

Pleural effusion in the cat: a focus on laboratory diagnosis



> Abstract

Pleural effusions constitute a common entity in feline medicine. Laboratory evaluation of pleural fluids remains the cornerstone of a proper diagnosis. Total nucleated cell count, total protein concentration and haematocrit values are the most important indices, which, along with cytological findings, are used for the classification of an effusion (transudate, modified transudate, exudate, haemorrhagic effusion). Occasionally, the biochemical examination of effusions is necessary in order to determine the presence of fluid of a more specific aetiology (chyle, feline infectious peritonitis effusions, septic exudates), while microbiological examination remains a standard procedure in suspected septic effusions. After having considered the obtained information, clinicians are usually able to understand the aetiology behind cavitory fluid accumulation and thus make the best therapeutic decision for the cat patient.

> Introduction

Amongst the most common pathologic conditions of the pleural cavities, as those are defined by the serous membranes that cover the lungs and the inner surface of the thoracic walls, are the accumulation of either abnormal quantities of fluid (termed as pleural effusion) or air (termed as pneumothorax). In feline medicine, pleural effusions are the second most frequent cause of respiratory distress, exceeded only by heart disease.^{1,2,3} The diagnosis of an effusion is considered challenging in cat patients as cats tend to compensate and quickly resume their normal activity. The presence of a pleural fluid is confirmed by diagnostic imaging while in cases of acute respiratory distress, stabilizing measures such as oxygen supply and alleviating thoracocentesis are required. Fluid aspiration is performed with the cat placed in sternal recumbency by inserting either a butterfly needle (20-22 G) or an over-the-needle catheter (20-22 G) connected with a 3-way tap and a 10-50 ml syringe, into the 7th or 8th intercostal space.⁴ Laboratory investigation of feline pleural effusions is essential and is not greatly differentiated from that of dog effusions, with the exception of feline infectious peritonitis (FIP). The laboratory evaluation of feline pleural fluids offers valuable information on the pathogenesis of effusion formation which subsequently facilitates final diagnosis or narrows down the differential diagnosis. However, the in-clinic evaluation of effusions requires certain basic laboratory equipment, such as an automated haematology analyser or haemocytometer, a centrifuge and a light microscope.

The present study is a review of routine laboratory investigation conducted in cats with pleural effusions.

> Sample handling

The fluid aspirated via thoracocentesis is separated into different aliquots which are subsequently placed in K3-Ethylene Diamine Tetraacetic Acid (K3-EDTA) tubes, serum tubes and/or sterile plain tubes.^{5,2} Samples should be placed in EDTA tubes shortly after aspiration in order to prevent their clotting and should then be submitted for total nucleated cell count (TNCC), total protein (TP) and specific gravity (SG) evaluation, as well

Kritsepi-Konstantinou Maria

DVM, PhD, Associate professor,
Diagnostic Laboratory,
School of Veterinary Medicine,
Faculty of Health Sciences,
A.U.Th., Greece

Tsouloufi Theodora K.

DVM, PhD candidate,
Diagnostic Laboratory,
School of Veterinary Medicine,
Faculty of Health Sciences,
A.U.Th., Greece

Corresponding author:

Maria Kritsepi-Konstantinou,
Diagnostic Laboratory,
School of Veterinary Medicine,
Aristotle University of Thessaloniki,
11 Stavrou Voutyra St.,
54627 Thessaloniki, Greece
E-mail: mkritsep@vet.auth.gr
Tel: + 30 2310 994523
FAX: + 30 2310 994511

Keywords



- Body fluids
- Exudates
- Feline
- Pyothorax
- Transudate



as for smear preparation. Failure to prevent sample clotting might lead to false TNCC and cytological smears of poor quality.⁵ Other coagulants, such as Lithium-Heparin tubes should not be used as alternatives since certain cytological features of the sample might be altered.⁶ At this point, it must be noted that direct smears should always be prepared within 30 minutes of fluid collection in order to avoid sample deterioration and potential cytological misinterpretation.⁷

Aliquots placed in serum tubes are submitted for biochemical examination of the fluid. Samples should be centrifuged at 3,000 rpm for 5 minutes and the supernatant is then submitted for evaluation. It should be noted that when glucose (GLU) concentration needs to be determined (see *Biochemical analysis*), the supernatant should be separated within 30-60 minutes of sample collection.⁸ Hazy supernatants may interfere with the results obtained for several analytes. Sterile plain tubes are used for microbiological examination (bacterial/fungal cultures, susceptibility tests) in cases suspected of septic inflammation or microbial contamination. In the event of suspected anaerobic infection, the sample should be collected with minimum oxygen contamination and subsequently placed in an anaerobic bacteria transport medium.⁹ Samples placed in EDTA tubes are inappropriate for bacteriological culture due to the bacteriostatic and bactericidal properties of EDTA.⁸ Cytological examination of the fluid requires a minimum of two smears per sample, direct or sediment; the latter pertains to fluids of low cellularity (recommended in fluids with less than 3×10^9 cells/L). Instead of sediment smears (by means of sample centrifugation or a crafted sample chamber), cytopspin smears could be prepared. Buffy-coat smear preparation might be proven useful in the evaluation of haemorrhagic or neoplastic effusions.⁸ On the other hand, fluids of high cellularity are strictly examined as direct smears.⁸

Technically, smears from fluids are either prepared as line or feathered-edge smears. In line smears, the slide is lifted up abruptly covering a short distance allowing a line of fluid to be created that is expected to have a profusion of cells.^{7,8} After being air-dried and fixed with common fixatives (e.g. methanol), the smears are either stained with a Romanowsky-type stain (e.g. Giemsa) if they are to be evaluated in-clinic, or are preferably saved unstained if they are to be submitted to an external laboratory.

> Clinicopathological investigation of feline pleural effusions

Taking into account the physical and biochemical properties of the sample, as well as the absolute and the differential cell count along with the TP and SG values, pleural effusions are classified into pure or

modified transudates, septic or non-septic exudates, of lymph, blood and neoplastic origin (Table 1).⁸ Biliothorax has been previously reported in a Siamese cat as a post-thoracostomy complication.¹⁰ While this classification discloses the pathogenesis of the fluid accumulation, little information is gained in terms of aetiological diagnosis.¹¹

Macroscopic evaluation

Initially, fluid samples are evaluated macroscopically as to their colour, turbidity, odour and clot formation.⁵ As a general rule, colourless and clear samples are suggestive of fluids of low cellularity (transudates), yellowish to pink samples with a clear to slightly turbid appearance are usually indicative of fluids of low to moderate cellularity (modified transudate), whereas a turbid to opaque appearance suggests fluids of moderate to high cellularity (exudates).⁸ A green-brown sample, generally accompanied by a foul smell, is typical of a septic exudate, although odourless samples are not always bacteria-free.^{5,12,13} Tints of red colour suggest blood contamination of the sample, while serosanguinous or pure red colour is compatible with haemothorax.^{5,11,14}

A lactescent fluid is highly suggestive of chylothorax.⁵ In some cases of confirmed chylothorax, colourless to serosanguinous fluid has also been obtained.¹¹ The differentiation of chylous from pseudo-chylous effusions rests mainly on a biochemical and cytological basis (see *Biochemical analysis* and *Cytological evaluation*), although pseudo-chylous effusions are usually not milky white but opaque, because of cell debris content.⁵ However, it should be noted that chylous effusions from anorectic animals might be opaque rather than milky.⁷ To define the presence of fibrinogen in effusions, a rather simple test can be performed by placing a small amount of sample into a serum tube and observing any clot formation.¹⁵ Threads or flakes of fibrin may be reported in exudates, chylous or blood effusions, indicating elevated TP concentration.^{5,14} Notably, bloody samples rarely clot.^{12,14}

Gross evaluation of supernatant after centrifugation is also important, especially in turbid, bloody or lactescent samples.¹² In cases of turbid or milky samples, if turbidity dissolves after centrifugation, cells or debris are considered to have imparted this turbid appearance to the sample (pseudo-chylothorax). On the other hand, if turbidity persists after centrifugation, a triglyceride-rich effusion is most likely (chylothorax).^{12,16} Additionally, in chylothorax cases after centrifugation, a characteristic "creamy" layer of chylomicrons may develop on top of the fluid.⁵ In bloody samples, the supernatant may present either a typical plasma appearance, clear or with a grade of a haemolysis if acute or chronic haemorrhage is the primary cause, or a yellowish hue in chronic effusions

**Table 1.** Classification of pleural effusions¹

Pleural fluid	Transudate	Modified transudate	Non-septic exudate	Septic exudate	Lymph	Blood	Neoplastic effusion
Colour	Clear to yellowish	Yellowish to pink	Yellowish to pink	Yellow to brown-red/green	Opalescent white (occasionally yellow, pink or red, depending on diet)	Red	Variable
Turbidity	Transparent	Transparent to slightly turbid	Transparent to turbid	Turbid to opaque	Opaque (even after centrifugation)	Opaque	
Odour	No	No	No	Putrid	No	No	No
Presence of fibrin	No	No	Yes (thread or flake shape)	Yes (thread or flake shape)	Yes (variable)	Yes	Variable
Total Nucleated Cell count (TNCC)	<1.5 x10 ⁹ cells/L	<5-7x10 ⁹ cells/L	>5x10 ⁹ cells/L (FIP <5x10 ⁹ cells/L)	>5x10 ⁹ cells/L	<10x 10 ⁹ cells/L	Same as peripheral blood	Variable
Total Proteins (TP)	<25 g/L	>25 g/L (reported values 25-75 g/L)	25-60 g/L (FIP ≤ 85g/L)	30-70 g/L	25-65 g/L	>30 g/L	Variable (usually >25 g/L)
Specific Gravity (SG)	<1,015	1,015-1,040	1,015-1,032	1,017-1,035	1,015-1,035	> 1,018	Variable
Triglyceride content	No	No	No	No	Yes (levels higher than in serum)	No	No
Presence of bacteria	No	No	No	Yes	No	No	No
Cytology	Neutrophils, macrophages, some mesothelial cells, occasionally lymphocytes	Macrophages and mesothelial cells predominate, elevated number of neutrophils and small lymphocytes	Non-degenerated neutrophils and macrophages predominate, absence of bacteria	Degenerated neutrophils and macrophages predominate, intracellular/extracellular bacteria	Small lymphocytes, occasionally neutrophils and macrophages	Red blood cells predominate with some white blood cells/erythrophagocytosis, haemosiderin granules, haematoidin crystals	Neoplastic cells, macrophages, neutrophils, reactive mesothelial cells

¹ Modified from Cowell and Tyler's Diagnostic Cytology and Hematology of the dog and cat. Valenciano AC, Cowell RL (eds). 4th edn. Elsevier Mosby: St. Louis, 2014

or if red blood cell breakdown has occurred.¹⁷

Feline infectious peritonitis effusions are characterized by specific macroscopic features common to effusions with elevated TP values: frothing when agitated, clot formation even in EDTA tubes, high viscosity and clear yellow colour.^{11,13,17,18} Nonetheless, other pathologic conditions should be excluded before making an FIP diagnosis, even though the fluid seems typical.¹⁸

Cell counts

Cell counts in effusions are typically performed on automated haematology analysers. Some in-clinic haematology analyzers can yield TNCC with considerable accuracy.^{19,20} Nevertheless,

automated cell counts cannot replace the cytological examination of the fluid as results do not always correlate well with cytological appearance.⁵ Alternatively, a haemocytometer might be used.^{5,16}

The absolute cell count comprises TNCC, red blood cell count (RBCC) and platelet count (PLTC).¹⁷ The TNCC, along with TP/SG values and the respective cytological features allow a primary classification of effusions into transudates, modified transudates and exudates.⁷ In general terms, transudates and modified transudates are characterized by low TNCC (TNCC <1.5 × 10⁹cells/L), whereas exudates consistently exhibit high TNCC (TNCC >5 × 10⁹cells/L).⁵ Feline infectious peritonitis effusions are mostly considered as exudates, although they have low TNCC.⁵ When performed on a haematology



analyser, TNCC not only includes WBC but also cells like mesothelial cells, macrophages and neoplastic cells.^{1,12} Thus, microscopic examination of the sample is necessary in order to identify the cells present. Red blood cell counts, haematocrit (Hct) values and PLTC depict the presence of blood in the sample. In the event that an automated method is unavailable, packed cell volume (PCV) might be performed by means of Wintrobe or microhaematocrit tubes.²¹ If the presence of blood is due to acute haemorrhage, the Hct or PCV of fluid is similar to the respective of the peripheral blood.⁷ However, in chronic haemorrhages, Hct or PCV values usually decline.²¹ Effusions with peripheral blood contamination due to accidental laceration of capillaries during fluid aspiration are characterized by low Hct or PCV values.⁵

Automated or manual differential cell count yields information concerning the presence and type of inflammation. The respective cell categories that are included in the count are all nucleated cells (mononuclear cells, polymorphonuclear cells) and they are usually the same as in CBCs. Consequently, an acute inflammatory process is characterized by high neutrophil counts, while chronic effusions mostly yield high large mononuclear (macrophages) and small lymphocyte counts. Blood contamination of the sample should be considered before a differential cell count is performed.

Total Proteins (TP) and Specific Gravity (SG)

Determination of TP values in feline effusions by means of refractometry is considered a reliable method, although dry chemistry analyzers generally afford higher accuracy for TP determination.^{5,22} Urine strips have also been used for in-house determination of TP concentrations below or above 20 g/L.²³ Total protein and SG constitute indices of ongoing inflammation and its intensity.¹¹ Elevated TP values (>25 g/L) usually suggest modified transudates or exudates, whereas low TP values (<25 g/L) are indicative of transudates. Should an effusion not clearly fall into one of the three main categories, TP concentration is considered more reliable for the differentiation of transudates from modified transudates, while TNCC is considered more reliable for the differentiation of modified transudates from exudates.¹⁵ Feline infectious peritonitis effusions are mostly considered as exudates.⁵

Specific gravity values are determined by means of refractometry and roughly depict the solute concentration of the fluid. However, SG values typically apply to urine analysis and are not diagnostically sensitive in the case of pleural effusions.²¹ Determination of TP and SG values should be performed in the sample's supernatant, as the fluid's turbidity might lead to falsely elevated

TP values by refractometry or spectrophotometry.^{5,11} Lactescent samples are frequently unsuitable for TP and SG evaluation for the same reason.^{11,13,17} Bloody samples should be handled as above, while relatively transparent samples can be measured without former centrifugation.⁵ Regarding effusions with suspected FIP, TP values above 35 g/L (>50% globulins) have been reported to attain sensitivity of 100% for FIP diagnosis.²⁴

Biochemical analysis

The biochemical analytes usually evaluated in effusions include levels of pH, GLU, albumins (ALB), globulins (GLOB), triglycerides (TG), lactate dehydrogenase (LDH) and cholesterol (CHOL). These analytes are concurrently determined in the animal patient's fluid and serum and subsequently compared.

The pH determination of pleural fluids is a valuable prognostic tool for parapneumonic effusions in human medicine.²⁵ It is usually performed by the introduction of a pH-meter sensor in the collected sample or a blood gas analyzer. However, precision in pH values is rarely attained as the sample to be evaluated should be air-free, following collection using a heparinised syringe, and subsequently examined rapidly. As a general rule, pH values decline in exudative effusions as the result of lactic acid produced by the bacterial population.¹²

Lactate dehydrogenase values in pleural effusions demonstrate the degree of inflammation in the pleural cavity, as LDH is released from damaged or destroyed cells.^{12,13,25} In humans, high LDH values are usually encountered in exudates.¹³ Lactate dehydrogenase values have also been determined in feline pleural effusions. Indicatively, in cases of FIP, pleural effusions have LDH values over 300 IU/L, while LDH values in septic exudates usually exceed 200 IU/L. Lactate dehydrogenase levels in the effusion, along with the pleural fluid/serum TP ratio, have been found to attain sensitivity of 100% and 91% and specificity of 100%, respectively, for the differentiation between transudative and exudative effusions in cats.¹³

The determination of lactate values for the differentiation of septic from non-septic pleural effusions has not been adequately evaluated in dogs and cats. Conversely, the concentration of lactate in feline peritoneal effusions was not considered reliable for the diagnosis of septic effusions.²⁶

Concentrations are usually low in exudates, possibly as a result of the metabolic consumption of GLU from leucocytes, neoplastic cells and/or pleura, as well as their irregular transportation from inflamed pleural membranes.¹² Some studies relate the pH values in pleural effusions to their respective GLU concentration.¹³ In septic exudates, GLU





concentration is usually below 1.7 mmol/L, while in neoplastic effusions it ranges between 0.5 and 4.5 mmol/L.¹³

Albumin-globulin ratio is mostly used in pleural effusions with suspected FIP and is considered to possess the highest diagnostic value compared to other tests for FIP.¹⁸ As FIP effusions are typically characterized by high TP concentrations, with GLOB being at least 50%, an ALB-GLOB ratio below 0.81 and especially below 0.4 is suggestive of the disease.^{5,24} In-house dry chemistry analyzers have been considered to lack precision for ALB determination in feline effusions in contrast to wet chemistry analysers.²²

Triglycerides and CHOL concentrations in pleural effusions are considered the best markers for the differentiation of chylous from pseudo-chylous effusions (rarely reported in dogs and cats) or for the diagnosis of atypical chylous effusions.⁵ In particular, chylous effusions have higher triglyceride and lower cholesterol concentrations than those in serum, while the opposite applies to pseudo-chylous effusions.⁵ A CHOL-TRIG ratio <1 is suggestive of chylous effusions.²⁷ It should be noted that pleural effusions due to feline cardiac disease are considered chylous.⁷

N-terminal pro-B-type natriuretic peptide values have been found to be elevated in both sera and pleural effusions from cats with cardiac disease. However, further studies are needed for the evaluation of this biomarker.²⁸

Microbiological examination

Microorganisms found in pleural effusions are either due to an endogenous inflammatory process (pyothorax) or iatrogenic contamination.¹⁴ In cats, obligate anaerobes and facultative anaerobes such as *Pasteurella spp.* are most commonly isolated.³ In a study of cats with pyothorax, 89% of samples yielded obligate anaerobes, while 44% yielded a mixture of obligate anaerobes and facultative bacteria.²⁹ Regarding fungi, *Histoplasma capsulatum* and *Cryptococcus neoformans* are mostly considered to be related to pleural effusion formation in cats,³⁰ although they do not constitute common entities for veterinary practice in Greece.

Chylous samples, in which bacterial growth normally is not facilitated because of the presence of fatty acids, might also be submitted for microbiological examination, especially when repeated thoracocenteses have been performed.³ In a study of 37 cats with chylothorax, microbiological cultures were positive in 13.5% of cases (iatrogenic pyothorax).³¹ It should be noted that previous antibiotic or antifungal therapies administered must always be considered when submitting samples for microbiological examination.

> Other tests

Additional diagnostic procedures available for pleural effusion investigation not only include plain methods like the Rivalta test but also advanced laboratory techniques such as histopathology, immunocytochemistry, immunofluorescence, reverse transcription-polymerase chain reaction/enzyme linked immunosorbent assay (RT-PCR/ELISA) and electrophoresis. The above tests are performed especially in cats with suspected FIP in order to establish diagnosis.

Rivalta's test

On a primary basis, the Rivalta's test has been used for the differentiation of transudative from exudative effusions. The test has been most commonly used for the diagnosis of FIP effusions, for which it has been reported to yield specificity of 80% and sensitivity of 98%.¹⁸ However, Fischer et al. have recently reported 91% sensitivity, 66% specificity and a positive predictive value (PPV) of 58% for the Rivalta's test.³² Positive results are linked to high concentrations of protein, fibrin and inflammatory mediators, while false positive results may arise in patients with bacterial peritonitis or lymphoma.¹⁸ The procedure is described as follows: 5 ml of distilled water is placed in a sample tube along with 1 drop of acetic acid (98% v/v). Subsequently, 1 drop of the effusion under study is placed on the upper surface of the solution. The test is characterized as "positive" when the drop remains on the solution surface or sinks slowly to the bottom of the tube, or "negative" when the drop dissipates in the solution (Figure 1).

Direct immunofluorescence

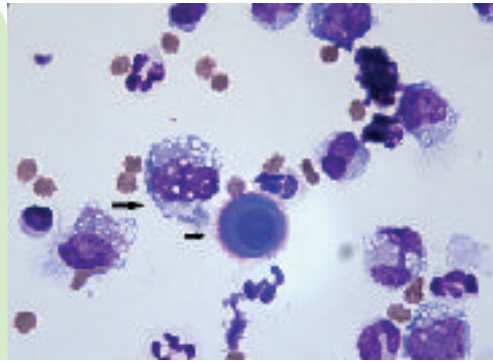
Direct immunofluorescence (DIF) is performed either on cytological or biopsy preparations for the detection of Feline Coronavirus (FCoV) antigen. The method has been reported to have sensitivity of 100% and specificity of 71.4% in effusion samples.³²



Figure 1. Positive Rivalta reaction.



Figure 2. Mesothelial cell with a distinct eosinophilic fringe (short arrow). A moderate number of activated macrophages (long arrow) is present, as well as a few red blood cells and neutrophils (Giemsa, 100x).



In biopsy samples, the method possesses sensitivity and specificity 100% for FIP diagnosis. However, it attains low negative predictive value (NPV).³³

Electrophoresis

Electrophoresis is mostly used in effusions highly suggestive of FIP, when α_2 - and γ - globulins concentrations rise. This method has been reported to have 100% positive predictive value (PPV) when γ -globulin content was >32%.³⁴ Lipoprotein electrophoresis has also been performed in pleural effusions from cats for the differentiation of chylous from non-chylous effusions.³⁵

> Cytological evaluation

Cytology attains sensitivity of 61% and specificity of 100% in the diagnosis of neoplastic body cavity effusions in cat patients; thus, along with histopathology, it remains a valuable tool for establishing diagnosis.³⁶

Cells types

The cell types that might be encountered in cat pleural effusions are mesothelial cells, RBCs, inflammatory cells such as mature, non- and degenerated or hypersegmented neutrophils, non- and activated macrophages, small lymphocytes and eosinophils, as well as neoplastic cells.^{5,7,11} Rarely, mast cells may also be encountered.^{7,11} Apart from cells, other features may also be observed such as bacteria, fungi and plant fibres.

As to mesothelial cells, these normally line the pleural cavities and are either naturally exfoliated in the pleural fluid or dislodged during sample collection.¹¹ Additionally, mesothelial cells are considered to play a role in the inflammatory process by means of cytokine production and antigen presentation.⁵ In terms of morphology, mesothelial cells are large cells with a round to oval nucleus which is typically centrally located and exhibits a uniformly-distributed-chromatin pattern, and basophilic cytoplasm. Occasionally, an

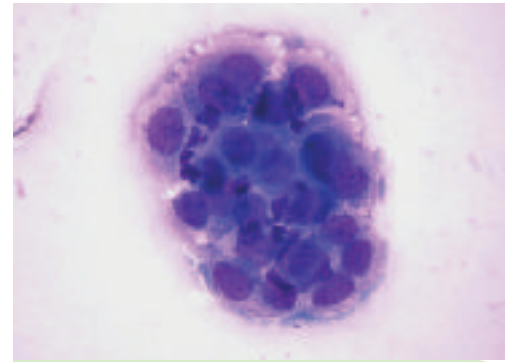


Figure 3. A sheet of reactive mesothelial cells with evidence of neutrophil emperipolesis (Giemsa, 100x).

eosinophilic fringe may be present peripherally.⁸ Mesothelial cells can be found either as single cells or in sheets (Figure 2-3).⁸

Eosinophilic pleural effusions in cat patients have previously been linked to pneumothorax, feline immunodeficiency virus infection, visceral mast cell tumours, hypereosinophilic syndrome and pulmonary parasitic infections.^{37,38,39,40} Eosinophil percentages above 10% of TNNC in effusions may be indicative of a neoplastic disease.

Transudates (true and modified)

In true transudates, cytological findings are characterized as non-typical and usually involve low counts of mature, non-degenerated neutrophils, mesothelial cells, macrophages at various stages of activation and small lymphocytes.^{5,11}

The most distinctive cytological feature of modified transudates is reactive mesothelial cells.¹¹ Due to effusion chronicity, mesothelial cells usually tend to react and thus become binucleated, with multiple, distinct nucleoli, and increased phagocytic activity.¹⁹ Moreover, high counts of non-degenerated neutrophils are commonly observed as well as activated macrophages (Figure 4).^{7,11}

Exudates (septic and non-septic)

Septic exudates are typically characterised by the predominance of degenerated neutrophils and the presence of intracellular and/or extracellular bacteria. Against the smear background, eosinophilic proteinaceous material is frequently observed. Degenerated neutrophils have been subjected to hydropic degeneration due to their exposure to bacterial toxins, and exhibit a swollen nucleus with less noticeable lobulation which is stained as eosinophilic with Romanowsky-type stains.⁷ In the presence of degenerated neutrophils, the smear should be extensively examined for bacteria, whilst

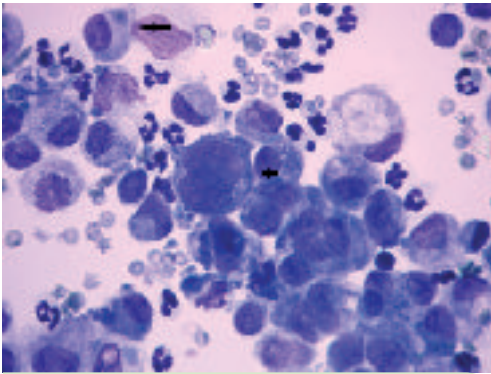


Figure 4. Granulomatous inflammation. Macrophages with signs of activation and erythrophagocytosis (long arrow). A binucleated mesothelial cell is also present (short arrow) (Giemsa, 100x).

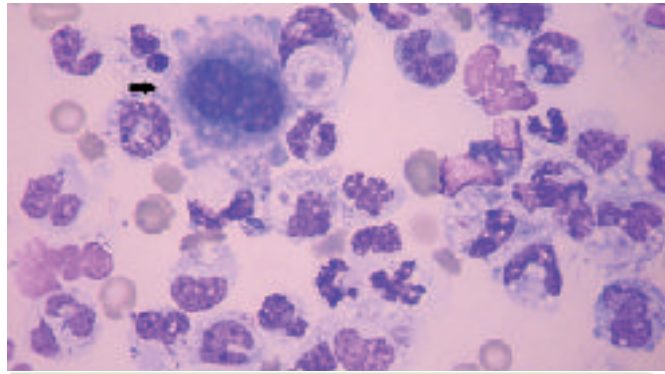


Figure 5. Septic inflammation. Degenerated neutrophils with phagocytized bacterial rods and cocci predominate. A binucleated mesothelial cell is also present (arrow) (Giemsa, 100x).

when degenerated neutrophils are not observed, a bacterial infection should not be excluded as toxin quality and quantity vary according to the species and number of bacteria present.⁷

Nonetheless, the key to cytological diagnosis of septic exudates is the observation of intracellular bacteria, while observation of extracellular bacteria might be due to a contaminated stain. Bacteria usually appear light pink to deeply basophilic when a Romanowsky-type stain is used and are most commonly found phagocytised by neutrophils and sometimes by macrophages (Figure 5).⁵

In non-septic exudates, non-degenerated neutrophils mainly predominate, while bacteria are not observed.^{5,8} Activated macrophages may also be present, as well as a low number of small lymphocytes.^{5,8}

Chylous and pseudochylous effusions

In chylous effusions, small lymphocytes usually predominate in the overall cell population, while



Figure 6. Chylous effusion contaminated with peripheral blood. Note the milky white appearance of the supernatant.

lipid droplets might be found in the background.^{5,8} However, as the effusion becomes chronic, pleura may exhibit signs of inflammation and thus, the predominant cell types are mature, non-degenerated neutrophils and/or macrophages (mostly activated, containing cytoplasmic vacuoles, which are related to lipids).⁷ Moreover, reactive mesothelial cells may also be reported, due to the irritation of pleura, as well as reactive lymphocytes (Figure 6-7).⁵

On the other hand, in pseudochylous effusions, the cytology is variable depending on the primary cause of the effusion. Hence, in pseudochylous effusions of neoplastic origin, neoplastic cells prevail, while in pseudochylous effusions of inflammatory origin, non-degenerated neutrophils and /or macrophages predominate.⁵

Haemorrhagic effusions

In haemorrhagic effusions, blood cells typically prevail as they exist in the peripheral blood of the cat patient. Macrophages and mesothelial cells may occasionally be observed. However, the presence of platelets varies, depending on whether there is

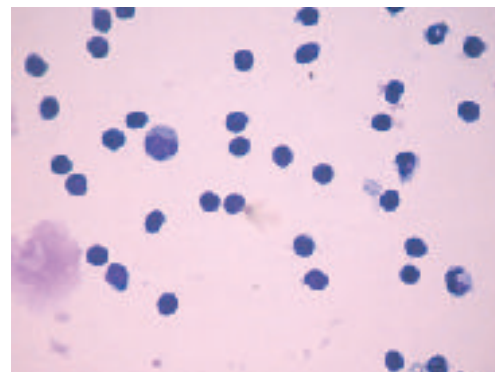
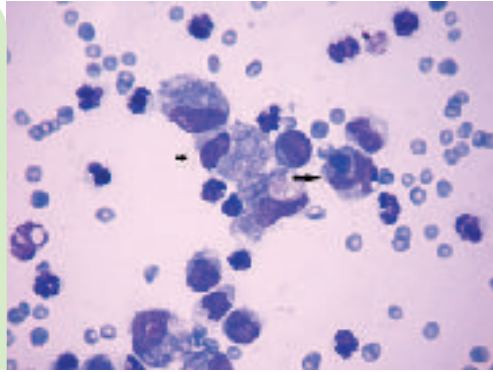


Figure 7. Chylothorax. Small lymphocytes are the predominant cell population. (Giemsa, 100x).



Figure 8. Erythrophagocytosis in a haemothorax (short arrow). A few phagocytes are also present (long arrow).



chronic haemorrhage or peripheral contamination and/or per-acute haemorrhage.^{5,8} In the first case, platelets are usually absent as they tend to form aggregations, become degranulated and thus, rapidly disappear.^{5,8} On the other hand, in cases of peripheral blood contamination and/or per-acute haemorrhage, platelets are usually present in the smear.^{5,8}

Chronic haemorrhages are also accompanied by the remarkable presence of activated erythrophages and signs of haemoglobin decomposition. The latter is marked by the presence of black-green granules (haemosiderin), mainly in the cytoplasm of macrophages and also in the background, as well as by the presence of rhomboid, yellow to orange crystals (haematoidin) in the cytoplasm of macrophages, especially when the haemorrhage has progressed in chronicity (Figure 8).⁵

Neoplastic effusions

The diagnostic value of cytology when neoplastic effusions are examined basically depends on the propensity of the neoplasm for exfoliation. In particular, neoplasms of epithelial origin, as well as round-cell tumours, tend to become easily exfoliated into body effusions, thus yielding samples of moderate to high cellularity.⁵ Signs of co-existent inflammation may also be present.^{7,8}

Intrathoracic tumours, whether primary or metastatic, are commonly involved in pleural effusion formation in cats.⁴⁷ Primary tumours in cats involve mesotheliomas and pulmonary tumours such as bronchial adenoma/adenocarcinoma and bronchioalveolar carcinomas. Mediastinal lymphomas are more often encountered from round-cell tumours, whereas sarcomas, both metastatic and primary, are rarely found in pleural cavities.^{7,8}

Neoplastic effusions usually fall into the modified transudate or exudate categories, although chylous or haemorrhagic effusions may also occur.^{5,7}

The most distinctive feature about carcinomas is that

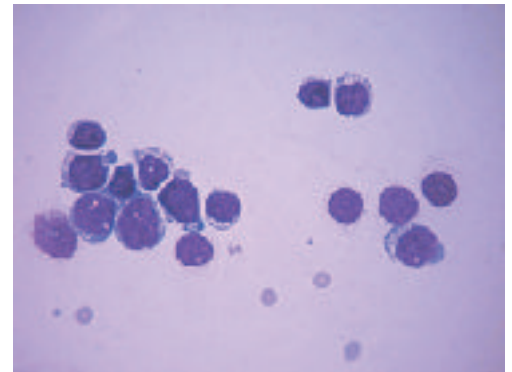


Figure 9. Neoplastic pleural effusion due to mediastinal lymphoma. Medium-sized lymphocytes with marked cytoplasmic basophilia and vacuolation predominate (Giemsa, 100x).

the epithelial cells are typically presented in clusters or sheets, while acinar patterns may sometimes be noted (adenocarcinomas).^{7,8} Neoplastic epithelial cells mainly exhibit anisokaryosis, multinucleation, high nuclear-cytoplasmic ratio, coarse nuclear chromatin pattern, multiple and prominent nucleoli, with variable appearance and abnormal mitoses.⁷ Basophilia and vacuolation of the cytoplasm may also be reported. In mesotheliomas, cells are characterized by the aforementioned criteria of malignancy. Common misinterpretations occur with cytology between hyperplastic or reactive mesothelial cells, neoplastic mesothelial cells and carcinomas, as the first tend to have features that assimilate malignancy.^{5,7,8}

In terms of mediastinal lymphomas, medium-sized lymphocytes and lymphoblasts are predominant in the cell population (usually, over 50% of the population).^{7,8} These cells may exhibit multiple, irregularly-shaped nucleoli and cytoplasmic basophilia and vacuolation. Moreover, several mitotic figures and lymphoglandular bodies might be present.⁸ Scarcely, small cell lymphomas occur in which small lymphocytes predominate (Figure 9).^{8,11,14}

> Conclusion

Whether performed in-clinic or by an external laboratory, the clinicopathological investigation of feline pleural effusions is an essential component of routine diagnosis. The categorisation of effusions into transudates, modified transudates and exudates, as well as into haemorrhagic and chylous effusions, is vital to the understanding of the aetiopathogenesis underlining their formation. Once the latter is established, a more focused therapeutic management is made possible.



> References

- Nelson LO, Sellon RK. Pulmonary parenchymal diseases. In: Textbook of Veterinary Internal Medicine. Ettinger SJ, Feldman EC (eds). 6th edn. Saunders Elsevier: St. Louis, 2004, pp. 1239-1266.
- Fossum TW, Miller MW, Rogers KS, Bonagura JD, Meurs KM. Chylothorax associated with right-sided heart failure in five cats. *J Am Vet Med Assoc* 1994, 204(1): 84-89.
- Moore LE, Biller DS. Mediastinal disease. In: Textbook of veterinary internal medicine. Ettinger SJ, Feldman EC (eds). 6th edn. Saunders Elsevier: St. Louis, 2004, pp. 1266-1283.
- Bexfield N, Lee K. Thoracocentesis. In: BSAVA Guide in Procedures in Small Animal Practice. Bexfield N, Lee K (eds). British Small Animal Veterinary Association: Gloucester, 2011, pp. 195-197.
- Papasouliotis K, Dewhurst E. Body cavity effusions. In: BSAVA manual of canine and feline clinical pathology. Villiers E, Blackwood L (eds). 2nd edn. BSAVA: Gloucester, 2005, pp. 340-354.
- Reece WO. The Respiratory System. In: Functional anatomy and physiology of domestic animals. Reece WO (ed). 4th edn. Wiley-Blackwell: Iowa, 2009, pp. 269-311.
- Valenciano AC, Arndt TP, Rizzi TE. Effusions: Abdominal, Thoracic and Pericardial. In: Cowell and Tyler's Diagnostic Cytology and Hematology of the dog and cat. Valenciano AC, Cowell RL (eds). 4th edn. Elsevier Mosby: St. Louis, 2014, pp. 244-265.
- Rebar AH, Thompson CA. Body Cavity Fluids. In: Canine and feline cytology. Raskin RE, Meyer DJ (eds). edn. Saunders Elsevier: St. Louis, 2009, pp. 171-190.
- Stillion JR, Letendre JA. A clinical review of the pathophysiology, diagnosis and treatment of pyothorax in dogs and cats. *J Vet Emerg Crit Care* 2015, 25(3): 113-129.
- Wustefeld-Janssens BG, Loureiro JF, Dukes-McEwan, German AJ, Burrow RD. Biliothorax in a Siamese cat. *J Fel Med Surg* 2011, 13(12): 984-987.
- Rebar AH, DeNicola DB. Cytology of body fluids. In: Congress proceedings of the North American Veterinary Conference. Orlando, Florida, 2007, pp. 248-252.
- Light RW. Pleural effusion. In: Textbook of Respiratory Medicine, Murray JF, Nadel JA (eds). 2nd edn. WB Saunders: Philadelphia, 1994, pp. 2164-2192.
- Zoia A, Slater LA, Heller J, Connolly DJ, Church DB. A new approach to pleural effusion in cats: markers for distinguishing transudates from exudates. *J Feline Med Surg* 2009, 11(10): 847-885.
- De Nicola DB. Feline thoracic and abdominal effusion evaluation. Common presentations. In: Congress proceedings of the international congress of the Italian Association of Companion Animal Veterinarians. Rimini, Italy, 2008, pp. 139-140.
- Baker R, Lumsden JH. Pleural and peritoneal fluids. In: Color atlas of cytology of dog and cat. Baker R, Lumsden JH (eds). Mosby: St. Louis, 2000, pp. 159-176.
- Smith RIE. The cat with a cloudy eye. In: Problem-based feline medicine. Rand J (ed). 1st edn. Saunders Elsevier: St. Louis, 2006, pp. 1254-1277.
- Kerr MG. Non-blood body fluids. In: Veterinary laboratory medicine. Kerr MG (ed). 2nd edn. Blackwell Science Ltd: Oxford, 2002, pp. 169-180.
- Hartmann K, Binder C, Hirschberger J, Cole D, Reinacher M, Schroo S, Frost J, Egberink H, Lutz H, Hermanns W. Comparison of different tests to diagnose feline infectious peritonitis. *J Vet Intern Med* 2003, 17(6): 781-790.
- Giannasi C, Brown A and Skeldon N. Evaluation of HemoCue WBC as a bedside analyser in characterising abdominal effusions. In: BSAVA Congress Scientific Proceedings Abstracts. Quedgeley, Gloucester, 2013, pp. 558.
- Welles EG, Oller E, Spangler EA, Suddeth J. Validation of an in-office automated haematology instrument, the Heska CBC-Diff, for total nucleated cell counts in body cavity effusions and comparison of differential cell counts with manual observations from prepared smears. In: ASVCP Annual Meeting Abstracts. Nashville, TN, 2011, p. 597.
- Stockham SL, Scott MA. Cavitary effusions. In: Fundamentals of Veterinary Clinical Pathology. Stockham SL, Scott MA (eds). 2nd edition. Blackwell Publishing: Iowa, 2008, pp. 831-868.
- Papasouliotis K, Murphy K, Dodkin S, Torrance AG. Use of the Vettesh 8008 and Refractometry for Determination of Total Protein, Albumin and Globulin Concentrations in Feline Effusions. *Vet Clin Path* 2002, 31(4): 162-166.
- Braun JP, Guelfi JF, Pages JP. Comparison of four methods for determination of total protein concentrations in pleural and peritoneal fluid from dogs. *Am J Vet Res* 2001, 62(3): 294-296.
- Sparkes AH, Gruffydd-Jones TJ, Harbour DA. An appraisal of the value of laboratory tests in the diagnosis of feline infectious peritonitis. *J Am Anim Hosp Assoc* 1994, 30(4): 345-350.
- Herrtage ME. Respiratory disorders. In: Clinical medicine of dog and cat. Schaer M (ed). 2nd edn. Manson Publishing: 2003, pp. 183-188.
- Bonczynski JJ, Ludwig LL, Barton LJ, Loar A, Peterson ME. Comparison of peritoneal fluid and peripheral blood pH, bicarbonate, glucose, and lactate concentration as a diagnostic tool for septic peritonitis in dogs and cats. *Vet Surg* 2003, 32(2): 161-166.
- Fossum TW, Jacobs RM, Birchard SJ. Evaluation of cholesterol and triglyceride concentrations in differentiating chylous and non-chylous pleural effusions in dogs and cats. *J Am Vet Med Assoc* 1986, 188(1): 49-51.
- Hassdenteufel E, Henrich E, Hildenbrandt N, Stosic A, Schneider M. Assessment of circulating N-terminal pro B-type natriuretic peptide concentration to differentiate between cardiac from noncardiac causes of pleural effusions in cats. *J Vet Emerg Crit Care* 2013, 23(4): 416-422.
- Love DN, Jones RF, Bailey M, Johnson RS, Gamble N. Isolation and characterization of bacteria from pyothorax (empyema) in cats. *Veterinary Microbiology* 1982, 7: 455-461.
- Sauve V. Pyothorax in cats: a Review. *Full Circle Forum* 2011, 4: 1-2.
- Forester SD, Troy GC, Fossum TW. Pleural effusions: pathophysiology and diagnostic considerations. *Comp Cont Educ* 1988, 10: 121-137.
- Fischer Y, Sauter-Louis C, Hartmann K. Diagnostic accuracy of the Rivalta test for feline infectious peritonitis. *Vet Clin Path* 2012, 41(4): 558-567.
- Paltrinieri S, Parodi Cammarata M, Camarata G. In vivo diagnosis of feline infectious peritonitis by comparison of protein content, cytology and direct immunofluorescence test on peritoneal and pleural effusions. *J Vet Diagn Invest* 1999, 11(4): 358-361.
- Shelly SM, Scarlett-Kranz J, Blue JT. Protein electrophoresis on effusions from cats as a diagnostic test for feline infectious peritonitis. *J Am Anim Hosp Assoc* 1988, 24(5): 495-500.
- Waddle JR, Giger U. Lipoprotein Electrophoresis Differentiation of Chylous and Nonchylous Pleural Effusions in Dogs and Cats and Its Correlation with Pleural Effusion Triglyceride Concentration. *Vet Clin Path* 2009, 19(3): 80-85.
- Hirshberger J, Koch S. Validation of the determination of the activity of adenosine deaminase in the body effusions of cats. *Res Vet Sci* 1995, 59(3): 226-229.
- Miller BH, Roudebush P, Ward HG. Pleural effusion as a sequela to aelurostrongylosis in a cat. *J Am Vet Med Assoc* 1984, 185(5): 556-557.
- Fossum TW, Wellman M, Relford RL, Slater MR. Eosinophilic pleural or peritoneal effusions in dogs and cats: 14 cases (1986-1992). *J Am Vet Med Assoc* 1993, 202(11): 1873-1876.
- Peaston AE, Griffey SM. Visceral mast cell tumour with eosinophilia and eosinophilic peritoneal and pleural effusions in a cat. *Aust Vet J* 1994, 71(7): 215-217.
- Saxon B, Hendrick M, Waddle JR. Restrictive cardiomyopathy in a cat with hypereosinophilic syndrome. *Can Vet J* 1991, 32(6): 367-369.

