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Dyspnea secondary to pleural effusion is a common presenting complaint in cats, and can be the result of a variety of disease processes, including neoplasia, cardiac disease, pyothorax, and feline infectious peritonitis. Differentiation of the underlying causes can be challenging in dyspneic cats. These cats might require supplementary oxygen and be too unstable to tolerate extensive diagnostic testing. Measurement of NT-proBNP using a first-generation feline-specific quantitative ELISA in plasma and pleural fluid differentiates cardiac from noncardiac causes of pleural effusion.

**Background:** Pleural effusion is a common cause of dyspnea in cats. N-terminal pro-B-type natriuretic peptide (NT-proBNP) measurement, using a first-generation quantitative ELISA, in plasma and pleural fluid differentiates cardiac from noncardiac causes of pleural effusion.

**Hypothesis/Objectives:** To determine whether NT-proBNP measurements using second-generation quantitative ELISA and point-of-care (POC) tests in plasma and pleural fluid distinguish cardiac from noncardiac pleural effusions and how results compare to the first-generation ELISA.

**Animals:** Thirty-eight cats (US cohort) and 40 cats (UK cohort) presenting with cardiogenic or noncardiogenic pleural effusion.

**Methods:** Prospective cohort study. Twenty-one and 17 cats in the US cohort, and 22 and 18 cats in the UK cohort were classified as having cardiac or noncardiac pleural effusion, respectively. NT-proBNP concentrations in paired plasma and pleural fluid samples were measured using second-generation ELISA and POC assays.

**Results:** The second-generation ELISA differentiated cardiac from noncardiac pleural effusion with good diagnostic accuracy (plasma: sensitivity, 95.2%, specificity, 82.4%; pleural fluid: sensitivity, 100%, specificity, 76.5%). NT-proBNP concentrations were greater in pleural fluid (719 pmol/L (134–1500)) than plasma (678 pmol/L (61–1500), P = 0.003), resulting in different cut-off values depending on the sample type. The POC test had good sensitivity (95.2%) and specificity (87.5%) when using plasma samples. In pleural fluid samples, the POC test had good sensitivity (100%) but low specificity (64.7%). Diagnostic accuracy was similar between first- and second-generation ELISA assays.

**Conclusions and clinical importance:** Measurement of NT-proBNP using a quantitative ELISA in plasma and pleural fluid or POC test in plasma, but not pleural fluid, distinguishes cardiac from noncardiac causes of pleural effusion in cats.

**Key words:** Biomarker; Blood testing; Cardiomyopathy; Dyspnea; Natriuretic peptide.
The aims of this study were to determine whether measurement of NT-proBNP in plasma or pleural fluid samples using either the second-generation quantitative ELISA or POC assay could distinguish cardiac from noncardiac causes of pleural effusion, and how these results compared to the results of a previous study using the first-generation quantitative ELISA assay.

Materials and Methods

The study was approved by the respective institutional animal use and care committees and informed owner consent was obtained. Thirty-eight cats presenting to one of two US teaching hospitals (Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, Philadelphia PA and the Foster Hospital for Small Animals, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA) with pleural effusion undergoing thoracocentesis were prospectively recruited (US cohort). Forty cats presenting to a UK teaching hospital (Queen Mother Hospital for Animals, Royal Veterinary College, North Mymms, Hatfield) with pleural effusion undergoing thoracocentesis were prospectively recruited, as previously described (UK cohort). For both cohorts, cats were recruited and the cause of the pleural effusion was determined to be either cardiac or noncardiac by a board-certified cardiologist on the basis of the echocardiogram, history, physical examination, and results of any additional diagnostic tests performed. The cardiologist was blinded to all quantitative and POC measurements of NT-proBNP.

Pleural fluid samples were obtained at the time of therapeutic or diagnostic thoracocentesis. Plasma samples were collected when venipuncture for diagnostic sampling was performed during the same hospital visit and as close to the time of therapeutic thoracocentesis as was clinically practicable on the basis of feline stability. One milliliter samples of pleural fluid and 2 milliliter samples of blood were collected in K2-EDTA treated tubes and were centrifuged at 3000 × g for 5 minutes within 15 minutes of collection. For the US cohort, NT-proBNP was measured in plasma using the POC assay within 30 minutes of centrifugation and the remaining plasma and pleural fluid samples were each transferred to a cryotube and frozen at −80°C for batched analysis. NT-proBNP was measured at the reference laboratory in plasma and pleural fluid samples using the second-generation ELISA and in pleural fluid samples using the POC assay. For the UK cohort, which was originally recruited for assessment of the first-generation NT-proBNP ELISA assay, NT-proBNP was measured at the reference laboratory in plasma and pleural fluid samples using the second-generation ELISA and POC assay in samples collected in a protease inhibitor tube and frozen at −80°C for batched analysis.

The second-generation NT-proBNP in cat assays incorporate a set of antibodies different from the first-generation assay that attempt to target stable epitopes of NT-proBNP in cats. The lower and upper limits of detection of the reference laboratory assay are 24 pmol/L and 1500 pmol/L, respectively. Values of NT-proBNP less than the lower limit of detection were assigned values of 24 pmol/L. Values of NT-proBNP greater than the upper limit of detection were assigned values of 1500 pmol/L. All reference laboratory samples were assayed in duplicate and the mean of the two values used.

The POC test is a bidirectional flow ELISA that uses the same pair of antibodies for the detection of NT-proBNP in cats as the second-generation reference laboratory test, and its use in detecting occult cardiomyopathy has been previously described. Briefly, to perform the POC assay, 3 drops of plasma or pleural fluid were mixed with 5 drops of assay conjugate and then added to the sample well of the test device. The conjugate-diluted sample was allowed to flow across the device. Once the sample reached the indicator window, the device was activated by the operator. This initiated the wash and color development steps of the assay. The relative color densities of the sample and reference spots were evaluated visually after 10 minutes of incubation. Positive results were recorded when the density of the sample spot appeared equal to or greater than that of the reference spot. Negative results were recorded when the density of the sample spot appeared less than that of the reference spot. The transition from negative to positive on the POC test occurs in an NT-proBNP range of 150–200 pmol/L.

Statistical Analysis

Statistical analysis was performed using commercially available software. Data were examined graphically for normality of distribution. Group-wise comparisons were performed using Mann–Whitney tests, Wilcoxon signed-rank tests, or Fisher’s exact test, as appropriate. Receiver operator characteristic (ROC) curves were constructed to derive cut-offs for differentiation of cardiac from noncardiac causes of pleural effusion in plasma and pleural fluid samples based on results from the US cohort. Sensitivity, specificity, and positive and negative likelihood ratios were calculated. The positive likelihood ratio is the ratio of true positives to false positives and the negative likelihood ratio is the ratio of false negatives to true negatives. A positive likelihood ratio <5 is considered a reasonable diagnostic test for ruling in a condition and a negative likelihood ratio less than 0.2 is considered a reasonable diagnostic test for ruling out a condition. By prespecified design, the cut-off values generated from the US cohort were subsequently tested in the UK cohort. Spearman’s rank order correlation coefficients (rho) and Bland–Altman plots were used to compare quantitative ELISA measurements of NT-proBNP between plasma and pleural fluid samples and the first- and second-generation assays.

Results

Thirty-eight cats with pleural effusion were enrolled in the US cohort between February and November 2014. Twenty-one cats were assigned to the cardiac group and 17 cats were assigned to the noncardiac group. The population characteristics of the US cohort are presented in Table 1. No differences were detected between groups for age, breed, body weight, heart rate, respiratory rate, or diastolic measurements of interventricular septum and left ventricular free wall thickness (IVSd and LVFWd, respectively). Cats in the cardiac group had significantly greater left atrial to aortic root ratios (LA/Ao), and were significantly more likely to be
Twenty-two cats were assigned to the cardiac and noncardiac groups using 2 assay methods for continuous variables. NB sex was not recorded for one cat in the cardiac group. ROC curve analysis in the US cohort revealed an optimal plasma NT-proBNP cut-off of ≥199 pmol/L with a sensitivity of 95.2% (95% CI 77.3–99.2%), specificity of 82.4% (95% CI 59.0–93.8%), positive likelihood ratio of 5.40 (95% CI 1.92–15.14), and negative likelihood ratio of 0.06 (95% CI 0.01–0.40). The area under the ROC curve was 0.952 (95% CI 0.891–1.00). A pleural fluid NT-proBNP cut-off of ≥240 pmol/L yielded a sensitivity of 100% (95% CI 84.5–100%), specificity of 76.5% (95% CI 52.7–90.4), and positive likelihood ratio of 4.25 (95% CI 1.80–10.01). The negative likelihood ratio could not be calculated because all 21 cats with pleural effusion of cardiac origin had pleural fluid NT-proBNP ≥240 pmol/L. The area under the pleural fluid ROC curve was 0.923 (95% CI 0.832–1.00). There was no significant difference between the area under the pleural fluid ROC curve and the area under the plasma ROC curve (P = 0.26).

In the US cohort, the POC test using plasma samples differentiated the cardiac and noncardiac groups with a sensitivity of 95.2% (95% CI 77.3–99.2%), specificity of 87.5% (95% CI 64.0–96.5%), positive likelihood ratio of 7.62 (95% CI 2.08–27.95), and negative likelihood ratio of 0.05 (95% CI 0.01–0.37). The area under the ROC curve for the POC assay using plasma samples was 0.914 (95% CI 0.818–1.00). The POC test using pleural fluid samples differentiated the cardiac and noncardiac groups with a sensitivity of 100% (95% CI 84.5–100%), specificity of 64.7% (95% CI 41.3–82.7%), and positive likelihood ratio of 2.83 (95% CI 1.49–5.39) in the US cohort. The negative likelihood ratio could not be calculated because all 21 cats with pleural effusion of cardiac origin had a positive POC assay. The area under the ROC curve for the POC assay using pleural fluid samples was 0.824 (95% CI 0.706–0.941) and was not statistically different from the area under the ROC curve for plasma samples (P = 0.086).

Results of NT-proBNP measurements in the UK cohort using the second-generation reference laboratory ELISA and the POC test are presented in Table 3. Insufficient plasma sample volume was available to measure NT-proBNP using the second-generation version of the ELISA in 3 cats and using the POC test in 6 cats. When the cut-off values derived from the US

Table 2. Results of NT-proBNP measurements for the cardiac and noncardiac groups using 2 assay methods for the US cohort. The median and interquartile ranges are shown for continuous variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Noncardiac (n = 17)</th>
<th>Cardiac (n = 21)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>12.0 (8.5–16.0)</td>
<td>12.0 (1.5–18.0)</td>
<td>0.86</td>
</tr>
<tr>
<td>Pedigree breed (yes/no)</td>
<td>2/15</td>
<td>1/20</td>
<td>0.58</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>6/11</td>
<td>16/4</td>
<td>0.008</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.1 (2.1–8.8)</td>
<td>4.9 (2.7–6.9)</td>
<td>0.74</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>200 (160–260)</td>
<td>180 (130–300)</td>
<td>0.27</td>
</tr>
<tr>
<td>Respiratory rate (breaths per minute)</td>
<td>68 (44–92)</td>
<td>60 (30–88)</td>
<td>0.17</td>
</tr>
<tr>
<td>Murmur (yes/no)</td>
<td>5/12</td>
<td>15/6</td>
<td>0.021</td>
</tr>
<tr>
<td>Gallop (yes/no)</td>
<td>1/16</td>
<td>13/8</td>
<td>0.001</td>
</tr>
<tr>
<td>LA/Ao</td>
<td>1.3 (1.0–1.9)</td>
<td>2.6 (1.6–3.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>3.5 (3.5–7.5)</td>
<td>6.2 (3.7–10.0)</td>
<td>0.37</td>
</tr>
<tr>
<td>LVFWd (mm)</td>
<td>5.8 (2.9–11.0)</td>
<td>7.2 (3.8–18.6)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Table 1.** Characteristics of the cardiac and noncardiac groups in the US cohort. The median and ranges are shown for continuous variables. NB sex was not recorded for one cat in the cardiac group.
cohort were applied to plasma and pleural fluid measurements of NT-proBNP using the second-generation assay from the UK cohort, plasma NT-proBNP ≥199 pmol/L yielded a sensitivity of 95.0% (95% CI 76.4–99.1%), specificity of 82.4% (95% CI 59.0–93.8%), positive likelihood ratio of 5.40 (95% CI 1.92–15.11), and negative likelihood ratio of 0.06 (95% CI 0.01–0.42). Pleural fluid NT-proBNP ≥240 pmol/L yielded a sensitivity of 100% (95% CI 85.1–100%), specificity of 66.7% (95% CI 43.8–83.7%), and positive likelihood ratio of 3.00 (95% CI 1.56–5.77). The negative likelihood ratio could not be calculated because all 22 cats with pleural effusion of cardiac origin had pleural fluid NT-proBNP ≥240 pmol/L. For both plasma and pleural fluid samples, the 95% CI of the sensitivity and specificity of the second-generation ELISA assay overlapped with reported confidence intervals using the first-generation ELISA assay.4 In the UK cohort, the POC test using plasma samples differentiated cardiac from noncardiac causes of pleural effusion with a sensitivity of 88.2% (95% CI 65.7–96.7%), specificity of 88.2% (95% CI 65.7–96.7%), positive likelihood ratio of 7.50 (95% CI 2.02–27.89), and negative likelihood ratio of 0.13 (95% CI 0.04–0.50). The POC test using pleural fluid samples differentiated cardiac from noncardiac causes of pleural effusion with a sensitivity of 100% (95% CI 85.1–100%), specificity of 77.8% (95% CI 54.8–91.0%), and positive likelihood ratio of 4.50 (95% CI 1.89–10.68). The negative likelihood ratio could not be calculated because all 22 cats with pleural effusion of cardiac origin had a positive POC assay.

NT-proBNP concentrations in paired plasma and pleural fluid samples from the US cohort were measured using the second-generation ELISA assay, and were significantly correlated (Spearman rho = 0.969; P < 0.0001); however, the limits of agreement between the two assay generations were wide, and NT-proBNP concentrations in plasma assayed using the second-generation assay were systematically greater than those in plasma samples assayed using the first-generation assay (plasma second-generation, 531 pmol/L [84–874] versus plasma first-generation, 254 pmol/L [50–620]; P = 0.002), with a mean bias of +156.1 pmol/L (Fig 2). NT-proBNP concentrations in pleural fluid samples from the UK cohort using the first- and second-generation ELISA assay were significantly correlated (Spearman rho = 0.966; P < 0.0001). Pleural fluid NT-proBNP concentrations were not significantly different between the two different generations of NT-proBNP ELISA assay (pleural second generation, 603 pmol/L [121–135] versus pleural first generation 542 pmol/L [142–1374]; P = 0.95).

Table 3. Results of NT-proBNP measurements for the cardiac and noncardiac groups using 2 assay methods for the UK cohort. The median and interquartile ranges are shown for continuous variables.

<table>
<thead>
<tr>
<th></th>
<th>Cardiac (n = 22)</th>
<th>Noncardiac (n = 18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Second-generation ELISA (pmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>849 (590–1500)</td>
<td>73 (33–138)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>1001 (640–1500)</td>
<td>98 (45–273)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Point-of-care test (no. positive/no. negative)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>15/2</td>
<td>2/15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>22/0</td>
<td>4/14</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Discussion

This study utilized data from two separate cohorts to investigate the diagnostic characteristics of a quantitative ELISA and semiquantitative POC test to differentiate cardiac and noncardiac causes of pleural effusion in cats. Our results demonstrate that measurement of NT-proBNP in plasma samples using either a quantitative ELISA or POC test differentiates these etiologies of pleural effusion with good diagnostic accuracy. Measurement of NT-proBNP concentration in pleural fluid samples using the quantitative ELISA also had good diagnostic accuracy as long as a higher cut-off value is used for pleural fluid samples (≥240 pmol/L) compared to the cut-off value used for plasma samples (≥199 pmol/L). In the emergency setting, POC testing of either plasma or pleural fluid is attractive because of the immediacy of assay results. Testing of pleural effusion, specifically, is attractive in instances in which cats with significant pleural effusion would undergo therapeutic thoracocentesis but might not be stable enough for venipuncture. However, the results of this study suggest that the diagnostic value of POC testing of pleural effusion samples is limited. Using pleural fluid samples, a negative POC assay result helped to rule out cardiac causes of pleural effusion, but a positive result was associated with a relatively low positive predictive value, and the likelihood ratio associated with a positive pleural fluid POC test from either the US or UK cohort (i.e., 2.83 and 4.5) was lower than the level (i.e. 5.0) generally considered to indicate a useful diagnostic test.7 Thus, a positive POC result using pleural fluid samples frequently represents a false positive result, and this limits its diagnostic value. The discrepancy in performance between testing plasma and pleural fluid samples using the POC test probably reflects the NT-proBNP concentration at which the POC device transitions from a negative to positive result. A previous study has shown this to occur at a NT-proBNP concentration between 150 and 200 pmol/L,6 which approximates the cut-off value in plasma (199 pmol/L) but is lower than the optimal cut-off for pleural fluid samples (240 pmol/L) found in this study.
Likelihood ratios can be used to estimate post-test probability of pleural effusion of cardiac cause. In this study, plasma POC test results were associated with approximate positive and negative likelihood ratios of 7.50 and 0.10, respectively. These values roughly translate into an absolute increase or decrease in the post-test probability for a cardiac cause of pleural effusion of 40% compared to the pretest probability. For example, in a population of cats whose pretest probability of pleural effusion because of a cardiac cause is

**Fig 1.** Bland–Altman plot illustrating agreement between measurements of NT-proBNP concentrations in pleural fluid and plasma samples in cats using the second-generation assay in samples from the US cohort. The mean bias and the dash-dot lines representing the 95% confidence intervals for the bias indicate that the NT-proBNP concentration of pleural fluid samples was on average 67.9 pmol/L greater than NT-proBNP concentration from paired plasma sample. The 95% limits of agreement between the two sample types are displayed as ±1.96 times the standard deviation of the difference.

**Fig 2.** Bland–Altman plot illustrating agreement between measurements of NT-proBNP concentrations in plasma samples from cats using the first- and second-generation assay in samples from the UK cohort. The mean bias and the dash-dot lines representing the 95% confidence intervals for the bias indicate that the NT-proBNP concentration of plasma samples using the second-generation assay was on average 156.1 pmol/L greater than NT-proBNP concentration from paired plasma sample using the first generation assay. The 95% limits of agreement between the two sample types are displayed as ±1.96 times the standard deviation of the difference.
The first-generation assay, but are in contrast to
pared pleural fluid and plasma samples in cats using
These findings agree with a previous study that com-
two different cut-off values depending on sample type.
measurements of NT-proBNP were found to be higher
using the first-generation ELISA. Given that plasma
measurements of NT-proBNP in Pleural Fluid 541
This study revealed that NT-proBNP concentrations
were significantly higher in pleural fluid samples than in plasma samples when measured using the quantita-
tive second-generation ELISA, resulting in the need for
two different cut-off values depending on sample type.
These findings agree with a previous study that com-
pared pleural fluid and plasma samples in cats using
the first-generation assay, but are in contrast to human patients, in whom pleural fluid and plasma NT-
proBNP concentrations are reported to be almost identi-
cal. The potential reasons for this discrepancy be-
 tween species are not immediately clear. In this study,
plasma NT-proBNP concentrations were signifi-
cantly higher using the second-generation versus the
first-generation assay, whereas in contrast NT-proBNP
concentrations in pleural fluid samples were not signifi-
cantly different between assay generations. One possible explanation is that the rate of postsample degradation is lower in pleural fluid than in plasma samples, per-
haps because of lower concentrations of protease enzymes. Further studies are necessary to investigate
this possibility.

This study has a number of important limitations. While attempts were made to collect plasma and pleu-
ral fluid samples at the same time, feline instability might have caused pleural fluid and blood samples to
be obtained hours apart from each other. Cats were assigned to the cardiac or noncardiac groups based on
the opinion of the blinded cardiologist, and misclassifi-
cation of cases could have occurred. This study involved use of different sample collection, handling
and storage protocols; however, samples collected for ELISA from the US cohort were handled according to
current manufacturer recommendations and results between the US and UK cohorts were comparable.
The pleural fluid POC tests from both the US and UK cohorts were performed on batched samples which had
been stored at -80°C, and further studies that utilize the POC test in pleural fluid samples in a truly pet-side
manner, including comparison of assay performance
when used immediately, compared with on previously
frozen samples, should be performed. Pleural fluid sample POC testing was not performed immediately in
this study because the POC device had not been vali-
dated for use with pleural fluid samples at the time of
sample collection. One disadvantage of using the POC
device is that the result is either positive or negative;
this is in contrast to the quantitative ELISA, which
allows for interpretation of the results throughout a
range of possible values. The POC device is not specifi-
cally designed to differentiate causes of pleural effusion
in cats, but rather to detect occult cardiomypathy in
cats without obvious respiratory signs, and the device’s
cut-off value between a negative and positive result is
relatively low for differentiation of cardiac versus non-
cardiac etiologies of pleural effusion. Nevertheless, the
POC assay when used with plasma was able to differ-
entiate cause of pleural effusion with good diagnostic
accuracy.

In conclusion, the results of this study suggest that
quantitative measurements of NT-proBNP in plasma
and pleural fluid samples using a second-generation
assay allow differentiation of cardiac from noncardiac
causes of pleural effusion with good diagnostic accu-
racy. Cut-off values specific for sample type should be
used. Measurement of NT-proBNP in plasma samples
using a POC test also allows differentiation of cardiac
from noncardiac causes of pleural effusion with good
diagnostic accuracy; however, although sensitivity is
excellent and a negative POC result in pleural fluid samples helps rule out a cardiac cause of pleural effu-
sion, the specificity of the POC test is low, resulting in
a low positive predictive value. Measurements of NT-
proBNP in plasma fluid samples obtained using the
second-generation assay are systematically greater than
those obtained using the first-generation assay, and cut-
offs specific to the second-generation assay should be
used.

Acknowledgments

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Conflict of Interest Declaration: Dr. Hezzell’s residency
is partly funded by IDEXX Laboratories.
Off-label Antimicrobial Declaration: Authors declare
no off-label use of antimicrobials.
Footnotes

a Cardiopet® Feline proBNP, IDEXX Laboratories Inc., Westbrook, ME
b SNAP® Feline proBNP, IDEXX Laboratories Inc., Westbrook, ME
c Cardiopet® proBNP specimen tubes, IDEXX Laboratories Inc., Westbrook, ME
e SPSS 22; IBM, Armonk NY; MedCalc 15.2.2; MedCalc Software, Ostend, Belgium

References