pancreatitis and ultrasound-guided fine-needle aspiration (FNA) cytology of the pancreas and peripancreatic fluid accumulations.\textsuperscript{7,14,34–38} Ultrasonographic changes associated with pancreatitis include pancreateomegaly, hypoechoic pancreatic parenchyma, hyperechoic peripancreatic fat/mesentery, irregular pancreatic border, dilated pancreatic or bile duct(s), gall bladder enlargement, thickened gastric wall, and corrugated, thickened duodenal wall.\textsuperscript{7,14,34–38} The minimum number of changes needed to make an ultrasonographic diagnosis of pancreatitis has not been determined. Pancreatitis has also been documented in the absence of sonographic abnormalities of the pancreas.\textsuperscript{29} Moreover, certain abnormalities are often associated with pancreatitis, such as enlargement of pancreatic ducts and pancreatic parenchyma hyperechochogenicity, which may also be seen in aged cats without pancreatitis.\textsuperscript{36,38–42}

The goals of this study were to establish the reference interval for the Spec fPL test in apparently healthy cats and determine statistical measures of performance (sensitivity, specificity, and both positive and negative predictive values) in a large number of ill cats with clinical signs consistent with pancreatitis (clinical cats). We hypothesized that the Spec fPL test would prove useful as a diagnostic test to help distinguish between cats with similar clinical signs that do or do not have pancreatitis.

**Methods**

**Animals**

Clinical cats—Client-owned cats with clinical signs consistent with pancreatitis (eg, inappetence, lethargy, vomiting, diarrhea, and/or weight loss) were evaluated at MedVet, Medical and Cancer Center for Pets (MMCC), Worthington, Ohio, between April 2007 and March 2008. Exclusion criteria were owner declining enrollment or a primary nonabdominal disease cause for the clinical signs, such as congestive heart failure or others.

Apparantly healthy control cats—Cats owned by employees of MMCC were evaluated during the same time period. All healthy cats had no history of gastrointestinal disease; were not being treated with any medications besides heartworm, tick, or flea preventative; and underwent the same diagnostic evaluation as the clinical cats.

All cats were entered into the study with informed owner consent. The study protocol was approved by the research board of MMCC. For all cats, a complete medical history, physical examination, CBC, serum biochemical profile, urinalysis, and serum fPLI concentration with the Spec fPL test were obtained. Within 12 hours of obtaining serum for fPLI concentrations with the Spec fPL test, pancreatic images were acquired ultrasonographically. When indicated in clinical cats, ultrasound-guided FNA or biopsy of the pancreas was performed to obtain samples for cytologic or histologic examination. No pancreatic aspirations or biopsies were performed in healthy cats. Additional diagnostic testing was performed as indicated for clinical cats under the direction of an internal medicine specialist (MAF) board-certified by the American College of Veterinary Internal Medicine (ACVIM).

**Spec fPL test**

The Spec fPL test is a sandwich ELISA utilizing a mouse monoclonal capture antibody coated on a 96-well microtiter plate and a second enzyme-labeled mouse monoclonal detection antibody. The anti-feline pancreatic lipase mouse monoclonal antibodies were developed using native feline pancreatic lipase purified from feline pancreata as described previously.\textsuperscript{31} Calibrators containing recombinant feline pancreatic lipase are used to construct a dose-response curve for each assay that is used to determine the concentration of pancreatic lipase in feline serum samples within the range of 0.5 to 50 µg/L. The Spec fPL test requires 25 µL of serum/test (run in duplicate fashion). Serum was obtained from serum separator tubes or red serum tubes.

The precision of serum fPLI concentration, as measured by the Spec fPL test, used 786 stored sera with known fPLI concentrations and was determined by a dose-response curve for the precision of serum fPLI concentration was determined by assaying each of 7 feline serum samples with doses spanning the assay range in 8 replicates across each of 6 plates. The SD and coefficient of variation were calculated to determine the total variation for all individual replicates across the 6 plates \((n = 48)\). In addition, the intra-assay and interassay components of precision were determined for each sample.

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**Transabdominal ultrasonography**

All ultrasonographic studies were performed utilizing the same ultrasound system (Technos MP
8.5-MHz convex array and 13-MHz linear array transducers; Biosound Esaote Inc) by 1 of 2 radiologists board certified by the American College of Veterinary Radiology. Multiple still images of the pancreas (a goal of 1 image from each pancreatic lobe and body) were acquired from each cat, as well as any pertinent images of nonpancreatic abnormalities. A complete abdominal ultrasonographic examination and a written report were generated at the time of the study for all clinical cats. Following completion of the study, a single radiologist (JTS) reviewed all images in a masked fashion. The following criteria were considered: visualization of the majority of the pancreas, smooth or irregular pancreatic borders, echogenicity of the pancreas relative to the liver, pancreatic duct size, peripancreatic fluid accumulation, the presence of pancreatic nodules or cysts, and the presence of enlarged peripancreatic lymph nodes. The distribution of abnormalities within the pancreas was also noted.

A grading scale for findings on transabdominal pancreatic ultrasonography (Supplementary Table S1) was designed a priori. The pancreas was judged to be enlarged if the left lobe was thicker than 9.5 mm, the body > 8.5 mm, or the right lobe > 6 mm. The degree of enlargement was assessed by measurement of the pancreatic regions by ultrasonography (pancreatic body and both left and right lobes), being classified as mildly, moderately, or severely enlarged, according to the grading scale used in the present study. Moreover, the classification comprised a subjective classification, considering it was dependent on the operator’s ultrasonographic still images and from the radiologist’s analysis. The pancreatic duct was considered enlarged if it measured > 2.5 mm in diameter and was also graded as mildly, moderately, or severely dilated.

**Cytology and histopathology**

As clinically indicated, pancreatic tissue was evaluated by cytologic and/or histologic examination. Cytologic samples were obtained by a board-certified radiologist using a nonaspiration, freehand technique. The cats were sedated as needed if patient movement limited the safe acquisition of a sample. A 25-gauge 1.5-inch needle with an attached 3-mL syringe drawn to the 1-mL mark with air was inserted via ultrasound guidance into a sonographic landmark, and gentle preparation by slide-over-slide technique was performed. Smears were air-dried and stained with Wright-Giemsa stain. Cytologic evaluation was performed by a single board-certified clinical pathologist (SCC) who had access to the clinical history but was masked to the PLI concentration. Surgical pancreatic biopsy samples were obtained in anesthetized cats using a ligature technique or laparoscopic biopsy using a clam-shell biopsy device and were evaluated by a single board-certified pathologist (SJN), also masked to the PLI results. Histopathology was also performed on the entire pancreas of a subset of cats that died or were euthanized. With the owner’s consent, the pancreata were removed in their entirety and fixed in neutral-buffered 10% formalin within 30 minutes of death. Pancreata were sectioned every 1 cm, and sections were routinely processed and stained with H&E stain. All postmortem pancreatic histopathology samples were reviewed and scored by a single board-certified pathologist (SJN) in a blinded fashion, using a modification of the semiquantitative histopathology grading scheme by De Cock et al. For each section, the following parameters were scored: fibrosis, necrosis, edema, neutrophilic inflammation, lymphocytic inflammation, atrophy, nodules, fluid accumulations, and the presence or absence of amyloid and cancer.

**Clinical diagnostic criteria**

A 6-category classification scheme was developed a priori to classify each cat’s likelihood of having pancreatitis (Supplementary Table S2). The categories were as follows: definitely pancreatitis (DP), probably pancreatitis (PrP), possibly not pancreatitis (PoP), possibly not pancreatitis (PoNP), probably not pancreatitis (PrNP), and definitely not pancreatitis (DNP). For category assignment, clinical review for all 182 cats included the entire medical history, physical examination findings at enrollment including a semiquantitative assessment of the presence of abdominal pain, all laboratory data, the sonographic report generated when the patient was entered into the study and the graded ultrasonographic criteria, cytologic and histopathologic reports, therapies provided, and outcome including postmortem examination findings (if performed). The ACVIM diplomate (MAF) supervising the clinical care of all cats presented each case to 2 ACVIM diplomates (PJA and JER) who had no prior knowledge of any of the cats (clinical or healthy controls). All available information except the fPLI results was reviewed.

Histologic evidence of pancreatitis or lack of pancreatitis was weighted most heavily, and a cat was not classified as DP unless there was histopathologic evidence of acute or chronic inflammation and/or necrosis in at least 1 section of the pancreas. The absence of the above lesions was evidence that the cat did not have pancreatitis (classified as DNP), although a cat was also classified as DNP on the basis of normal pancreatic ultrasonography with an absence of supportive clinical evidence even if histopathology of the pancreas was not available.

Convincing ultrasonographic evidence of pancreatitis, along with supportive clinical evidence, needed to be present for a cat to be classified as PrP. Cats with weak or no evidence of pancreatitis could be classified as PoP, PoNP, or PrNP on the basis of overall clinical assessment (Supplementary Table S2). Pancreatic fluid accumulations, nodules and/or masses, and documented cancers were classified on the basis of the likelihood of underlying or concurrent pancreatitis.

Clinical signs considered consistent with pancreatitis included lethargy, inappetence or anorexia, apparent abdominal pain, weight loss, dehydration, hypothermia or fever, jaundice, vomiting, and/or diarrhea. Laboratory findings considered consistent with pancreatitis included leukocytosis or leucopenia,
high liver enzyme activity, hyperbilirubinemia, hypoalbuminemia, hypocalcemia, hypokalemia, prerenal azotemia, hypercholesterolemia, and/or hyper- or hypoglycemia. For a cat to be categorized as DP or PrP, compatible clinicopathologic findings of disease were required to be present. For a cat to be classified as PoP or PoP, there needed to be compatible clinical signs but weak or no laboratory support for pancreatitis. For a cat to be classified as PrNP, clinical and laboratory evidence of pancreatitis had to be absent or weak.

A convincing presence or absence of another disease that explained a cat’s clinical signs was used to categorize cats as PoNP or PoP, respectively, such as when a clinical suspicion of pancreatitis existed but the cat had no histopathology available, ultrasonographic findings that were not convincing or clinically normal, and laboratory results within reference ranges, not convincing, or compatible with another disease process. In the absence of pancreatic histopathology being available, the presence of another convincing disease was used in conjunction with clinically normal findings on pancreatic ultrasonography to classify a cat as DNP.

Statistical analysis

In an effort to maximize group size and recognizing the difficulty of clinical classification of pancreatitis, the DP and PrP categories were considered a single group and termed the pancreatitis group, the DNP and PrNP categories were termed the not pancreatitis group, and the PoP and PoNP categories were termed the indeterminate pancreatitis group. Considering the relatively weak diagnostic evidence to support or eliminate pancreatitis in the indeterminate pancreatitis group and to avoid a type 1 error of misclassifying a cat, these 2 categories were not included in the calculation of sensitivity, specificity, and positive and negative predictive values (and their respective 95% CIs).

Comparisons were performed between the pancreatitis, not pancreatitis, and indeterminate pancreatitis groups for the following variables: median age, sex, breed, frequency of occurrence of diarrhea, vomiting, anorexia, weight loss, lethargy, abdominal pain, dehydration, cranial abdominal mass, body condition score, selected CBC results (Hct, WBC count, and platelet count), serum biochemical analysis (total calcium, BUN, creatinine, glucose, albumin, globulin, total bilirubin, and cholesterol concentrations and AST, ALT, ALP, and GGT activities), urinalysis (urine specific gravity), Spec fPL test results for fPLI concentration, findings on pancreatic ultrasonography (size, contour, duct size, cysts, nodules, fluid accumulation, and enlarged lymph node), and histologic examination (fibrosis, necrosis, edema, acute inflammation, chronic inflammation, atrophy, nodules, cysts, amyloid, and cancer).

For continuous variables, descriptive data were reported as means, medians, SDs, IQR, and ranges as appropriate. For categorical variables, descriptive data were reported as frequencies and counts. For all hypothesis tests, $P < .05$ was considered statistically significant. Spec fPL test measurements of fPLI concentrations were nonnormally distributed; therefore, nonparametric statistical methods were performed. The Spearman rank correlation was calculated to assess the correlation between Spec fPL test concentration and selected blood biochemical analyses and hematologic results. A 1-way ANOVA on the ranks of Spec fPL test results was performed among the 3 diagnostic groups; this is equivalent to the Kruskal-Wallis test. Using this approach, a contrast was constructed to test for an increasing linear trend in the ranks of Spec fPL across the diagnostic groups.

Utilizing the 95% CI of Spec fPL test results for 41 healthy cats, the normal reference interval for the Spec fPL test was established as fPLI < 3.5 µg/L. A receiver operator characteristic analysis was performed (Figure 1) comparing the DP-PrP groups as pancreatitis positive versus the DNP-PrNP groups as pancreatitis negative to select the optimal cutoff for Spec fPL test measurement of fPLI, giving equal weight to sensitivity and specificity. On the basis of this analysis, a Spec fPL measurement of fPLI concentration of $\geq 5.4$ µg/L was determined to be most accurate for diagnosing and ruling out pancreatitis.

To accurately demonstrate the diagnostic utility of the Spec fPL test, multiple estimates of sensitivity and specificity, along with likelihood ratios, were calculated at the cutoff of fPLI of 5.4 µg/L for various combinations of the PoP and PoNP categories into the negative (not pancreatitis) and positive (pancreatitis) diagnoses. The 95% Wilson-score CIs were calculated for all estimates of sensitivity and specificity. Statistical analyses were performed using SAS (version 9.2; SAS Institute Inc). Positive and negative predictive values were calculated; however, they are highly influenced by the prevalence of the disease.
**Results**

A total of 182 cats were entered into the study. Of the 182 cats, 141 had clinical signs consistent with pancreatitis (clinical cats) and 41 had no clinical signs (healthy controls). In the clinical group, 77 were male castrated, 64 were female spayed, and 21 were male castrated; in the control group, 20 were female spayed. No cats in the study were male or female intact. In the clinical group, 108 cats were domestic shorthair cats, 14 domestic long hair, 5 Siamese, 3 Maine coon cats, 2 domestic medium hair, 2 not classified in the medical record, and 1 each was a Rag Doll, Scottish Fold, Himalayan, Exotic shorthair, Abyssinian, Manx, and American shorthair. The healthy control cats had a similar breed distribution, with 26 domestic short-hair, 8 domestic long hair, 4 domestic medium hair, and 1 each of Siamese, Maine coon, and Tonkinese. The cats' ages ranged from 0.6 to 18 years (median, 11 years) for the clinical group and from 1 to 13 years (median, 5 years) for the healthy group. This age difference between groups was significantly different.

Using the Clinical Diagnosis Criteria Classification Scheme (Supplementary Table S2), the likelihood of pancreatitis was determined for all cats (clinical and healthy). For the clinical cats, 34 cats were determined to have pancreatitis (9 DP and 25 PrP), 59 cats were determined to not have pancreatitis (26 DNP and 33 PrNP), and 48 cats were classified as indeterminate pancreatitis (29 PoP and 19 PoNP). All of the healthy cats were determined to not have pancreatitis (41 DNP).

Of the 141 clinical cats, 5 underwent ultrasound-guided pancreatic FNA for cytologic examination and 17 underwent pancreatic biopsy for histologic examination. Procedures for biopsy included a ligature technique (n = 2) or laparoscopic biopsy using a clam-shell biopsy device (1). Additionally, histologic examination was performed on the entire pancreas of each cat that died or was euthanized (n = 14).

Of the 34 cats with pancreatitis, 4 (12%) had pancreatic FNA cytologic examination and 9 (26%) had pancreatic histologic examination performed by post-mortem evaluation of the entire pancreas. A cellular sample was obtained via FNA cytology in 3 of 4 cases (1 was inconclusive; too few tissue cells were present to evaluate). Acute inflammation (n = 2 cats), hyperplastic pancreatic epithelial cells (1), and carcinoma with acute inflammation (1) were described. All 9 cats classified as DP by histopathology had evidence of acute inflammation and necrosis in at least 1 section of the pancreas. Other pancreatic lesions present were chronic inflammation (n = 9 cats), nodules (8), cysts (8), fibrosis (6), atrophy (3), and edema (2). Pancreatic islet amyloid and pancreatic adenocarcinoma were detected in 4 and 2 cats, respectively. In 1 cat with pancreatic adenocarcinoma, intestinal histopathology also revealed adenocarcinoma. Similar clinical signs were noted in the 3 cats diagnosed with pancreatic carcinoma. The Spec fPL test results were fPLI concentrations of 5.4, 25, and 36 µg/L. Pancreatic ultrasonographic abnormalities noted included pancreatic echogenicity and hyperechoic peripancreatic echogenicity (3) and mild (1) to moderate (2), irregular pancreatic capsular margin. No pancreatic nodules or masses were present in this group.

In 2 of the 48 cats with indeterminate pancreatitis, pancreatic FNA cytology revealed well-differentiated pancreatic epithelial cells with no cytoplasmic abnormalities (n = 1 cat) and surgical pancreatic biopsy histopathology revealed nodular hyperplasia (1). Intestinal and hepatic histopathology in the latter cat revealed mild to moderate lymphocytic enteritis of the duodenum and jejunal, minimal biliary hyperplasia, and multifocal lymphocytic portal hepatitis.

In the 59 clinical cats without pancreatitis, pancreatic biopsies were performed in 7 cases by surgery (n = 1), laparoscopy (1), and postmortem examination (5). No pancreatic cytology was performed in this group. All pancreatic biopsy samples were diagnostic. Histopathology of the 2 cats with premortem biopsies revealed pancreatic nodular hyperplasia. Of the 5 cats with postmortem histopathology, none had acute inflammation or necrosis present in any section. Lesions described were chronic inflammation (n = 5 cats), nodules (5), pancreatic islet amyloid (4), fibrosis (3), cysts (3), edema (1), and atrophy (1). All pancreatic histopathology findings in this group were mild except for the presence of nodules. Mild hepatic lipodisosis was detected in 2 of 2 cats with liver histopathology. Low-grade small jejunal lymphoma was detected in a single cat with intestinal histopathology.

Similar to previous studies, lethargy (91% of the 34 cats in the pancreatitis group), anorexia (82%), and weight loss (74%) were commonly noted clinical signs (Table 1). No differences were noted between cats in the pancreatitis and not pancreatitis groups. Abdominal pain (68%) and dehydration (50%) were noted on physical examination of the pancreatitis group cats. No differences were noted between cats in the pancreatitis versus not pancreatitis groups for these parameters, nor was there a difference between the 2 groups for the presence of a cranial abdominal mass on physical examination or in body condition score.

Selected CBC, serum biochemical, urinalysis, and fPLI concentrations in healthy and clinical cats were compiled (Table 2). Multiple significant but weak Spearman rank correlations with Spec fPL, respectively, were noted: creatinine (P = .010, 19), BUN (P = .004, .21), AST (P = .001, .238), GGT (P < .001, 283), albumin (P < .001, .245), glucose (P = .004, 211), calcium (P = .049, 147), total carbon dioxide (P = .027, .165), WBC (P = .009, .197), HCT (P < .001, .309), and urine specific gravity (P < .001, .334). The Spearman rank correlates various biochemical analyses and hematologic measures with fPLI, with the strongest correlation to fPLI being urine specific gravity, a weak negative correlation. Significant differences (P < .001) were noted for Spec fPL concentrations between normal cats, cats with pancreatitis, indeterminate pancreatitis, and cats without pancreatitis. There was a clear increasing trend in fPLI concentrations as the disease grade progressed from normal to DP.

Abdominal ultrasonography scores (Table 3) for pancreatic size (pancreatomegaly) and contour
Table 1—Results of key clinical signs, physical examination findings, and concurrent diseases diagnosed in healthy cats (n = 41) and ill cats (clinical cats; 141) evaluated for pancreatitis, with clinical cats grouped on the basis of a clinical diagnosis criteria classification scheme into the pancreatitis group (definitely pancreatitis [DP] or probably pancreatitis [PrP]), intermediate pancreatitis group (possibly pancreatitis [PoP] and possibly not pancreatitis [PoNP]), or not pancreatitis group (definitely not pancreatitis [DNP] and probably not pancreatitis [PrNP]).

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of healthy controls (n = 41)</th>
<th>No. of DP and PrP (n = 34)</th>
<th>No. of PoP and PoNP (n = 48)</th>
<th>No. of PrNP and DNP (n = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical signs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lethargy</td>
<td>0</td>
<td>31</td>
<td>38</td>
<td>47</td>
</tr>
<tr>
<td>Anorexia</td>
<td>0</td>
<td>28</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>Weight loss</td>
<td>0</td>
<td>25</td>
<td>35</td>
<td>41</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0</td>
<td>23</td>
<td>37</td>
<td>30</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
<td>4</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Physical examination</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1</td>
<td>23</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>Dehydration</td>
<td>0</td>
<td>17</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Cranial abdominal mass</td>
<td>0</td>
<td>8</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>BCS*</td>
<td>5 (4–9)</td>
<td>4.5 (1–8)</td>
<td>4 (3–9)</td>
<td>5 (2–9)</td>
</tr>
<tr>
<td>Concurrent diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute on CKD</td>
<td>0</td>
<td>9</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Hepatic lipidosis</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>DM or DKA</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>0</td>
<td>9</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Probable or confirmed cancer</td>
<td>0</td>
<td>4</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>IBD</td>
<td>0</td>
<td>6</td>
<td>15</td>
<td>6</td>
</tr>
</tbody>
</table>

BCS = Body condition score. CKD = Chronic kidney disease. DM = Diabetes mellitus. DKA = Diabetic ketoacidosis. IBD = Inflammatory bowel disease.

*Data reported as median and range.

Table 2—Median (range) selected CBC, serum biochemical analysis, urinalysis, and feline pancreatic lipase immunoreactivity (fPLI) concentration (measured with Spec fPL test; Idexx Laboratories Inc) for 41 healthy and 137 ill (clinical) cats described in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Healthy controls (n = 41)</th>
<th>DP and PrP (n = 34)</th>
<th>PoP and PoNP (n = 47)</th>
<th>PrNP and DNP (n = 56)</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct (%)</td>
<td>40.9 (34.5–50.1)</td>
<td>33.1 (17.7–85.3)</td>
<td>35.6 (16.2–58.6)</td>
<td>34.0 (11.0–50.7)</td>
<td>29%–45%</td>
</tr>
<tr>
<td>WBC (X 10^3 cells/µL)</td>
<td>8.0 (4.6–25.7)</td>
<td>11.3 (2.8–34.4)</td>
<td>12.3 (3.60–87.0)</td>
<td>10.6 (3.30–30.50)</td>
<td>4.2–15.6 (X 10^3 cells/µL)</td>
</tr>
<tr>
<td>Platelets (X 10^3 platelets/µL)</td>
<td>209 (7–572)</td>
<td>205 (76–845)</td>
<td>251 (52–632)</td>
<td>278 (22–595)</td>
<td>170–600 (X 10^3 platelets/µL)</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.7 (8.3–11.6)</td>
<td>9.0 (6.3–11.5)</td>
<td>9.2 (4.7–12.1)</td>
<td>9.4 (6.7–14.0)</td>
<td>8.2–11.8 mg/dL</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>25 (18–46)</td>
<td>29 (7–277)</td>
<td>28 (8–200)</td>
<td>26 (5–326)</td>
<td>15–34 mg/dL</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.4 (1–2.8)</td>
<td>1.3 (0.7–16.1)</td>
<td>1.4 (0.5–10.3)</td>
<td>1.6 (0.4–29.2)</td>
<td>0.8–2.3 mg/dL</td>
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<tr>
<td>Albumin (g/dL)</td>
<td>3.5 (2.8–4.10)</td>
<td>2.9 (1.5–3.8)</td>
<td>3.1 (1.1–4.0)</td>
<td>3.0 (1.6–4.2)</td>
<td>2.3–3.9 g/dL</td>
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<tr>
<td>Globulin (g/dL)</td>
<td>3.6 (2.9–4.8)</td>
<td>3.3 (1.6–6.8)</td>
<td>3.6 (1.5–5.9)</td>
<td>3.4 (2.1–6.4)</td>
<td>3.0–5.6 g/dL</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.0 (0.0–0.2)</td>
<td>0.2 (0.0–41)</td>
<td>0.1 (0.0–5.3)</td>
<td>0.1 (0.0–31.8)</td>
<td>0.0–0.4 mg/dL</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>160 (67–272)</td>
<td>148.5 (19–564)</td>
<td>133 (65–255)</td>
<td>157 (1.4–262)</td>
<td>82–218 mg/dL</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>29 (17–105)</td>
<td>68 (17–1212)</td>
<td>45 (16–1186)</td>
<td>58 (21–779)</td>
<td>5–55 U/L</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>23.5 (7–68)</td>
<td>30.5 (3–842)</td>
<td>25 (0.0–345)</td>
<td>23 (0.0–518)</td>
<td>0–62 U/L</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>0.0 (0–1)</td>
<td>0.0 (0–23)</td>
<td>0.0 (0–5)</td>
<td>0.0 (0–271)</td>
<td>0–7 U/L</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>53 (33–146)</td>
<td>80.5 (27–1365)</td>
<td>77 (7–1257)</td>
<td>54 (20–970)</td>
<td>28–100 U/L</td>
</tr>
<tr>
<td>USG</td>
<td>1.063 (1.018–1.097)</td>
<td>1.030 (1.010–1.080)</td>
<td>1.030 (1.010–1.060)</td>
<td>1.030 (1.010–1.080)</td>
<td>1.010–1.060</td>
</tr>
<tr>
<td>fPLI (µg/L)*</td>
<td>1.40 (0.61–4.40)</td>
<td>1.19 (0.44–11.22)</td>
<td>3.35 (0.5–46)</td>
<td>2.30 (0.8–1200)</td>
<td>0.7–3.5 µg/L</td>
</tr>
</tbody>
</table>

fPLI = Feline pancreatic lipase immunoreactivity concentration as measured with the Spec fPL test. USG = Urine specific gravity.

*n = 41, 34, 48, and 59 for the groups in order across the columns.
but not for fluid accumulations, nodules, peripancreatic fluid accumulation, or enlarged peripancreatic lymph nodes were different between the pancreatitis and not pancreatitis groups but not between indeterminate pancreatitis and not pancreatitis groups. Pancreas parenchyma and peripancreas mesentery were isoechoic in all healthy cats and abnormal (hypoechoic, hypoechoic, and mixed; hypoechoic and hyperechoic) for cats in the pancreatitis (26, 0, and 4; 0 and 26, respectively), not pancreatitis (5, 3, and 1; 0 and 3, respectively), and indeterminate (18, 1, and 4; 0, 12, respectively) groups. The distribution of ultrasonographic lesions were none (no lesions were noted) in all the healthy cats and abnormal (focal, regional, multifocal, and diffuse) for cats in the pancreatitis (10, 14, 3, and 3, respectively), not pancreatitis (12, 2, 0, and 0, respectively), and indeterminate groups (16, 11, 2, and 5, respectively). The most common ultrasonographic pancreatic abnormalities detected in the pancreatitis group of cats were a hypoechoic pancreas (76%), hyperechoic peripancreas mesentery (76%), pancreatomegaly (74%, 44% moderate) with an irregular contour (82%, 46% mild), and regionally distributed (47%). No concurrent disorders were noted in the healthy cats. Numerous concurrent disorders were noted in the clinical cats, including acute and chronic kidney disease, hepatic lipidosis, diabetes mellitus or diabetic ketoacidosis, hyperthyroidism, probable or confirmed cancer, and inflammatory bowel disease (Table 2). Diagnoses of ill cats with Spec fPL test measurements fPLI concentrations within the reference range in the present study included cholangiohepatitis, hepatic lipidosis, trichobezoar, colonic mass, feline infectious peritonitis, bartonellosis, chronic kidney disease, interstitial cystitis, and chronic rhinitis. Screening for primary liver, intestinal, or thyroid disease was performed at the discretion of the attending clinician and with the consent of the owner and was not performed in all cats; therefore, it is likely concurrent disorders are underestimated.

The Spec fPL results correlated highly with radioimmunoassay (Texas A&M University) measurements of fPLI concentration in 786 stored sera samples (Figure 2). Spec fPL assay precision values are shown (Table 4). Using the Spec fPL concentration of 5.4 µg/L as the diagnostic cutoff and excluding cats with indeterminate pancreatitis, the sensitivity and specificity (and their respective 95% CIs) were 79.4% (63.2% to 89.7%) and 79.7% (67.7% to 88.0%). The likelihood of a positive test was 3.9 and of a negative test was 0.3. Considering all 182 cats in the study and grouping PoP with the pancreatitis group and PoNP with the not pancreatitis group, the sensitivity and specificity (and their respective 95% CIs) were 65.1% (52.8% to 75.7%) and 82.1% (72.1% to 89.0%). The likelihood of a positive test was 3.6 and of a negative test was 0.4, and positive and negative predictive values were 69% and 87%, respectively. The ability of the test to correctly identify a cat without pancreatitis (negative predictive value) was high (87%), with a lower ability to
and cats with clinical signs that could be attributable to pancreatitis were evaluated to determine the diagnostic cutoff for serum fPLI concentration that maximized the test’s sensitivity and specificity. Signalment, clinical signs, physical examination findings, biochemical and hematologic abnormalities, and concurrent disorders were similar to those in previous studies of cats with pancreatitis. 

The cutoff that maximized the diagnostic accuracy of the measurement of serum fPLI concentration as measured by Spec fPL was determined to be \( \geq 5.4 \) µg/L, and using this cutoff, the Spec fPL assay was 79.4% sensitive for pancreatitis when results were below 5.4 µg/L and 79.7% specific in cats whose Spec fPL concentration was \( \geq 5.4 \) µg/L. It is important to note that if the Spec fPL concentration was within the normal reference interval in ill cats, the sensitivity of the Spec fPL concentration for excluding pancreatitis increased to 85.3%, but the specificity decreased to 64.4% for diagnosing pancreatitis in cats whose Spec fPL concentration was above normal. The ability of the test to correctly identify a cat without pancreatitis (negative predictive value) was high (87%), with a lower ability to correctly identify a cat with pancreatitis, a positive predictive value (69%).

**Discussion**

In this study, the reference interval for serum fPLI concentration as measured by the Spec fPL test was determined to be 0.7 to 3.5 µg/L, based on measurements in a group of healthy cats with clinically normal routine laboratory results and clinically normal results on abdominal ultrasonography. A group of ill cats with clinical signs that could be attributable to pancreatitis were evaluated to determine the diagnostic cutoff for serum fPLI concentration that maximized the test’s sensitivity and specificity. Signalment, clinical signs, physical examination findings, biochemical and hematologic abnormalities, and concurrent disorders were similar to those in previous studies of cats with pancreatitis.

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In a previous study, the specificity for fPLI was determined to be 100% in 8 cats with a histopathologically normal pancreas but only 67% in 3 cats with clinical signs of pancreatitis yet a normal pancreas on histopathology. The overall specificity was 91%. By comparison, the specificity of the Spec fPL test in the current study was 79.7% based on 59 cats with clinical signs compatible with pancreatitis that were clinically categorized as not pancreatitis (DNP and PrNP) and was 82.1% when considering the pancreatitis and PoNP groups. In the current study, the normal healthy control cats (n = 41) were not included in the specificity calculations since this population was utilized to determine the reference interval. Discrepancies between results of the current and prior studies may also be partly explained by the small sample size in the prior study (n = 12). In a prior study evaluating feline PLI concentration, the sensitivity was determined to be higher for cats with histopathologically graded severe pancreatitis (100%) versus cats with mild pancreatitis (54%) with an overall sensitivity of 67%. By comparison, in the current study, the sensitivity was 79.4% for all cats with pancreatitis (DP and PrP) and was 65.1% when considering the pancreatitis and PoP subgroups. Using primarily clinically defined groups in the current study did not permit subclassification of mild or severe pancreatitis.

Wide and conflicting sensitivities for the diagnosis of pancreatitis by abdominal ultrasonography have been reported, ranging between 20% to 35% and 62%, with a specificity of 73% based on limited numbers of clinical cats without pancreatitis. Advances in ultrasonographic equipment, ultrasonographer training, and study methodology may have contributed to a wide range of sensitivities. In the current study, efforts were made to maximize the accuracy of the ultrasonographic diagnosis by utilizing advanced ultrasonography equipment, utilizing a limited number of board-certified radiologists with an interest in feline ultrasonography, incorporating advances in knowledge of normal ultrasonographic aging changes in the feline pancreas into the grading scheme, and utilizing an initial clinical ultrasonography report and follow-up graded ultrasonography criteria. The goal of this study was not to determine the sensitivity and specificity of abdominal ultrasonography for the diagnosis of pancreatitis but to utilize ultrasonography, in addition to other parameters, to classify the cats in the study. Similar abnormalities were detected in cats with and without pancreatitis. It is important to note that during the classification stage, the 2 board-certified internists made an effort to classify cats on the basis of all available criteria and not solely on the ultrasonographic diagnosis. As reported in a previous study, despite no ultrasonographic abnormalities, 1 cat was classified in the pancreatitis group on the basis of clinical signs and histopathology.

Limitations to this study included the substantial age difference between healthy control cats and clinical cats and reliance on a clinical identification...
and classification scheme for the diagnosis of pancreatitis rather than pancreatic histopathology. Despite efforts to obtain an older population for the healthy control cats, a significant age difference existed between the clinical, ranging from 0.6 to 18 years (median, 11 years), and healthy control cats, ranging from 1 to 13 years (median, 5 years). The rigid selection criteria for the healthy control cats to have no current or historical gastrointestinal clinical signs potentially eliminated cats that would have been appropriate for the healthy control population. The ability to definitively include and exclude pancreatitis by clinical evaluation in cats, dogs, and humans is challenging.\textsuperscript{14,45,46} Pancreatic histopathology or cytology was only obtained in 5 (4%) and 17 (12%) cats, respectively, of the clinical cats and none of the healthy control cats in this study. Although this is to be expected in a clinical study, it would have been ideal to have obtained pancreatic histopathology (from multiple biopsy sites) in all cats. Histopathology, despite being the gold standard for many disorders,\textsuperscript{47} has limitations in regard to the diagnosis of pancreatitis. Pancreatitis can be multifocal and locally severe, and therefore pancreatic inflammation can be missed without serial sections of the pancreas,\textsuperscript{48} which is not possible in clinical cases. Additionally, the degree and type of pancreatic inflammation that correlates with clinical signs of pancreatitis have not been definitively established.\textsuperscript{9} Similarly, the clinical significance of foci of inflammation in the pancreata, especially in aged cats, needs to be established. In this study, we did not attempt to classify cats as acute (neutrophilic/necrotizing) or chronic (lymphocytic/plasmacytic) pancreatitis, either clinically or when histopathology/cytology was available. It is possible this parameter influences the diagnostic parameters of the Spec fPL.\textsuperscript{4} It is unknown whether the presence of pancreatic carcinoma changes serum fPLI concentrations independent of the presence of inflammation. Intestinal and hepatic inflammation commonly occurs concurrently with pancreatitis\textsuperscript{5,9,14,48} and can cause similar clinical, physical examination, and biochemical and hematologic abnormalities and confuse/confound the diagnosis of pancreatitis. We cannot comment on the presence or absence of concurrent gastrointestinal or hepatic inflammation in most of the clinically ill cats in this study.

Given the challenges in making a clinical diagnosis of pancreatitis in cats, we proposed an a priori likelihood classification scheme of clinically defined groups incorporating the clinical signs, laboratory evidence, ultrasonographic imaging, cytology, and/or histopathology. For the purpose of this study, the measurement of serum fPLI concentration was not included in the laboratory evidence portion. However, it would be appropriate for future studies to include this parameter in the classification scheme. Likelihood classification schemes have been utilized in other difficult-to-diagnose disorders, including feline infectious peritonitis and hypertension.\textsuperscript{39,50} Our hope was that the classification scheme would identify cats with clinically significant pancreatitis from those without pancreatitis.

Feline pancreatitis was historically considered rare\textsuperscript{14}; however, with the developments and advances of noninvasive techniques to diagnose pancreatitis, including serologic testing and pancreatic imaging, it is now recognized as more common than previously believed. Considering this, it is possible that feline pancreatitis is underdiagnosed rather than overdiagnosed, which would result in a higher specificity.

In summary, the high sensitivity for the measurement of serum fPLI concentration, as measured by the Spec fPL suggests it is an appropriate screening test for cats with clinical signs compatible with pancreatitis. Additionally, the high specificity for Spec fPL suggests it is an appropriate test to diagnose pancreatitis when results are being integrated with other clinical findings, such as abdominal ultrasonography and other additional diagnostic testing to exclude other causes of similar clinical signs. Additional clinical studies, ideally with concurrent pancreatic histopathology, are indicated to confirm the results of this study. Although the majority of cats diagnosed with pancreatitis do not have an identified underlying condition,\textsuperscript{2} cats in the present study were determined to have pancreatic carcinoma, likely causing pancreatitis. Increased screening for this etiology of pancreatitis should be considered.

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**Disclosures**

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**References**

5. Pratschke KM, Ryan J, McAlinden A, McLauchlan G. Pancreatic surgical biopsy in 24 dogs and 19 cats: postoperative complications and clinical relevance of histo-

**Supplementary Materials**

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