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Differentiating between feline pleural effusions of cardiac and non-cardiac origin using pleural fluid NT-proBNP concentrations

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Objective: To assess whether pleural fluid and urine amino terminal proB-type natriuretic peptide (NT-proBNP) can distinguish cardiac from non-cardiac causes of pleural effusion.

Methods: Blood, urine and pleural fluid were prospectively collected from cats presenting with pleural effusion categorised as cardiac or non-cardiac in origin. NT-ProBNP concentrations were measured using a feline-specific enzyme-linked immunosorbent assay. Groups were statistically compared and receiver operating characteristic curves constructed to determine cut-offs to distinguish cardiac from non-cardiac pleural effusion in plasma, pleural fluid and urine.

Results: Forty cats with pleural effusion (22 cardiac and 18 non-cardiac) were studied. NT-proBNP concentrations in plasma and pleural fluid were strongly correlated. Plasma (P<0·001) and pleural fluid (P<0·001) NT-proBNP concentrations and urinary NT-proBNT/creatinine ratios (P=0·035) were significantly higher in the cardiac group. After receiver operating characteristic curve analysis a plasma NT-proBNP cut-off of 214·3 pmol/mL was suggested [sensitivity=86·4% (95% CI: 66·7 to 95·3%), specificity=88·9% (95% CI: 67·2 to 96·9%)] and a pleural fluid NT-proBNP cut-off of 322·3 pmol/mL was suggested [sensitivity=100% (95% CI: 85·1 to 100%), specificity=94·4% (95% CI: 74·2 to 99·0%)]. No cut-off with adequate sensitivity and specificity for urinary NT-proBNP/creatinine ratios was suggested.

Clinical Significance: Measurement of NT-proBNP in pleural fluid distinguishes cardiac from non-cardiac causes of pleural effusion in cats.

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INTRODUCTION

Cats frequently present to veterinary practitioners with dyspnoea secondary to pleural effusion. Disease processes causing pleural fluid accumulation include neoplasia, cardiac disease, pyothorax and feline infectious peritonitis (Waddell & King 2007). Various tests are available to aid the clinician in elucidating the underlying cause, including cytological examination of the pleural fluid, fluid culture and echocardiography (Beatty & Barrs 2010). In humans, the measurement of amino terminal proB-type natriuretic peptide (NT-proBNP) in pleural fluid has been found to be useful in distinguishing between cardiogenic and non-cardiogenic causes of pleural effusion (Porcel *et al.* 2004; Kolditz *et al.* 2006; Janda & Swiston 2010). NT-proBNP has also been measured in the urine of human patients with heart failure and has been found to correlate well with plasma concentrations (Cortés *et al.* 2007). Serum or plasma measurements of NT-proBNP have been shown to be beneficial in aiding identification of cardiac

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disease in cats (Connolly *et al.* 2009; Fox *et al.* 2009) and this has led to the development of a commercially available test for feline NT-proBNP (Vetsign Feline Cardiopet proBNP; IDEXX Laboratories).

Performing venepuncture on a cat in respiratory distress due to pleural fluid can be challenging and the stress it causes can be deleterious. As therapeutic thoracocentesis is mandatory in such cases and the collection of voided urine involves no contact with the animal, the value of NT-proBNP measurement in these fluids over serum is clear if they were to be diagnostic of cardiac disease.

The aims of this study were to determine whether NT-proBNP was detectable in the pleural fluid and urine of cats presenting with pleural effusion arising from a variety of causes, to assess whether pleural fluid and urine NT-proBNP correlate with plasma NT-proBNP and finally to determine whether pleural fluid and urine NT-proBNP concentrations could distinguish cardiac from non-cardiac causes of pleural effusion. The null hypothesis was that pleural and urinary NT-proBNP would not distinguish cardiac from non-cardiac causes of pleural effusion.

MATERIALS AND METHODS

The study was approved by the institutional ethics and welfare committee of the Royal Veterinary College (RVC) and informed owner consent was obtained. Cats with pleural effusion requiring thoracocentesis consecutively presenting to the RVC as first opinion emergencies or as referral cases were prospectively recruited to the study. Forty cats were recruited as this number was deemed to be possible within a 2-year period given the historical frequency of cats presenting with pleural fluid to the hospital. The signalment of the cats and their final diagnoses were recorded. Body condition score and systolic blood pressure were also recorded. All cats received a thorough physical examination and appropriate diagnostic tests, including echocardiography (interpreted by a board-certified veterinary cardiologist), in order to determine the cause of the pleural effusion. Cats were placed in one of two groups, those having a cardiac cause and those having a non-cardiac cause of pleural effusion. Cats were classified as having a cardiac cause of their pleural effusion by the attending cardiologist after full analysis of their echocardiogram, history, physical examination and results of any other diagnostic tests performed.

Pleural fluid samples were obtained at the time of therapeutic or diagnostic thoracocentesis. Plasma samples were obtained when venepuncture was required for diagnostic samples. Urine samples were obtained either when diagnostic cystocentesis was performed or a free catch sample was obtained from the litter tray (filled with plastic beads) as soon as micturition was noted. One millilitre samples of pleural fluid and urine were collected and centrifuged at 3000 *g* for 5 minutes within 15 minutes of collection. The supernatant was transferred into commercially available tubes containing a proprietary protease inhibitor (PI tube) (Cardiopet proBNP specimen tubes; IDEXX Laboratories). Two millilitre samples of blood were collected into K3-EDTA treated tubes. Within 15 minutes of collection the samples were centrifuged at 3000 *g* for 5 minutes, separated, and 1 mL aliquots of plasma were transferred to PI tubes. Protease inhibited samples of pleural fluid, urine and plasma were stored at –80°C for batched analysis.

Samples were allowed to thaw at room temperature and plasma NT-proBNP (pmol/L) was measured within 1 hour of thawing using a commercially available enzyme-linked immunosorbent assay (ELISA) (Vetsign Feline Cardiopet proBNP; IDEXX Laboratories) previously validated for use with feline plasma (Connolly *et al.* 2008). The kit was used precisely according to the manufacturer's instructions. The kit incorporates two immunoaffinity-purified sheep antibodies specific for feline NT-proBNP. The plate consists of the capture antibody anti-NT-proBNP (1 to 20) bound to the wells of the plate. The tracer comprises the detection antibody, anti-NT-proBNP (60 to 80) conjugated to horseradish peroxidase. Incubation time was 5 hours at room temperature (mean temperature=22°C) and sample volume per well was 30 µL in all samples tested. Photometry was performed using a Wallac Victor 2 1420 Multilabel Counter. The lower and upper limits of detection used were those reported in the manufacturers' instructions, 24 and 1500 pmol/L, respectively. Values of NT-proBNP less than the lower limit of detection or greater than the upper limit of detection of the assay were assigned values of 24 or 1500 pmol/mL, respectively. All samples were assayed in duplicate and the mean of the two values used. Following initial analyses, samples were pooled from a number of cats with measurements at the lower, middle and upper ends of the range of assay detection. These pooled samples were subsequently used for validation purposes. Case acquisition was not completed before the expiration of assay test kits obtained in house; samples collected subsequently (54 of 109) were assayed by a commercial laboratory (IDEXX Laboratories). One millilitre samples of urine were also spun as described above and the creatinine concentration in the supernatant was measured by a commercial laboratory (Diagnostic Laboratories, Royal Veterinary College). The ratio of urinary NT-proBNP to creatinine was calculated.

Statistical analysis

Statistical analysis was performed using commercially available software (SPSS 20; IBM). Precision and reproducibility of measurements of NT-proBNP in pleural fluid and urine were assessed by calculation of intra- and inter-assay coefficients of variation (CV), respectively, in samples of low, medium and high NT-proBNP concentrations. 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. Correlations were assessed using Spearman's correlation coefficient. Receiver operating characteristic (ROC) curves were constructed to determine separate cut-offs to distinguish cardiac from non-cardiac causes of pleural effusion if significant differences between groups were detected. The areas under the ROC curves were compared using the method described by Hanley & McNeil (1983). The positive and negative likelihood ratios of these cut-offs were calculated for this population. The positive likelihood ratio is the ratio of true positives to false positives and the negative likelihood ratio is the ratio of true negatives to false negatives. A positive likelihood ratio

Table 1. Differences in variables between the cardiac and non-cardiac groups. The median and interquartile range are shown for continuous variables

Variable	Cardiac group	Non-cardiac group	P
Age (months) (n=40)	119·5 (51·0, 153·8)	108·5 (81·0, 146·5)	0·463
Pedigree breed (yes/no) (n=40)	6/ 22	3/ 15	>0·999
Sex (male/female) (n=40)	16/ 6	11/7	0·509
Body condition score (n=36)	4·5 (4·0, 5·5)	4·0 (3·3, 5·0)	0·219
Systolic blood pressure (mmHg) (n=35)	113·0 (117·5, 133·5)	125·0 (115·0, 137·0)	0·419
Plasma NT-proBNP (pmol/mL) (n=40)	565·5 (300·1, 1104·4)	51·8 (24·0, 120·3)	*<0·001*
Pleural fluid NT-proBNP (pmol/mL) (n=40)	1135·3 (621·5, 1500·0)	111·0 (25·5, 197·1)	*<0·001*
Urinary NT-proBNP to creatinine ratio (pmol/nmol) (n=28)	0·016 (0·005, 0·448)	0·004 (0·001, 0·018)	*0·035*

Continuous variables and median (25th, 75th percentiles) are reported. Significant differences are highlighted in italic text

greater than 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.

RESULTS

Forty cats with pleural effusion were enrolled in the study between February 2011 and June 2012. All cats that presented with a pleural effusion and that had thoracocentesis and venepuncture were recruited when the study personnel were available for sample collection and processing. Pleural fluid and plasma samples were obtained in all cases with urine samples obtained in 28 of the 40 cats (65%). The study population consisted of 26 domestic short-haired cats, five domestic long-haired cats, two Maine coon and two Burmese cats, and one each of the following breeds: Bengal, Birman, Persian, rag doll and Siamese. Twenty-four cats were male neutered, 12 were female neutered, 3 were male entire and 1 was female entire. The median age of the cats was 9·5 years (range: 3 months to 16 years 4 months). Median body condition score was 4 of 9 (range: 2 to 7) although it was not recorded in four cats. Median blood pressure was 120 mmHg (range: 60 to 180 mmHg) with measurements not recorded for five cats.

The cause of pleural effusion was determined as cardiac in 22 of the 40 cats. The echocardiographic diagnoses (as described by Bonagura (2010)) were hypertrophic cardiomyopathy (n=11), feline unclassified cardiomyopathy (n=5), restrictive cardiomyopathy (n=2), dilated cardiomyopathy (n=2) and arrhythmogenic right ventricular cardiomyopathy (n=2). The cause of pleural effusion was found to be non-cardiac in 18 cats, with underlying causes being neoplasia (n=8), idiopathic chylothorax (n=3), pyothorax (n=2) and traumatic chylothorax, aspiration pneumonia and feline infectious peritonitis (n=1 each). In two cats no cause for the pleural effusion could be determined; these cats were assigned to the non-cardiac group as echocardiographic findings were not suggestive of a cardiac cause of the effusion.

In pleural fluid samples assayed at the RVC, intra-assay CVs for samples of low (150 pmol/L), medium (609 pmol/L) and high (1332 pmol/L) NT-proBNP concentrations were 5·1, 8·6 and 2·1%, respectively. Inter-assay CVs for samples of low, medium and high NT-proBNP concentrations were 16·5, 10·3 and 10·0%, respectively. In urine, samples assayed by an external laboratory (IDEXX Laboratories), intra-assay CVs for samples of low (77 pmol/L), medium (115 pmol/L) and high (1620 pmol/L) NT-proBNP concentrations were 20·5, 3·9 and 7·6%, respectively. Inter-assay CVs for samples of low and medium NT-proBNP concentrations were 13·6 and 6·9%, respectively. Inter-assay CVs for high NT-proBNP concentrations could not be calculated as all results were greater than 1500 pmol/L. Urinary NT-proBNP was measured by both laboratories in five samples. No evidence of a difference in NT-proBNP measurements between laboratories was detected (P=0·500).

Summary statistics are provided in Table 1. No differences in age, breed, sex, body condition score or systolic blood pressure were detected between groups.

NT-proBNP concentrations were significantly higher in pleural fluid samples than in plasma (P<0·001). NT-proBNP concentrations were strongly correlated in plasma and pleural fluid samples (R_s=0·759, P<0·001). A moderate correlation between plasma NT-proBNP and urinary NT-proBNP/creatinine ratio (UBC) was detected (R_s=0·488, P=0·008). No correlations between plasma NT-proBNP and age (P=0·594), body condition score (P=0·606) or systolic blood pressure (P=0·409) were detected. NT-proBNP concentrations were significantly higher in the cardiac group in plasma (P<0·001) (Fig 1 and Table 1) and pleural fluid (P<0·001) (Fig 2 and Table 1); UBC was also

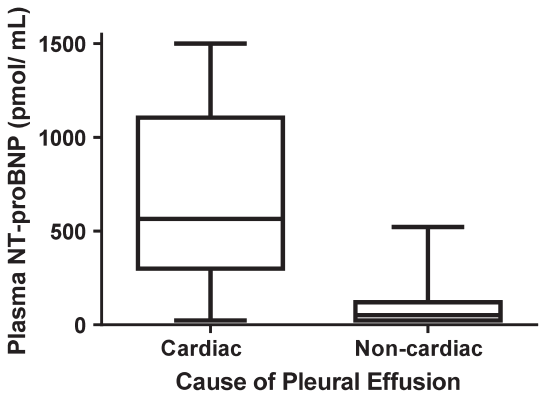

FIG 1. Box and whisker plots of plasma NT-proBNP for cats with cardiac and non-cardiac causes of pleural effusion. The lower and upper boundaries of the box represent first and third quartiles of the data respectively, with the line within the box representing the median. The whiskers represent the complete range of the data. A significant difference was detected between the groups (P<0·001)

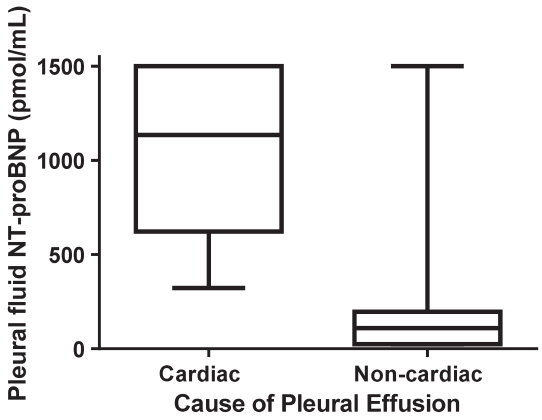

FIG 2. Box and whisker plots of pleural fluid NT-proBNP for cats with cardiac and non-cardiac causes of pleural effusion. The lower and upper boundaries of the box represent first and third quartiles of the data respectively, with the line within the box representing the median. The whiskers represent the complete range of the data. A significant difference was detected between the groups (P<0·001)

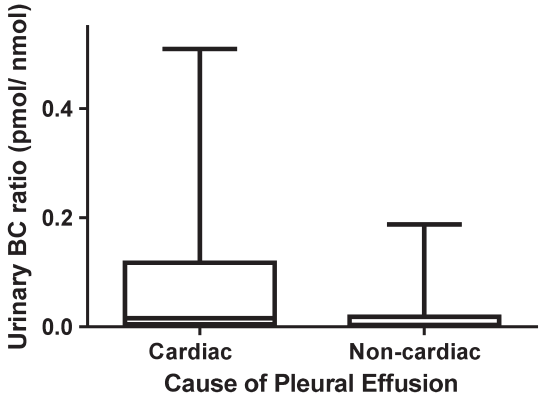

FIG 3. Box and whisker plots of urinary NT-proBNP to creatinine (BC) ratio for cats with cardiac and non-cardiac causes of pleural effusion. The lower and upper boundaries of the box represent first and third quartiles of the data respectively, with the line within the box representing the median. The whiskers represent the complete range of the data. A significant difference was detected between the groups (P=0·035)

significantly higher in the cardiac group (P=0·035) (Fig 3 and Table 1). Five cats with non-cardiac causes of pleural effusion had plasma NT-proBNP concentrations greater than 100 pmol/mL, two of which had plasma NT-proBNP concentrations greater than 360 pmol/mL.

ROC curves were constructed to determine separate cut-offs to distinguish cardiac from non-cardiac causes of pleural effusion for plasma and pleural fluid NT-proBNP and urinary NT-proBNP to creatinine ratio (Figs 4 and 5). ROC curve analysis for plasma NT-proBNP had an area under the curve (AUC) of 0·908 (95% CI: 0·810 to 1·000), and suggested an optimal cut-off of 214·3 pmol/mL [sensitivity, 86·4% (95% CI: 66·7 to 95·3%), specificity, 88·9% (95% CI: 67·2 to 96·9%)]. ROC curve analysis for pleural fluid NT-proBNP had an AUC of 0·953 (95% CI: 0·863 to 1·000), and suggested an optimal cut-off of 322·3 pmol/mL [sensitivity, 100% (95% CI: 85·1 to 100%), specificity, 94·4% (95% CI: 74·2 to 99·0%)]. There was no evidence of a significant difference between the areas under the curves. No cut-off with adequate sensitivity and specificity for UBC was suggested by ROC curve analysis.

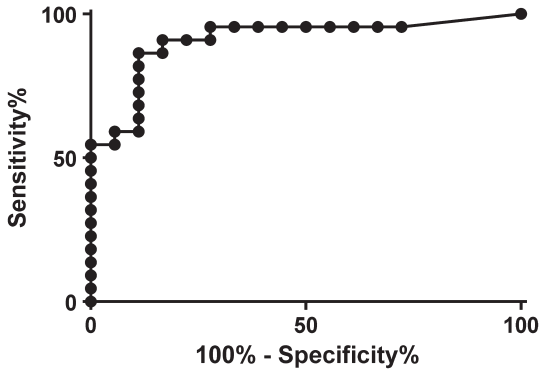

FIG 4. ROC curve for the prediction of cardiac cause of pleural effusion by plasma NT-proBNP. AUC=0·908 (95% CI: 0·810-1·000), P<0·001. NT-proBNP Amino terminal proB-type natriuretic peptide, ROC Receiver operating characteristic, AUC Area under the curve

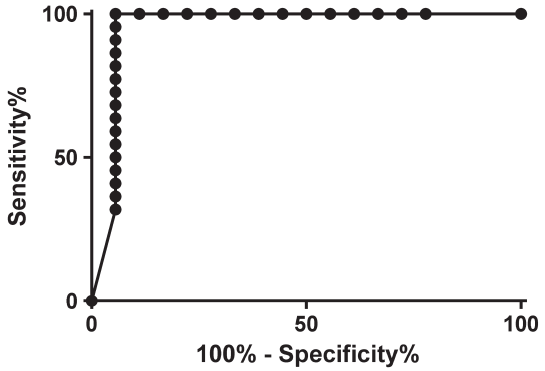

FIG 5. ROC curve for the prediction of cardiac cause of pleural effusion by pleural fluid NT-proBNP. AUC=0·953 (95% CI: 0·863-1·000), P<0·001. ROC Receiver operating characteristic, NT-proBNP Amino terminal proB-type natriuretic peptide, AUC Area under the curve

The number of animals with measurements of plasma and pleural fluid NT-proBNP above and below these cut-offs in each group, with associated positive and negative likelihood ratios, are listed in Table 2.

DISCUSSION

The results of this study suggest that pleural fluid NT-proBNP, measured using a feline-specific assay, can distinguish cardiac from non-cardiac causes of pleural effusion in cats with similar accuracy to plasma NT-proBNP concentrations. Thoracocentesis is usually indicated in cats with pleural effusion for diagnostic or therapeutic reasons. Measurement of NT-proBNP in pleural fluid rather than plasma may therefore be preferable, as this might remove the need to perform venepuncture, a procedure which can cause undesirable distress in cats with respiratory compromise.

Table 2. The receiver operating characteristic curve cut off levels applied to the data and their positive and negative likelihood ratios

	Cardiac group (Number above cut-off/number below cut-off)	Non-cardiac group (Number above cut-off/number below cut-off)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	P
Plasma NT-proBNP	19/3	2/16	7·773 (2·082-29·014)	0·153 (0·053-0·445)	<0·001
Pleural fluid NT-proBNP	22/0	1/17	18·000 (2·679-120·918)	0 (N/A)	<0·001

CI Confidence interval

Both plasma and pleural fluid NT-proBNP concentrations had good sensitivity and specificity for the diagnosis of cardiac disease in this study. The cut-off for plasma NT-proBNP concentration suggested by the ROC curve analysis of 214·3 pmol/L is similar to other reported cut-offs of 220 pmol/L with a sensitivity of 93·9% and specificity of 87·8% (Connolly *et al.* 2009) and 265 pmol/L with a sensitivity of 90·2% and specificity of 87·9% (Fox *et al.* 2009).

In humans, Kolditz *et al.* (2006) found that pleural fluid and serum concentrations of NT-proBNP were nearly identical. This led the authors of that study to question whether there was any benefit in measuring pleural fluid NT-proBNP concentrations as in humans venepuncture is generally preferable to thoracocentesis. However, as discussed above, this is not necessarily the case in cats. In contrast, in this study, NT-proBNP measurements were higher in pleural fluid than in plasma. This is interesting as pleural fluid NT-proBNP is hypothesised to be derived from plasma proBNP which has diffused into the pleural space before being cleaved into NT-proBNP and BNP (Zemans *et al.* 2004). Concentrations might therefore be expected to be lower in pleural fluid rather than higher. The reasons for the higher concentrations found in pleural fluid in this study are unknown.

Analysis of urine NT-proBNP to creatinine ratio in this study showed that it was not a useful indicator of whether cardiac disease was the cause of pleural effusion. This is unlike the findings in humans where urinary NT-proBNP has been shown to be a useful indicator of symptomatic heart failure (Cortés *et al.* 2007; Jungbauer *et al.* 2010). This may be because the samples in this study were not processed quickly enough after urine was voided as many samples were collected by free catch and processed only once noted in the cat's litter tray. NT-proBNP concentrations have been shown to decrease significantly faster in non-protease inhibited feline plasma than in samples mixed with PI (Connolly *et al.* 2011) and it is likely that this is also true for urinary NT-proBNP. Other possible explanations include the fact that pleural fluid, plasma and urine samples were all collected at different time points, although the investigators attempted to collect samples as close together in time as possible. It is also possible that feline and human excretion of NT-proBNP differs.

A limitation of this study is that plasma, pleural fluid and urine samples were not collected at the same time point, with the maximum time recorded between collection of samples being 42 hours. Echocardiography was also not performed at the same time as sample acquisition. Another limitation is that NT-proBNP measurements were performed in two separate laboratories, which may have increased the inaccuracy of measurements. This would reduce the probability of detecting differences between groups and correlations between measurements. Finally, cats were assigned to the cardiac or non-cardiac pleural effusion groups based on the opinion of the attending cardiologist. This decision was inherently subjective although echocardiography was performed in all cases and was interpreted by an experienced board-certified cardiologist decreasing the risk of misclassification. These limitations prevent a definitive conclusion that urinary NT-proBNP is not useful in the diagnosis of cardiac disease. However, they did not prevent the demonstration that pleural fluid concentration of NT-proBNP is a sensitive and specific indicator.

One case that was classified as having a non-cardiogenic pleural effusion had markedly elevated pleural fluid (>1500 pmol/L) and plasma (522 pmol/L) NT-proBNP concentrations compared to all the other non-cardiogenic cases. This cat had immune-mediated haemolytic anaemia and the underlying cause for the pleural fluid was not determined. The cat had a left atrial to aortic ratio of 1·15 (with <1·5 described as normal by Luis Fuentes (2010)), however, the left atrium appeared enlarged in long axis at 21·5 mm (with a normal value of <16 mm described by Luis Fuentes (2010)) and the left ventricular internal diameter in diastole was 21·3mm (upper range of 20 mm reported by Luis Fuentes (2010)) indicating moderate dilation. The attending cardiologist felt that cardiac disease was unlikely to be the cause of the pleural fluid and so the cat was classified as having non-cardiogenic pleural fluid. The echocardiographic results in this case were equivocal and it is possible that the cat was misclassified. This represents a potential limitation of the gold standard applied, but it is also possible that the cat's underlying disease process or therapy administered could have resulted in the elevated NT-proBNP concentrations.

General practitioners are able to diagnose cardiac disease in cats with significantly improved accuracy and confidence when the results of plasma NT-proBNP measurements are available (Singletary *et al.* 2012). It is therefore likely that measurement of NT-proBNP in pleural fluid would similarly assist practitioners. This study analysed samples from clinical cases rather than from animals in which disease was induced, making the results relevant to practicing veterinarians. The diagnosis of cardiogenic pleural effusion on the basis of NT-proBNP measurements should be accompanied by careful assessment of the patient's full clinical history and physical examination to decide whether further investigation of potential exacerbating factors is necessary.

The intra- and inter-assay CV for pleural fluid were acceptable for medium and high concentration samples, but the inter-assay CV was higher than desirable at 16·7% for low concentration samples. This suggests that the ELISA is less consistent at lower

concentrations of pleural fluid NT-proBNP, but concentrations at this level are distant from the cut-off reported making this finding clinically less significant.

In conclusion, the results of this study suggest that NT-proBNP can be measured in feline pleural fluid with good precision and acceptable reproducibility. Measurement of NT-proBNP in plasma or pleural fluid, but not urine, allows differentiation of cardiac from non-cardiac causes of pleural effusion with similar accuracy in cats. These findings should be validated by prospective testing of the suggested cut-off values in a separate population.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

References

Beatty, J. & Barrs, V. (2010) Pleural effusion in the cat: a practical approach to determining aetiology. *Journal of Feline Medicine and Surgery* **12**(9), 693-707

Bonagura, J. D. (2010) Feline cardiomyopathies. In: BSAVA Manual of Canine and Feline Cardiorespiratory Medicine. 2nd edn. Eds V. Luis Fuentes, L. R. Johnson and S. Dennis. BSAVA, Gloucester, MA, USA

Connolly, D. J., Magalhaes, R. J., Syme, H. M., *et al.* (2008) Circulating natriuretic peptides in cats with heart disease. *Journal of Veterinary Internal Medicine* **22**, 96-105

Connolly, D. J., Soares Magalhaes, R. J., Fuentes, V. L., *et al.* (2009) Assessment of the diagnostic accuracy of circulating natriuretic peptide concentrations to distinguish between cats with cardiac and non-cardiac causes of respiratory distress. *Journal of Veterinary Cardiology* **11**, S41-S50

Connolly, D. J., Hezzell, M. J., Fuentes, V. L., *et al.* (2011) The effect of protease inhibition on the temporal stability of NT-proBNP in feline plasma at room temperature. *Journal of Veterinary Cardiology* **13**, 13-19

Cortés, R., Rivera, M., Salvador, A., *et al.* (2007) Variability of NT-proBNP plasma and urine levels in patients with stable heart failure: a 2-year follow-up study. *Heart* **93**(8) 957-962

Fox, P. R., Oyama, M. A., Reynolds, C., *et al.* (2009) Utility of plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) to distinguish between congestive heart failure and non-cardiac causes of acute dyspnea in cats. *Journal of Veterinary Cardiology* **11**, S51-S61

Hanley, J. A. & McNeil, B. J. (1983) A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* **148**, 839-843

Janda, S. & Swiston, J. (2010) Diagnostic accuracy of pleural fluid NT-pro-BNP for pleural effusions of cardiac origin: a systematic review and meta-analysis. *BMC Pulmonary Medicine* **10**, 58

Jungbauer, C. G., Buchner, S., Birner, C., *et al.* (2010) N-terminal pro-brain natriuretic peptide from fresh urine for the biochemical detection of heart failure and left ventricular dysfunction. *European Journal of Heart Failure* **12**, 331-337

Kolditz, M., Halank, M., Schiemanck, C.S., *et al.* (2006) High diagnostic accuracy of NT-proBNP for cardiac origin of pleural effusions. *The European Respiratory Journal* **28**, 144-150

Luis Fuentes, V. (2010) Echocardiography. In: *BSAVA Manual of Canine and Feline Cardiorespiratory Medicine.* 2nd edn. Eds V Luis Fuentes, L. R. Johnson and S. Dennis. BSAVA, Gloucester, MA, USA. pp 79-97

Porcel, J. M., Vives, M, Cao, G., *et al.* (2004) Measurement of pro-brain natriuretic peptide in pleural fluid for the diagnosis of pleural effusions due to heart failure. *The American Journal of Medicine* **115**, 417-420

Singletary, G. E., Rush, J. E., Fox, P. R., *et al.* (2012) Effect of NT-pro-BNP assay on accuracy and confidence of general practitioners in diagnosing heart failure or respiratory disease in cats with respiratory signs. *Journal of Veterinary Internal Medicine* **26**, 542-546

Waddell, L. S. & King, L. G. (2007) General approach to dyspnoea. In: *BSAVA Manual of Canine and Feline Emergency and Critical Care.* 2nd edn. Eds L. G. King and A. Boag. BSAVA, Glouceste, MA, USA. pp 85-113

Zemans, R. L., Chatterjee, K. & Matthay, M. A. (2004) Diagnostic utility of b-type natriuretic peptide in patients with acute dyspnea or pleural effusions. *The American Journal of Medicine* **116**, 424-426