

The Cytological Evaluation of Effusions: What's the confusion?

A. Rick Alleman, DVM, PhD, Diplomate ABVP, ACVP
Lighthouse Veterinary Consultants, LLC

Introduction

Normally, only a small amount of fluid is present in the body cavities of dogs and cats. This fluid provides lubrication that allows the frictionless movement of adjacent organ surfaces and the body cavity walls. Effusions are the abnormal or increased accumulation of this fluid in any of the body cavities that are lined by mesothelial cells. These include the thoracic, pericardial, and abdominal cavities. Effusions are commonly encountered in veterinary practice. These body fluids accumulate as a result of one or more of a number of disease conditions including, but not limited to, trauma, neoplasia, cardiovascular compromise, metabolic disorders (hypoalbuminemia), and infectious/ inflammatory diseases. Proper collection and evaluation of body cavity fluids can provide diagnosticians with valuable information that will assist in identifying the disease process responsible for the fluid accumulation. In this session, the methods for fluid collection and evaluation are detailed. Practical application of diagnostic cytology in the evaluation of effusions is emphasized in an effort to guide the practicing veterinarian toward determining the underlying etiology and a more efficacious therapeutic intervention.

Fluid Collection and Processing

Thoracic and abdominal fluid may be collected using a 20-22 gauge needle and a 6 cc syringe. The needles are best inserted at an angle to avoid damage to any vital structures and prevent leakage of fluid into surrounding tissues. A specimen containing 2 ccs of fluid is usually adequate for all diagnostic procedures. A small portion of the fluid ($\frac{1}{2}$ to 1 cc) should be placed in a red-top tube in the event culture and sensitivity are required. The remaining fluid is placed in EDTA-containing tubes for cytologic analysis.

Pericardiocentesis is best approached on the right side, with the animal in left lateral recumbency, at the 5th or 6th intercostal space. The procedure is usually performed under local anesthesia, but sedation may be required in some cases. The area is clipped and scrubbed and a small stab incision is made in the skin. A 16 gauge, $3\frac{1}{4}$ inch, radiopaque, Teflon catheter is inserted at a 45° angle until fluid flows. The stylet is then removed and the catheter is advanced into the pericardial sac.

Classification of Effusions in Dogs and Cats

Effusions may be classified as a **pure transudate**, a **modified transudate**, an **exudate**, a **hemorrhagic effusion**, or a **neoplastic effusion**. Exudates may be subdivided into septic or non-septic exudates. The classification of these fluids is based on 3 parameters; **total protein**, **cell counts**, and **cytologic appearance**. A refractometer is usually adequate for measurement of the total protein in most fluids. If protein content is very low, spectrophotometry may be necessary for accurate determination. White blood cell counts are easily determined by an automated cell counter, or manually by using a hemocytometer. The cytologic appearance of the fluid can then be evaluated by smearing a drop of fluid between 2 glass slides and staining the material with

a Romano sky-type stain such as Wright-Giemsa or Diff-Quik. After staining the material is then ready for microscopic evaluation.

Pure Transudate

Pure transudate formation is considered to be a passive process because fluid accumulation is not the result of altered capillary bed permeability. Pure transudates most frequently form as a result of hypoproteinemia from either increased loss or decreased production of albumin. Albumin maintains the plasma colloidal osmotic pressure within the vascular system, preventing leakage of fluid into and promoting reabsorption of fluid from the extravascular compartments such as the body cavities. Transudates resulting from hypoalbuminemia alone usually require plasma albumin concentrations to be < 1.0 g/dl. However, if hypertension is also present, as is sometimes seen associated with liver disease, transudates may accumulate when albumin concentrations are above 1.0 g/dl.

Parameters: Transudative fluids are typically clear and colorless, with protein concentrations of less than 2.5 g/dl, and less than 500 nucleated cells / μ l. Cytologically these fluids contain low numbers of nondegenerate neutrophils, monocytes, lymphocytes and occasional reactive mesothelium.

Clinical conditions resulting in pure transudates: Most pure transudates accumulate in conditions that result in increased loss or decreased production of albumin.

Decreased production is seen in patients with liver failure such as cirrhosis. Decreased synthesis of proteins may also result from malnutrition, maldigestion, or malabsorption.

Increased loss may be seen in protein losing nephropathies which may result from a number of conditions including glomerulonephritis, nephrotic syndrome, or renal amyloidosis. Albumin may also be lost in the gastrointestinal tract as a result of a protein losing enteropathies such as lymphangiectasia or lymphocytic/plasmacytic enteritis, chronic hemorrhage, intestinal parasitism, or intestinal neoplasia. In protein losing enteropathies globulins may also be lost resulting in a concurrent hypoglobulinemia.

A relative decrease in plasma albumin may result in a transudative fluid from overzealous fluid administration, particularly in patients whose albumin is approaching 2 g/dl or less.

Modified Transudates

A modified transudate is an effusion that occurs by transudative mechanisms where vascular fluids leak out of "normal" or "noninflamed" vessels (e.g. via increased capillary hydrostatic pressure or lymphatic obstruction). However, leakage of fluid from these vessels carries high protein content and is therefore "**modified**" by the addition of protein and/or cells. Although most modified transudates result from some type of obstruction to venous or lymphatic drainage, or more uncommonly vascular anomalies such as lymphangiectasia, the clinical conditions that can result in these obstructions are numerous. Because of this, modified transudates are probably the most difficult of the fluid types to define clinically.

Parameters: Modified transudates are seldom clear, as are transudates, but the color may vary from slightly turbid to pink, to white, depending on the etiology. The protein content usually ranges between 2.5 g/dl to 5.0 g/dl. The cell counts may vary from 500 cells to 8,000 cells / μ l. In a modified transudate, the majority of these cells will be mononuclear cells, either macrophages, lymphocytes, or a combination of both. Mesothelial cells and nondegenerate neutrophils will make up a much smaller percentage. A modified transudate may be a transitory stage of an effusion, and if the fluid remains in the body cavity long enough, the protein content and degenerating cells will result in chemotaxis of neutrophils into the area. This will alter the classification of these fluids from a modified transudate to a nonseptic exudate. Therefore, some of the conditions which are described here as causing a modified transudate may also produce a nonseptic exudate if fluids are long-standing.

Clinical conditions resulting in modified transudates:

Congestive heart failure: The most common cause of a modified transudate is congestive heart failure. In the dog, effusions resulting from congestive heart failure are usually serous to serosanguinous and slightly turbid. This fluid initially will contain variable numbers of erythrocytes, macrophages, and lymphocytes, with low numbers of mesothelial cells and nondegenerate neutrophils. In the cat, chylous effusions typically result from congestive heart failure (See below).

Chylous effusions: Chylous effusions result from leakage of lymphatics into the thoracic and/or abdominal cavity. They are opaque white to pink with cell counts and protein content similar to other modified transudates. Accurate protein concentrations may be difficult to obtain using a refractometer due to the high turbidity. Chylous effusions are easily recognized by their color and can be confirmed by finding an opaque effusion with a triglyceride concentration of > 100mg/dl.

Chylous effusions are placed in the modified transudate category because initially these fluids contain a predominant population of mononuclear cells (lymphocytes) and the fluid accumulation results from leakage of lymphatics from noninflamed vessels. The lymphocytes seen in chylous effusions are small and well-differentiated. These cells contain nuclei that are approximately the size of erythrocytes with dense dark nuclear chromatin. The cytoplasm is scant. The exception would be the chylous effusion that results from lymphoma where some reported cases will contain a significant population of large lymphoblasts. However, as with other modified transudates, over time macrophages and nondegenerate neutrophils will increase due to the inflammatory response associated with the fluid and/or repeated thoracocentesis.

As mentioned previously, a high number of thoracic chylous effusions in the cat are associated with cardiac diseases, including feline heartworm disease. Other causes of pleural chylous effusions in the dog and cat include mediastinal neoplasms (lymphoma, thymoma), diaphragmatic or peritoneopericardial hernia (cat), lung torsion, intestinal lymphangiectasia (dog), mediastinal cryptococcal granuloma, or idiopathic. Pure bred cats seem to be more affected than mixed breeds, and in one study Afghan hounds were considered to have a higher incidence of chylothorax than other breeds of dogs. Although originally thought to be a significant cause of pleural chylous effusions, trauma and resulting thoracic duct rupture results in relatively few chylous effusions.

Although less frequently encountered, chylous ascites has been reported in the dog and cat. Although intraabdominal neoplasia was the inciting cause in most of the cats, in the dog, most cases were associated with non-neoplastic causes of lymphatic obstruction and leakage.

Miscellaneous causes: Neoplastic conditions such as lymphoma, carcinoma, mesothelioma, and hemangiosarcoma may cause accumulation of modified transudates. In addition, hernias such as diaphragmatic hernias or peritoneal-pericardial hernias may cause thoracic or pericardial effusions respectively. Lung atelectasis or torsion of an organ such as lung lobe, liver lobe or loop of intestine may initially cause a modified transudate, however, these fluids usually progress rapidly to a nonseptic exudate. Other causes include diaphragmatic or peritoneal-pericardial hernias (thoracic or pericardial effusions respectively), partial or complete obstruction to the cranial vena cava (thoracic effusion), caudal vena cava (abdominal effusion), or hepatic vein (abdominal effusion) from neoplasia or trauma, restrictive pericarditis or pericardial effusions of any cause, or any disease resulting in intrahepatic portal hypertension.

Exudates

Exudates are the result of increased vascular permeability and inflammation. They are further classified as **septic or nonseptic** depending on whether or not infectious agents are identified in the fluid. Nonseptic exudates may result from conditions which cause long-standing modified transudates, as well as other more inflammatory disease conditions.

Parameters: Exudates may vary in color from white to amber to pink, but are usually turbid. The protein content is usually high (> 3 g/dl), and the cell counts are typically above 3,000 cells / μ l. In exudates, the neutrophil is the predominant cell population and they may be accompanied by variable numbers of macrophages, lymphocytes, and mesothelial cells.

Clinical conditions resulting in nonseptic exudates:

Modified transudates: Any condition listed above that can cause a modified transudate may eventually progress to a nonseptic exudate.

Uroperitoneum: The fluid may be clear to slightly amber. Cell counts and protein may sometimes be low due to the dilution effect of the urine. However, most of the nucleated cells will be neutrophils allowing classification as a nonseptic exudate. The neutrophils may rapidly degenerate in this environment, resulting in a characteristic nuclear membrane lysis that is prominent over curves in the segmented nucleus. The fluid is typically nonseptic, however, sepsis may occur with uroperitoneum if there is a concurrent urinary tract infection. Comparison of serum creatinine to the creatinine concentration in the fluid is an accurate means of identifying the etiology. Urea nitrogen measurements may also be similarly evaluated but may not be as useful as creatinine since urea equilibrates between the body cavity and blood more rapidly than creatinine. Serum electrolytes in patients with uroabdomen are also altered, resulting in hyperkalemia and hyponatremia.

Bile peritonitis: Bile peritonitis may result from obstructive rupture or injury to the common bile duct. Bile is a very irritating substance and causes an inflammatory exudate in the peritoneal cavity. This fluid is typically nonseptic in the acute stages, but may later become septic, particularly if there is concurrent infectious cholecystitis. Abdominal fluid usually has a yellow-green to brown tint. Characteristic dark green to black pigment is usually seen in the background and within the cytoplasm of phagocytes. A dense, granular, amorphous material is often seen in the background of slides prepared from fluid containing bile.

Feline Infectious Peritonitis: FIP is the classic example of a nonseptic exudate in the cat. This fluid usually has a very high protein content, cytologically appearing as a granular, eosinophilic background material. Protein

electrophoresis of the fluid may be useful in making a pre-mortem diagnosis. One report indicated that an effusion with a high total protein content (for example > 3.5 g/dl), of which greater than 32% is gamma globulins, was shown to have a 100% positive predictive value for FIP. The total nucleated cell count in fluids from patients with FIP can be quite variable and may range from 1,000 cells/ μ L to 30,000 cells/ μ L. The predominant cell type is the neutrophil, and they are usually nondegenerate. Numerous macrophages and variable numbers of lymphocytes and plasma cells may also be observed.

Infection/inflammation in internal organs: Torsion of internal organs such as lung lobes, liver lobes, or spleen may eventually result in the accumulation of a nonseptic exudate. In addition, infection in internal organs such as lymph nodes, pancreas, lung, or liver may result in an exudative effusion even though organisms may not be present in the fluid.

Miscellaneous causes: Conditions such as sterile foreign bodies and neoplasms may also induce an inflammatory response, resulting in a nonseptic exudate.

Septic exudates: The identification of phagocytized, intracellular organisms, usually bacteria, distinguishes a septic exudate from a nonseptic one. The predominant cell type in most septic exudates is the neutrophil. Many of these cells will be degenerate as evident by nuclear karyolysis (swollen pale nucleus) or karyorrhexis (nuclear fragmentation). Degenerate neutrophils in an effusion warrants suspicion of sepsis, but definitive identification relies on the presence of intracellular organisms.

Conditions resulting in septic exudates: Any number of conditions may result in a septic exudate. Many septic exudates occur by introducing organisms into the body cavity via traumatic puncture wounds, bite wounds, perforation of the intestinal tract, migrating foreign bodies, ruptured pulmonary, hepatic, or prostatic abscess, pyometra, pneumonia, or pleuritis. Many aerobic and anaerobic organisms have been identified in exudates. The classic example of a septic exudate is pyothorax in the cat. Some organisms such as *Actinomyces* spp. and *Nocardia* spp. may be recognized cytologically by the presence of microcolonies of long, filamentous, beaded rods.

Hemorrhagic effusions

Hemorrhagic effusions can result from ruptured vessels or alterations in vascular endothelial integrity that is normally maintained by the interaction of platelets and various clotting factors. Hemorrhagic effusions grossly and microscopically contain a certain amount of blood and the packed cell volume of the fluid should be at least 10% to 25% of the peripheral blood. These fluids must be distinguished from iatrogenic blood contamination that might occur during any sampling procedure. Several factors may help in distinguishing between these two processes, but peracute hemorrhage occurring less than 45 minutes of sampling may be impossible to distinguish from iatrogenic contamination. One distinguishing factor is that platelets are usually not seen in hemorrhagic effusions present for more than one hour before sampling. Similarly, because of the rapid mechanical defibrination that occurs after extravasation, blood that is the result of hemorrhage into a body cavity will not clot, even in a clot tube. Additionally, true hemorrhagic effusions will eventually contain reactive macrophages with phagocytized erythrocytes or intracytoplasmic hemosiderin and/or hematoidin. Conversely, iatrogenic contamination with peripheral blood during sampling will contain platelets and will usually clot after collection. The packed cell volume of this fluid will be equal to that of the peripheral blood if a vessel is punctured or greater than peripheral blood if the spleen is inadvertently aspirated.

Parameters: There are no specific numerical values that define a hemorrhagic effusion, however, hemorrhagic fluid with leukocyte counts higher than that seen in the peripheral blood should be considered inflammatory as well.

Clinical conditions resulting in hemorrhagic effusions:

Traumatic injury: Traumatic injury to any internal organs may result in a hemorrhagic effusion. The more vascular, parenchymal organs such as the liver, spleen, lung, and heart are particularly susceptible to laceration.

Rodenticide poisoning: Ingestion of vitamin K antagonistic, rodenticide poisons such as warfarin or indanedione may result in a hemothorax or hemoabdomen.

Neoplasia: Rupture or leakage of vessels in a neoplasm may result in the accumulation of a hemorrhagic effusion. The classic and perhaps most common example of this is a splenic or atrial hemangiosarcoma, resulting in hemoabdomen or hemopericardium respectively. Adrenal tumors (pheochromocytomas and adrenal adenocarcinomas) have recently been identified as potential causes on nontraumatic peritoneal and retroperitoneal hemorrhage in the dog (JAVMA 219:329-333, 2001).

Hemorrhagic pericardial effusions: As with any other fluid accumulation, pericardial effusions may be classified as transudates, modified transudates, etc. However, most pericardial effusions, particularly in the dog, are hemorrhagic. Hemorrhagic pericardial effusions may result from neoplasia, benign idiopathic pericardial hemorrhage, trauma, coagulopathies, or spontaneous rupture of the left atrium as a sequela to mitral insufficiency with severe left atrial dilatation. The vast majority of the hemorrhagic pericardial effusions in the dog are either due to neoplasia or benign idiopathic pericardial hemorrhage, and these occur in approximate equal frequency.

Benign pericardial effusion: Idiopathic benign pericardial disease is a pericardial disease of unknown etiology, most commonly affecting middle aged dogs.

Neoplasia: The two neoplasms commonly resulting in pericardial effusions are hemangiosarcomas and chemodectomas or heart base tumors (commonly seen in old brachycephalic dogs). Thymomas, thyroid carcinomas, metastatic adenocarcinomas, and mesotheliomas are encountered much less frequently. As with other hemorrhagic effusions that result from neoplasia, neoplastic cells are rarely identified in the pericardial fluid. Therefore, cytologic evaluation is of little value in distinguishing between hemorrhagic effusions resulting from neoplasia and those resulting from benign idiopathic pericardial hemorrhage. In addition, pericardial effusions may result in dramatic mesothelial cell proliferation, and exfoliated mesothelium may have characteristics that mimic malignancy.

pH values in differentiating causes for pericardial effusions: In a JAAHA article (Edwards, 1996, 32:63-67), 51 dogs with pericardial effusions were evaluated, it was found that the pH of the fluid could reliably distinguish benign pericardial effusion from effusions associated with a neoplastic process. However, a more recent article indicated that the measurement of pH is not a specific indicator of the etiology of pericardial effusion and that there is too much overlap between the groups to be of much use (JVIM, 17:525-529, 2003).

Neoplastic effusions

Neoplasia is a common cause of effusions in dogs and cats. In one report, 57% of pericardial effusions and 11% of peritoneal and pleural effusions in the dog were the result of neoplasia (O'Brien and Lumsden, Sem. Vet. Med. Surg. 3:140-156,1988). In the same study, neoplastic effusions accounted for 37% of the pleural effusions in cats. However, neoplastic cells are not always identifiable on cytologic preparations. Neoplastic processes occurring within the body cavities may result in various types of fluid accumulations including modified transudates, exudates, and hemorrhagic effusions. **The term "neoplastic effusion"** should only be used to describe effusions where a neoplastic cell population has been identified in the fluid. Making this determination is difficult because neoplastic cells are absent in many effusions caused by neoplasia and reactive mesothelial cells often have cytologic criteria that mimic malignancy. In one study involving over 400 peritoneal and pleural effusions in dogs and cats, even in the hands of experienced cytopathologists, cytologic evaluation for the detection of tumors had a sensitivity of 64% and 61% in dogs and cats respectively (Hirschberger, *et al.* Vet. Clin. Pathol., 28:142-146, 1999). These figures may be an overestimation since hemorrhagic effusions resulting from ruptured tumors were not included in this determination.

Carcinomas, round cell tumors, mesotheliomas, and sarcomas may result in neoplastic effusions. Carcinomas appear to be the most common cause of neoplastic peritoneal and pleural effusions in the dog and peritoneal effusions in the cat. However, round cell tumors appear to be the most common cause of neoplastic pleural effusions in the cat, probably due to the frequent occurrence of thymic lymphoma in this species and the relative ease of identifying neoplastic lymphoblasts in the fluid.

Parameters: There are no particular numerical parameters for neoplastic effusions; however, the total protein content of these fluids is typically high (> 3.0 g/dl). These fluids are often inflammatory and may also have evidence of hemorrhage.

Clinical conditions resulting in neoplastic effusions:

Lymphoma: Lymphoma involving the thymus, liver, or intestinal tract may eventually result in a neoplastic effusion. Lymphoma may account for up to ½ of the neoplastic effusions in cats. This most frequently occurs with thymic lymphoma. The neoplastic effusion will often contain a homogeneous population of large lymphoblasts with moderate amounts of basophilic cytoplasm and nuclei ranging from 1.5 to 5 times the size of erythrocytes. The chromatin pattern is typically diffuse and nucleoli may be prominent depending on the type of lymphoma.

Carcinoma/adenocarcinoma: Carcinomas that result in effusions may often contain a neoplastic cell population. Carcinomatosis (metastasis of tumor cells to serosal surfaces and/or omentum) typically results in neoplastic effusions. This usually occurs with aggressive tumors such as pancreatic adenocarcinomas or ovarian tumors. Carcinomas account for approximately ½ of the neoplastic effusions in dogs and cats. The difficulty is in distinguishing a malignant cell population from a population of reactive macrophages and mesothelial cells. This distinction is always difficult because of the bizarre features often seen in reactive cell populations. Cytologic features that may help distinguish reactive mesothelium and macrophages from carcinoma cells include the following malignant criteria: 1) marked pleomorphism, 2) macrokaryosis (giant nuclei), 3) large, angular, or multiple nucleoli, 4) multinucleation, and 5) increased mitotic activity.

Hemangiosarcomas: Dogs with HSA often have pleural and/or peritoneal effusions, the majority of which are hemorrhagic. More than ½ of the dogs with splenic hemangiosarcoma (HSA) have hemoperitoneum at the time of surgery. However, cytologic evaluation of the effusion usually **does not** result in the identification of

neoplastic cells. The literature suggests that only 25% of effusions associated with HSA in dogs yield a cytologic diagnosis. However, in our experience, the likelihood of making a cytologic diagnosis of HSA from evaluation of the abdominal effusion is less than the reported incidence. When present, the cells are typically very large compared to the surrounding blood cells and are easily identified. HSA cells will be seen individually and in small clusters containing 3 or more cells. The cytoplasm may be polygonal to spindle-shaped and often has a characteristic pale blue, almost veil-like appearance with small, punctate, clear cytoplasmic vacuoles. The nuclei are usually very pleomorphic (variably shaped) and have some of the most bizarre features seen on cytologic preparations. Nuclei are often very large (3 or more times the size of erythrocytes) with marked anisokaryosis (variable nuclear size), clumped chromatin, and prominent, multiple nucleoli. Megalocytosis (abnormally large cells), multinucleation, and mitotic figures are also frequently observed.

Mesothelioma: Mesotheliomas are uncommon tumors of dogs and cats. The neoplastic mesothelial cells will have cytologic features which are indistinguishable from those of a carcinoma or adenocarcinoma. Care must again be taken in over-interpretation of findings since reactive mesothelium can often have features which mimic malignancy.

Mast Cell Tumors: Mast cell tumors that are systemic and/or involve the spleen, liver or intestinal tract may result in a neoplastic effusion. The neoplastic mast cells will have the typical round cell appearance and contain variable numbers of metachromatic, cytoplasmic granules. Low numbers of mast cells may rarely be seen in inflammatory effusions.

Classifications of Effusions in Dogs and Cats

	Total Protein	Cells/ μ L	Cell Types	Special Features
Transudate	<2.5 g/dL	<500	Mononuclear	Low cellularity
Modified Transudate	2.5 – 5.0 g/dL	500 – 8000	Mononuclear	Cell type varies with etiology
Nonseptic Exudate	>3.0 g /dL	>3000	Neutrophils	Nondegenerate neutrophils
Septic Exudate	>3.0 g/dL	>3000	Neutrophils	Degenerate neuts with intracellular bugs
Hemorrhagic	>3.0 g/dL	Variable	Sim. to blood	Erythrophagia or hemosiderin in macrophages
Neoplastic	>2.5 g/dL	Variable	Tumor cells	Neoplastic population

