



A new approach to pleural effusion in cats: markers for distinguishing transudates from exudates

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Classification of pleural effusion (PE) is central to diagnosis. Traditional veterinary classification has distinguished between transudates, modified transudates and exudates. In human medicine PEs are divided into only two categories: transudates and exudates. The aim of this study was to evaluate, in 20 cats presented with PE, paired samples of serum and pleural fluid for the following parameters: Light's criteria (pleural fluid lactate dehydrogenase concentration (LDHp), pleural fluid/serum LDH ratio, pleural fluid/serum total protein ratio (TPr)), pleural fluid total protein, pleural fluid cholesterol concentration, pleural fluid/serum cholesterol ratio (CHOLr), serum-effusion cholesterol gradient (serum cholesterol minus PE cholesterol concentration (CHOLg)), PE total nucleated cells count (TNCCp) and pleural fluid glucose (GLUp). LDHp and TPr were found most reliable when distinguishing between transudates and exudates, with sensitivity of 100% and 91% and specificity of 100%, respectively. When conflict between the clinical picture and laboratory results exists, calculation of CHOLr, CHOLg and TNCCp measurement may help in the classification of the effusion. Measurement of serum albumin (in the case of a transudate) may provide additional information regarding the pathogenesis of the effusion.

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The aetiopathogenic classification of pleural effusion (PE) can be challenging.¹ In human medicine, PEs are categorised only as transudates, resulting from increased hydrostatic pressure or decreased osmotic pressure, or exudates, resulting from increased vascular permeability.^{2–5} Simultaneous evaluation of pleural fluid and serum protein, lactate dehydrogenase (LDH) and other biochemical parameters has been proven reliable and effective in identifying the pathophysiology of formation of a PE.^{2–4,6} In the case of a transudate, subsequent evaluation of the serum albumin will then clarify if the effusion formed is due to a decrease in colloid osmotic pressure or the result of an increase in hydrostatic pressure.

In veterinary medicine, PEs were originally classified as transudates or exudates and specific gravity, protein content and cellularity of the effusion were used to differentiate them.⁷ Due to the common overlap in values of these parameters between transudate and exudate Perman introduced the modified transudate group to veterinary medicine⁸ and defined it as

closely resembling an exudate based on protein content and cellularity, but resulting from increased hydrostatic pressure.^{8,9} Another commonly reported definition of modified transudate is a 'long standing transudate'.¹⁰ While the latter definition does not describe a pathophysiological mechanism of fluid formation, but instead an 'in vivo ageing sample artifact', Perman's definition, also called obstructive effusion,¹¹ gives information on the pathophysiological mechanism of the fluid formation. A survey of the literature neither reveals published evidence to support this classification nor studies showing how the cut-off values for the markers conventionally used to classify PEs were derived. Studies assessing the sensitivity and specificity of these markers in classifying PEs correctly are also lacking. The large and variable number of disorders associated with modified transudates and the fact that this category has overlapping protein content and cellularity with transudates and exudates, limit the current veterinary classification scheme of PEs.

The aim of this study was to determine whether the simultaneous evaluation of pleural fluid and serum protein, LDH and other biochemical parameters is

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useful in the classification of feline PE based on the pathophysiological mechanism of formation, as already demonstrated in human literature.²⁻⁵

Material and methods

This was a prospective study in which 20 consecutive cats presenting with PE between March 2002 and September 2003 were enrolled. Fifteen cats were referred to the Royal Veterinary College, Queen Mother Hospital for Animals (QMHA) and five cats were seen in private practices.

Inclusion criteria included the presence of PE, ability to collect a blood and a PE sample no more than 2 h apart from each other and determination of the aetiology of the PE. A complete history, including prior medical treatment, was obtained. Patients that received any pharmacological treatment, which could have altered the aetiological diagnosis prior to sampling (eg, glucocorticoids), were excluded. Animals that received diuretics before presentation were included. A full physical examination and complete blood count, serum biochemistry profile were performed on each cat. Pleural fluid samples, collected by thoracocentesis in all cats, were divided into three aliquots and stored in K₃-EDTA, plain and fluoride oxalate tubes. The serum and plain pleural fluid samples were centrifuged and separated within 30 min and processed within 48 h of collection. An automated cell count and a cytological examination by a board certified clinical pathologist were performed. All biochemical parameters from the blood and pleural fluid were measured with validated feline assays using an OpeRA Chemistry Analyzer (Bayer PLC, Berkshire, UK) at the QMHA. Thoracic radiographs were obtained from all cats. When PE volume precluded good thoracic radiological assessment they were repeated post-thoracocentesis. Further tests [eg, abdominal ultrasonography or radiography, echocardiography, electrocardiogram, serum total T₄, feline leukaemia and feline immunodeficiency virus status (by rapid immunochromatographic method, Speed Duo FeLV/FIV, Vetlab Supplies, UK), feline coronavirus (FCoV) antibody titre (by immunofluorescence as previously described)]¹² were performed as clinically indicated to achieve a definitive diagnosis. One cat underwent a full post-mortem examination.

The cause of PE was identified with reference to the following pre-determined criteria:

- a. Congestive heart failure (CHF) as a cause of PE was diagnosed if the patient had appropriate history, presence of cardiac murmur, severe structural heart disease diagnosed on echocardiography by a board certified cardiologist, moderately to severe enlarged left atrium (left atrial to aortic diameter ratio of >1.5 on 2D echocardiography from the right-parasternal short-axis heart base view)¹³ and/or right atrium and other causes of PE were excluded. In

one cat a full post-mortem examination confirmed *in vivo* findings.

- b. Malignant effusion required histopathological or cytological demonstration of neoplastic tissue in the pleural cavity or fluid.
- c. Pyothorax required positive bacterial culture, or presence of degenerate neutrophils and intracellular bacteria on cytological preparations of pleural fluid.
- d. Chylous effusion was characterised based on colour (milky), turbidity (opaque), fluid triglycerides > 100 mg/dl, fluid to serum triglyceride ratio > 1, fluid to serum cholesterol ratio < 1, fluid cholesterol to triglyceride ratio < 1, protein concentration > 30.0 g/l and recognition of lymphocytes or neutrophils as the predominant effusion cell type.¹⁴⁻¹⁶
- e. PE secondary to feline infectious peritonitis (FIP) was diagnosed when supportive history, signalment, clinical and clinicopathological data including non-regenerative anaemia, lymphopenia, predominance of non-degenerate neutrophils on cytological examination of the effusion, serum and fluid total protein concentration > 80 g/l, serum and fluid globulin concentration > 50 g/l, serum and fluid albumin:globulin ratio < 0.45, serum and fluid FCoV antibody titres > 1:1280 and increased serum α -1 acid-glycoprotein were present and no other causes of PE could be identified.

Effusions were then classified as transudates or exudates based on their pathophysiology. Transudates were the effusions from animals with CHF. Exudates were the effusions from animals with neoplasia, pyothorax and FIP. For the purposes of this study, chylous effusions were classified in accordance with human literature as exudates.^{17,18}

Parameters measured or calculated included: Light's criteria (namely pleural fluid LDH (LDHp), pleural fluid/serum LDH ratio (LDHr) and pleural fluid/serum total protein ratio (TPp)), pleural fluid total protein (TPp), PE total nucleated cells count (TNCCp), pleural fluid cholesterol (CHOLp), pleural fluid/serum cholesterol ratio (CHOLr), serum-effusion cholesterol gradient (serum cholesterol concentration minus CHOLp) (CHOLg), pleural fluid glucose (GLUp) and pleural fluid red blood cells (RBCp). Due to the clinical instability of the cats and the difficulties in collecting the samples, without further compromise to the clinical status of the patients, not all the above tests were available for each animal (Table 1).

For comparison the same effusions were also classified using traditional veterinary method based on TPp and TNCCp (transudate: TPp < 25 g/l, TNCCp < 1500 μ l; modified transudate: TPp = 25-75 g/l, TNCCp 1000-7000 μ l; exudate TPp > 30 g/l, TNCCp > 7000 μ l).¹⁵ The modified transudate was considered, as first suggested by Perman, an effusion resultant from an increase in hydrostatic pressure.^{8,9}

Table 1. Aetiology of PEs from 20 cats and values of the analytes measured and calculated in the PE and serum

Case <i>n</i>	Causes of PE	PE						Serum			Calculated values			
		LDHp (IU/l)	TPp (g/l)	GLUp (mmol/l)	RBCp $\times 10^{12}/l$	TNCCp $\times 10^9/l$	CHOLp (mg/dl)	LDH (IU/l)	TP (g/l)	CHOL (mg/dl)	TPr	LDHr	CHOLr	CHOLg
1	CHF	26	3.2	8.5	0	1.5	3	163	71.9	274	0.04	0.15	0.01	271
2	CHF	54	22.6	6.7	0.02	1.3	58	329	56	135	0.4	0.16	0.42	80
3	CHF	66	34.4	9.4	0.11	5.1	89	423	65.8	—	0.52	0.15	—	—
4*	CHF	79	35	12.8	0	3.6	81	402	68.4	—	0.51	0.19	—	—
5*	CHF	105	30.3	7.4	0	2.5	85	196	71	274	0.42	0.53	0.3	189
6	CHF	114	30.1	5.1	—	3.5	42	538	59.6	89	0.5	0.21	0.47	47
7	CHF	177	44.6	6.1	0	5.9	127	284	78.5	236	0.56	0.62	0.53	109
8	CHF	202	30.9	7.8	0	1.0	77	263	57.2	201	0.54	0.76	1.38	124
9*	CHF	226	13.8	10.2	0	0.3	27	1865	62.9	150	0.21	0.12	0.18	123
10	Carcinoma	258	43.3	9.6	—	0.85	116	215	64.9	189	0.67	1.2	0.61	73
11*	Idiopathic C	276	54.8	6	0	8.3	65	360	72.6	92	0.75	0.76	0.7	27
12	Lymphoma	363	29.9	5.2	0	9.2	46	200	59.7	108	0.5	1.81	0.42	62
13	Carcinoma	364	54.3	9.5	0.03	9.0	112	513	76	174	0.71	0.7	0.64	62
14	FIP	647	82.4	—	0.008	4.2	—	1499	82.4	208	0.69	0.43	—	—
15	Idiopathic C	949	51.2	7.5	0.09	6.7	85	479	64.3	112	0.79	1.98	0.75	27
16	Cardiogenic C	1701	62.3	10.3	—	6.9	89	266	52	185	1.19	6.39	0.48	96
17*	Carcinoma	2363	48.1	5	0.67	6.6	104	269	70.6	—	0.68	8.78	—	—
18	Carcinoma	5837	64.2	0	—	22.1	119	375	84.7	162	0.75	15.6	0.73	43
19	Pyothorax	13,010	27	0	—	297	34	3274	42.7	69	0.63	3.97	0.49	35
20	Pyothorax	20,156	27.6	0.1	0.56	81.2	58	4875	34.1	112	0.8	4.1	0.51	54

n = PE number; C = chylothorax; TP = total protein; GLU = glucose; CHOL = cholesterol; p = pleural effusion; r = ratio; g = gradient.

*Animals that received diuretics treatment before thoracocentesis.

Statistical analysis

Median values for TPp, TPr, TNCCp, LDHp, LDHr, CHOLp, CHOLr and CHOLg were compared between transudates and exudates using Mann–Whitney *U* tests. Using the cut-off value adopted in human medicine for each of the eight above mentioned biochemical parameters the accuracy $[(Tp + Tn)/(Tp + Tn + Fp + Fn)]$ of each test in distinguishing exudates and transudate was established, where *Tp* is the number of true positive diagnoses, *Tn* the number of true negative diagnoses, *Fp* the number of false positive diagnosis, and *Fn* the number of false negative diagnoses. In addition the utility of each biochemical parameter in identifying exudates was evaluated by calculating sensitivity $[Tp/(Tp/Fn)]$ and specificity $[Tn/(Tn + Fp)]$. As transudates and exudates are complementary terms, we must emphasise that for any given analyte, the specificity for exudates corresponds to the sensitivity for transudates.

Receiver operating characteristic (ROC) curve analysis, graphing sensitivity against (1-specificity) was then used to establish the optimal cut-off point of the above biochemical analytes measured and calculated. Optimal cut-off points (to maximise both sensitivity and specificity) were established by selecting the points of test values that provided the greatest sum of sensitivity and specificity, corresponding to the point closest to the top left hand corner of the ROC curve. The three cut-off values of the Light's criteria were also used in parallel with an 'or' rule as in the original study.² Performance of each test for diagnostic separation of transudates and exudates was assessed again using: sensitivity, specificity and overall accuracy at the optimal cut-off point for each parameter.

Median RBCp value between exudative neoplastic and non-neoplastic PE was compared using Mann–Whitney *U* tests.

Spearman's rank correlation tests were used to assess correlations between: LDH and RBC and glucose and TNCC in the PE.

For all analyses, *P* values <0.05 were considered significant.

Results

Based on pathophysiology of formation nine effusions were transudates (all secondary to CHF) and 11 were exudates (five caused by malignancies, three by chylothorax and three by infectious diseases) (Table 1). Five of the cats with transudates were male (one entire and four neutered) and four were female (one entire and three neutered), with an average age of 8.89 ± 4.58 years (range 3.0–14.41 years). Those with exudates were five male (all neutered) and six female (one entire and five neutered), with an average age of 9.07 ± 4.96 years (range 0.33–13.83 years).

Using the traditional veterinary classification^{8,9,15} of the nine modified transudates, TPp and TNCCp values caused misclassification of two effusions (effusion number: 2 and 9) as pure transudates and it was not

possible to clearly classify two more effusions (effusions number: 1 and 8) because of discordant information deriving from TPp and TNCCp (Table 1). Of the 11 exudates TPp and TNCCp results caused misclassification of four effusions. Three (effusion number: 15, 16 and 17) were incorrectly classified as modified transudates and one (effusion number: 10) was misclassified as intermediate between a pure transudate and a modified transudate. In addition TPp and TNCCp results prevented clear classification of four more exudative effusions (effusion number: 12, 14, 19 and 20) because of discordant information deriving from TPp and TNCCp (Table 1). The overall accuracy of the traditional veterinary classification was 40%.

TPp and TPr

TPp and TPr values were significantly different between transudate and exudates ($P = 0.0251$ and $P = 0.0002$, respectively).

TNCCp

TNCCp values were significantly different between transudate and exudates ($P = 0.003$).

LDHp and LDHr

LDHp and LDHr values were significantly different between transudates and exudates ($P = 0.0001$ and $P = 0.0002$, respectively).

LDHp and RBCp

RBCp count was available for 15/20 cats. Statistic analysis showed a statistically significant positive correlation between LDHp values and RBCp ($Rho = 0.54$, $P = 0.037$). RBCp count was available for 7/11 exudate and median RBCp value between neoplastic and non-neoplastic PE was not statistically different ($P = 0.95$).

CHOLp, CHOLr and CHOLg

CHOLp, CHOLr, and CHOLg were available for 19/20, 16/20 and 16/20 cats, respectively. CHOLp values were not significantly different between transudates and exudates ($P = 0.2864$). CHOLr and CHOLg values were significantly different between transudates and exudates ($P = 0.0251$ and $P = 0.0047$, respectively).

GLUp

GLUp was available in 19/20 cases and showed a statistically significant negative correlation with TNCC of the effusion ($Rho = -0.56$, $P = 0.012$).

Results for sensitivity, specificity, accuracy, number of misclassified transudate and exudate for TPp, TPr, TNCCp, LDHp, LDHr, Light's criteria (used in parallel with an 'or' rule), CHOLp, CHOLr and CHOLg calculated for each of the above parameters using

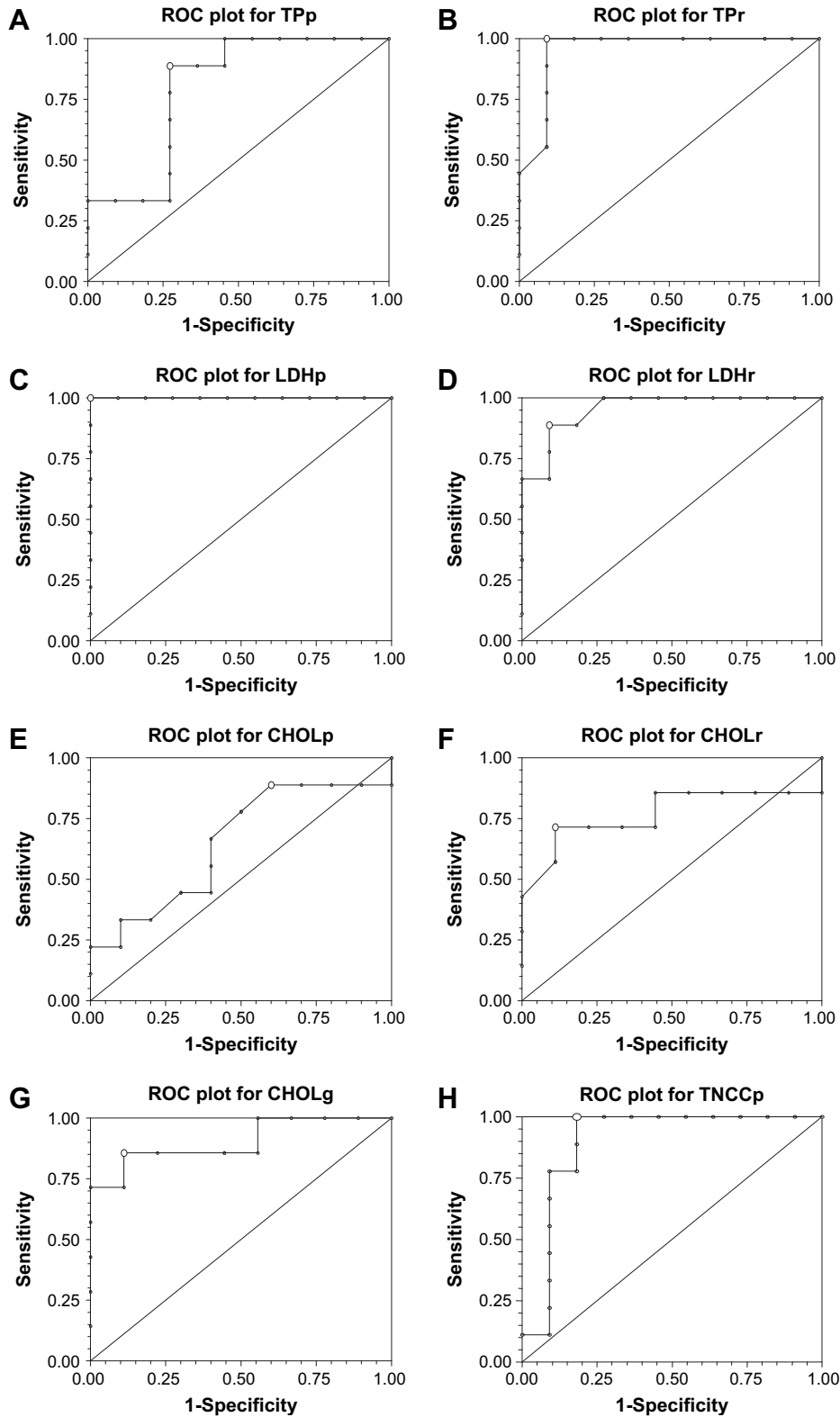


Fig 1. ROC plots of pleural fluid values for TPp, TPr, LDHp, LDHr, CHOLp, CHOLr, CHOLg and TNCCp. The optimum cut-off level (O) was determined by selecting points of test values that provided the greatest sum of sensitivity and specificity. The optimum cut-off levels for TPp, TPr, LDHp, LDHr, CHOLp, CHOLr, CHOLg, TNCCp were 35.0 g/l, 0.56, 226 IU/l, 0.62, 88 mg/dl, 0.47, 77.0 mg/dl and 5900 μ l, respectively. TP = total protein, LDH = lactate dehydrogenase, CHOL = cholesterol, TNCC = total nucleated cell counts, p = pleural effusion, r = ratio, g = gradient.

cut-off values obtained by ROC curve analysis of our data (Fig. 1) are reported in Table 2. For comparison sensitivity, specificity and accuracy for the same parameters using human cut-off values are also displayed in Table 2.

Discussion

Alterations of Starling's law on the pleural and/or pulmonary capillaries may lead to pleural fluid formation.¹⁹ A transudate occurs when factors influencing formation and reabsorption of pleural fluid, such as decreased plasma osmotic pressure or elevated systemic or pulmonary hydrostatic pressures are altered.¹⁹ By contrast, an exudate results from alterations in the permeability of these capillaries.^{2,20}

A Tpp cut-off level of 30.0 g/l in human medicine has an accuracy of about 90% in distinguishing a transudate from an exudate.^{2,21,22} In veterinary medicine, a Tpp cut-off value of 30.0 g/l has been arbitrarily chosen to separate exudates from transudates,^{9,11,15} but this cut-off value fails to distinguish between exudates and modified transudates,^{11,14,15} rendering it of little value in classifying PEs. In our study, classifying PEs into only exudate or transudate, according to the pathogenic process of formation, a Tpp cut-off value of 35.0 g/l

enabled separation of these two groups with similar accuracy to that reported in human studies.^{2,21,22} The reason for a higher cut-off value compared with that in humans may be due to the small number of animals enrolled in our study, the different level of serum protein of these two species, or the fact that 3/9 cats with a transudate received diuretic treatment prior to sampling (see later discussion). The same reasons could also explain why in our study a cut-off for TPr of 0.57 instead of 0.50 (cut-off used in human medicine)² for exudates was obtained. In the only other veterinary study²³ in which TPr was used to distinguish a transudate from an exudate a cut-off of 0.50 was used. No information was given regarding statistical method of selection of this value which enabled correct classification of 19/20 effusions for which this parameter was available.²³

In human medicine it has been noted that very few transudates have Tpp that fall into the exudative range.^{5,17,24} Most of these have been associated with administration of diuretics before sample collection, which leads to removal of the water component of the effusion resulting in relatively higher Tpp and TPr values.^{25,26} To by-pass this problem in human medicine it is recommended that if a patient is thought to have a transudative effusion by clinical criteria, but the fluid is identified as exudative by Light's

Table 2. Sensitivity, specificity, accuracy, number of misclassified transudate and exudate for Tpp, TPr, TNCCp, LDHp, LDHr, Light's criteria (used in parallel with an 'or' rule), CHOLp, CHOLr and CHOLg calculated using cut-off values obtained by ROC curve analysis of our data and also using human cut-off values reported in literature

Test	Cut-off value	Ss (%)	Sp (%)	Acc (%)	Transudates misclassified	Exudate misclassified
Tpp (g/l)	>35 by ROC	73	89	80	1/9	3/11
	>30 human value ¹⁹	73	33	55		
TPr	>0.56 by ROC	91	100	95	0/9	1/11
	>0.50 human value ⁸	100	44	75		
TNCCp (µl)	5900 by ROC	82	100	90	0/9	2/11
	Human value NA	—	—	—		
LDHp (IU/l)	>226 by ROC	100	100	100	0/9	0/11
	>200 human value ⁸	100	78	90		
LDHr	>62 by ROC	91	89	90	1/9	1/11
	>60 human value ⁸	91	78	85		
Light's criteria	See ROC values	100	78	90	2/9	0/11
	See human values ⁸	100	44	75		
CHOLp (mg/dl)	>88 by ROC	50	78	63	2/9	5/10
	>60 human value ⁹	70	44	58		
CHOLr	>0.47 by ROC	89	71	76	2/7	1/9
	>0.3 human value ⁹	100	28	69		
CHOLg (mg/dl)	<77 by ROC	86	89	87	1/7	1/9
	Human value NA	—	—	—	—	—

TP = total protein; CHOL = cholesterol; p = pleural effusion; r = ratio; g = gradient; Ss = sensitivity; Sp = specificity; Acc = accuracy.

criteria, a serum-effusion albumin gradient (serum albumin concentration minus PE albumin concentration) should be calculated.^{17,24,27} The albumin gradient may be more reliable than the TPr after diuretic therapy due to relatively increased non-albuminaemic protein that originates and then accumulates in the pleural space or due to the mathematics of a gradient that may be more representative of the TPr.⁵ In our study, the PE albumin value was not measured; it was, therefore, impossible to calculate the serum-effusion albumin gradient in the cats that received diuretic treatment before thoracocentesis.

Animal and human studies have shown that increases in hydrostatic pressure can increase TPr and, therefore, also TPr by the opening of larger pores in the endothelium with elevated hydrostatic pressure.²⁸ This situation may also occur secondary to CHF²⁸ and might explain why, in this study, one transudate associated with CHF that had not received diuresis prior to sampling had an increased TPr value.

In our study, three exudates (effusion number: 12, 19, 20; Table 1) had TPr less than the cut-off value of 35.0 g/l. Interestingly in all these patients, serum total proteins were also below the reference interval (reference interval 64–80 g/l). These results are not surprising as most of the protein content of the exudative effusion is derived from the serum protein leakage through the compromised pulmonary or pleural microvasculature⁵ and a positive correlation between serum and pleural fluid total protein have been previously demonstrated.²⁹ The importance of TPr should, therefore, be questioned when classifying effusions in dysproteinaemic patients. As the majority of, but not all proteins in the PE are derived from the serum, the TPr should not be affected to the same degree in the case of altered serum protein value. In fact only one of the three cats mentioned above had a low TPr. Similar conclusions were reported in a study in humans with PE.²⁹

Although in humans TNCCp has not been found useful to distinguish an exudate from a transudate,^{30,1,21} in veterinary medicine a TNCCp > 7000 μ l is one of the suggested cut-off values used for this purpose.¹⁵ In our study using this value to classify the effusion as an exudate, a sensitivity of 55% and a specificity of 100% were obtained with an accuracy of 75% (data not shown). Using ROC curve analysis we derived a cut-off value of 5900 μ l which decreased specificity, but increased sensitivity and overall accuracy of the test (Table 2). Although TNCCp does not appear to be as powerful as TPr and LDHp in correctly discriminating between a transudate and an exudate, cytological analysis and differential cell count of the fluid remains of paramount importance, as described in human medicine,²⁴ in investigating the cause of an exudate.

LDH (EC 1.1.1.27) is a cytoplasmic enzyme with a molecular mass of 140 kDa, present in essentially all organ systems and it is released from cells only after cell damage or death.^{31–33} LDH can, therefore,

serve as an indicator of pathological disruption of cellular integrity.³¹ Elevated pleural fluid LDH concentrations can arise from activated, injured or dead white blood cells, neoplastic cells or pleural mesothelial cells and represents a sensitive marker of an underlying exudative process. In Light's original study, LDHp was found significantly lower in human with transudates and a cut-off value of >200 IU/l was derived to separate transudates from exudates.² Also in our study, LDHp was found to be significantly lower in cats with a transudate and using LDHp > 226 IU/l as a cut-off value, sensitivity, specificity and accuracy were 100% in dividing transudates from exudates. The small difference in the optimum cut-off value between our study and Light's original study could be due to the small number of animals enrolled in our study, the use of different assays to measure LDH or the different inter-species levels of tissue LDH. To compare the LDHp value measured with different assays in human medicine, a pre-determined cut-off for LDHp is no longer used, but instead, two-thirds of the upper limit of 'normal' serum LDH have been suggested (modified Light's criteria).³⁴ A reference range for serum feline LDH was not available, therefore, this method could not be used.

Using the Light's criteria the simultaneous measurement of protein and LDH in effusion and serum, classifies an exudate as fulfilling one or more of the following: TPr > 0.50, LDHp > 200 UI/l, or LDHr > 0.60.² Light et al used these three cut-off values in parallel with an 'or' rule as criteria for segregating transudates from exudates with a sensitivity and specificity both near 100%. Using only TPr and LDHp, but not LDHr, with the same cut-off values to Light's original study a previous veterinary study classified all the 22 cats with PE in the study correctly.²³ In this study no explanation for the lack of use of LDHr was given neither was information given regarding how the cut-off value for LDHp was derived.²³ In our study, simultaneous use of these three tests failed to perform as well as in Light's study (Table 2). The compartmentalised elevation of LDH concentration in the exudative processes may explain why the LDHr has no role in the diagnostic separation of PE into transudate and exudate,^{29,35} and why when this parameter is used to separate an exudate from a transudate the sensitivity decreases, as also showed in more recent humans studies.^{3–5}

Although RBCs contain a large amount of LDH, one human study demonstrated no correlation between the pleural fluid LDH level and pleural fluid RBC,² while another supported a relationship between RBC and LDH.³⁶ In our study, a positive correlation was found between LDHp and RBC in the effusion, but correcting the LDHp value to the RBC number according the Eid formula³⁶ (corrected LDH = measured LDH – 0.0012 \times RBC count/ μ l) did not affect our results (data not shown). Nevertheless in cases of marked haemolysis use of LDHp should be questioned.

In human medicine increased RBCp ($>100,000/\mu\text{l}$) is associated with neoplastic effusion,²⁰ and also with trauma and pulmonary embolism²⁴ and in a veterinary article an RBCp $> 50,000/\mu\text{l}$ was said to be associated, in the absence of trauma, with malignancy.³⁷ In our study no effusion was caused by trauma or pulmonary embolism and neoplastic exudative effusions had a mean RBCp value similar to non-neoplastic exudative effusions with only three pleural fluids, one due to malignancy, one due to idiopathic chylothorax and one due to pyothorax, having an RBCp value above this cut-off.

The origin of cholesterol in PEs is still unclear. 'Serum leakage' or 'release' in loco after cellular degeneration has been proposed as possible mechanisms.³ In our study, CHOLp did not help in differentiating transudates from exudates and only CHOLr and CHOLg were useful for this purpose. Performance of the latter two tests in discriminating an exudate from a transudate is similar to human studies^{3,4,6} when cut-off values derived from our ROC curve are used. The difference in cut-off values between our study and the human studies may be due to the difference between lipoproteins in humans and cats or the small number of animals enrolled in our study.

Pleural fluid pH correlates closely with GLUp level^{38,39} and it may decrease in exudative processes.²⁷ These two values are often measured in cases of parapneumonic and neoplastic effusions in human medicine to gain information regarding treatment options and as prognostic indicators.^{40,41} In Stewart's study, pleural fluid pH and glucose values were helpful in distinguishing neoplastic from septic exudates,²³ but overlap between GLUp values between neoplastic and other non-infectious inflammatory processes and between pyothorax and granulomatous diseases existed. Moreover, no information regarding sample handling or methodology of pH measurement was given.

In our study, no pH measurement was made due to the inability to process samples immediately after collection and anaerobically and glucose value alone was acquired. Three animals (effusions number: 18, 19 and 20; Table 1) had a very low GLUp concentration. Proposed mechanisms to explain the low glucose in the PE include use of glucose by pleural fluid constituents including leukocytes and free malignant cells, pleural membrane metabolism, especially by malignant cells, and abnormal transfer of glucose across diseased pleural membranes.⁴⁰ Interestingly, all three effusions with low GLUp in our study had very high nucleated cell count (respectively 270.0, 81.2 and $22.1 \times 10^9/\text{l}$) compared to the other effusion samples (values from $0.3\text{--}9.2 \times 10^9/\text{l}$), and an inverse correlation between GLUp and TNCC was present. Therefore, the increased TNCC may have been responsible directly or indirectly for the low GLUp in these three effusions. Further studies should evaluate if GLUp and PE pH may be used for diagnostic or prognostic purposes.

Three major limitations were identified in our study. First the small number of cats enrolled could

have affected the cut-off values that we derived. Nevertheless, it probably did not affect our major findings that LDHp and TPr are the most reliable parameters in segregating a transudate from an exudate as supported by previous studies in humans and cats.^{27,34,23} Secondly, in the transudate group we did not have any cat which developed PE due to decreased colloid osmotic pressure. Unfortunately, this type of effusion seems to be extremely rare in the feline population as apparent from our data and three other feline studies^{23,42,43} on PE in which only one cat in a total of 226 could be identified with this type of effusion. Thirdly in the case of chylous effusions our classification scheme fails to identify the pathophysiology of pleural fluid formation. In fact, due to the inflammatory reaction of the pleura associated with this type of fluid,¹⁶ the resultant effusion will have exudative characteristics whether formed secondary to an increase in hydrostatic pressure of the lymphatic vessels or due to increased permeability.

In summary, based on the results of this preliminary study the first step in investigating PE should be its classification as either a transudate or an exudate. Measurement of LDHp and calculation of TPr (as in human medicine)^{2,29,35} allowed reliable classification of PEs into two categories: transudates and exudates. When conflict between the clinical picture and laboratory results exists, calculation of CHOLr, CHOLg and TNCCp measurement may help in the classification of the effusion. Measurement of serum albumin (in the case of a transudate) may provide additional information regarding the pathogenesis of the effusion.

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