Cytology and Fluid Analysis of the Acute Abdomen

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In patients with acute abdominal pain, abdominal paracentesis and diagnostic peritoneal lavage often yield fluid samples for cytologic and biochemical evaluation. Cytology of the effusion from a patient with acute abdominal disease can be a crucial tool for the rapid diagnosis necessary for initiation of timely and appropriate therapy. Appropriate sample collection, handling, and preparation are essential to obtain an accurate diagnosis. Analysis of the fluid sample should include gross examination of the effusion, measurement of total nucleated cell count, packed red blood cell volume, and protein concentration, as well as examination for the presence of other cells, bacteria, food particles, or plant material. Biochemical evaluation should proceed based on the clinician's index of suspicion for a particular disease process. Abdominal effusions are generally classified as transudate, modified transudate, or exudate, depending on the total nucleated cell count and protein concentration. Cytology of all fluids collected should be performed systematically, utilizing progressively higher magnifications with a microscope. Specific diseases with associated abdominal effusions include septic peritonitis, nonseptic peritonitis, hemoabdomen, uroabdomen, pancreatitis, bile peritonitis, chylous effusion, and neoplasia. A complete description of sample preparation and evaluation is reviewed.

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Performing cytologic evaluation of abdominal fluid from a patient with acute abdominal disease is essential for rapid determination of the disease etiology, often assisting the clinician in initiating appropriate therapy. In many cases, analysis and cytology of the abdominal fluid provide valuable information necessary for deciding whether medical or surgical intervention is most appropriate. The goal of performing cytology and fluid analysis of any effusion is to obtain a rapid and accurate diagnosis, such that appropriate therapy can be instituted within an acceptable period of time, thereby minimizing patient morbidity and mortality.

In the patient presenting with acute abdominal pain, one must first decide whether abdominocentesis is appropriate. A complete evaluation of the patient, including signalment, a thorough history, and physical examination, is an important first step that may help narrow the list of differential diagnoses in patients with acute abdominal disease.1 Given the extensive list of potential causes of acute abdominal pain, abdominocentesis can be helpful in making a definitive diagnosis more rapidly.

Fluid Analysis

Fluid analysis begins with appropriate sample collection. If abdominocentesis is indicated, but is negative after a complete four-quadrant paracentesis has been performed, diagnostic peritoneal lavage is indicated. Sample collection methodology is discussed elsewhere in this issue. Collected samples should be saved in a sterile ethylenediaminetetraacetic acid (EDTA) (lavender) tube to prevent clot formation that may alter cell count, regardless of whether hemorrhage is apparent grossly. The EDTA sample will undergo cytologic examination, and total protein content and total nucleated cell count will be determined. A portion of the sample should also be saved in a sterile tube with no additives (red top) for biochemical evaluation if indicated, and in culture media appropriate for aerobic and anaerobic culture and antibiotic susceptibility testing if bacterial culture is indicated. Samples should always be collected aseptically as possible to prevent sample contamination by nonoffending microorganisms.

On any sample collected via abdominocentesis, fluid analysis and cytology should be performed. Any fluid analysis includes a subjective description of the fluid, measurement of the total nucleated cell count using an automated analyzer or a hemocytometer, measurement of packed red blood cell volume, total protein, and ancillary biochemical testing, as indicated. The fluid should first be examined grossly. If the sample is completely clear and colorless, peritonitis, severe intraabdominal injury or perforation, and leakage from the gastrointestinal tract may be ruled out.2 Samples may also grossly appear clear and colorless if obtained within 3 hours of visceral perforation or in cases involving the retroperitoneal space. Fluid that is amber and clear to mildly opaque in color is suggestive of a modified transudate of low to moderate cellularity.3 Fluid that appears moderately to markedly turbid or flocculent is suggestive of a highly cellular effusion.3 Guidelines for sample preparation before performing cytologic examination are described below. Additional ancillary tests that can aid in obtaining a definitive diagnosis can be performed on the supernatant, including creatinine, urea nitrogen, lipase, amylase, cholesterol, triglyceride levels, albumin, globulin, and bilirubin. Table 1 lists the ancillary tests that may be performed on abdominal fluid, the indications for performing the test, the expected result of the test, and the corresponding diagnosis.4 In many cases, comparison of the abdominal fluid values with those of serum can assist in supporting a definitive diagnosis.

Abdominal fluid collected may be classified as a transudate, modified transudate, or exudate, depending on its cell count and protein content. Numbers vary slightly depending on the source referenced, but in general, a transudate contains fewer than 1,500 cells/μL and less than 2.5 g/dL protein, a modified...
transudate contains 1,000 to 7,000 cells/μL and/or a protein concentration of 2.5 to 7.5 g/dL, and an exudate contains greater than 5,000 cells/μL with greater than 3.0 g/dL protein. Transudates generally arise due to reduced absorption or increased production of fluid, secondary to hypoproteinemia, overhydration, or lymphatic or venous congestion. Transudates are generally not associated with acute abdominal pain. Any cell type may be present in an exudative effusion; however, the typical exudate contains primarily phagocytic cells. Exudates arise due to chemotaxis of inflammatory cells, altered vascular permeability, and leakage of plasma proteins. Other causes of exudative effusions include rupture or penetration of a visceral organ or vessel, exfoliation of neoplastic cells, and leakage of a chylous fluid.

### Sample Preparation for Evaluation

If a sample is relatively clear (generally when the total cell count is less than 10,000 cells/μL), a sediment smear should be made. The sample is centrifuged at a low rpm (165 to 360 G or in a centrifuge with a radial arm length of 14.6 cm at 1,000 to 1,500 rpm) for 5 to 10 minutes. The protein content is measured from the supernatant using a refractometer, spectrophotometer, or automated analyzer. If using a refractometer, it is important to determine the protein content of the supernatant because if the effusion is highly cellular, the refractometer of light by suspended particles or cells may result in an erroneously high total protein reading. Smears for cytologic evaluation can be made from the resuspended sediment utilizing one of several techniques. One technique is similar to that used to make a blood smear. The blood smear technique includes expelling a small portion of the fluid near one end of a glass microscope slide. A second slide is then held to the first at a 30 to 40 degree angle. The second slide is pulled backward along the first until it comes in contact with the sample. Once the sample fluid has spread sideways along the junction of the two slides, the second slide is rapidly and smoothly moved forward, producing a sample with a feathered edge, similar to a blood smear. A smear can also be prepared using the squash preparation technique in which a portion of the aspirate is expelled onto the center of a glass slide and a second clean slide is placed over the sample such that the two slides are perpendicular to each other. This method should sufficiently spread the sample. If it does not spread well, gentle digital pressure can be applied to the top slide, taking care to avoid placing excessive pressure on the slide, as cells may rupture. The second slide is then gently, smoothly, and quickly slid across the original slide. Yet another technique that can be used is the line smear technique. It is made similar to the blood smear technique, with the exception that the second slide is not moved forward to the end of the first slide to make a feathered edge. Instead, the second (spread-er) slide is only advanced about two-thirds to three-fourths the distance used to make a slide with a feathered edge, then the spreader slide is raised directly upward. This yields a line containing a higher concentration of cells than the rest of the slide; therefore, it is best utilized when the fluid cannot be concentrated with centrifugation or the centrifuged sample is of low cellularity. A direct smear can be made from samples with high cellularity using either the blood smear technique or the squash preparation. Smears may then be air dried to partially preserve the cells, and allow them to adhere to the slide such that they do not fall off during the staining process. Once dry, the slide may be stained with a Romanowsky-type stain. Romanowsky's stains are alcohol-based stains used in most veterinary practices and by most diagnostic laboratories to stain cells for hematologic as well as routine cytologic evaluation. They include Wright's stain, Giemsa stain, Wright's Giemsa stain, May-Grünwald-Giems stain, and the so-called "fast" stains used in private veterinary clinics such as Diff-Quick. Each stain has its own unique set of instructions with which the clinician should become familiar. In general, the thinner the smear and the lower the total protein content of the effusion, the less time required to stain the slide. Thus, smears made from fluids of low cellularity and protein content may stain better using one-half or less of the recommended staining time. Conversely, a smear made from a fluid of high protein content and cellularity may need to be stained at least twice as long as the recommended time. Also, if staining will not occur within a few days of slide preparation, cell preservation is enhanced by "fixing" the slide. This is done by submersing the slide for several minutes in methyl alcohol.

### TABLE 1. Specific Tests, the Indications for the Tests, the Expected Results, and Corresponding Diagnoses for Ancillary Tests That May Be Performed on Abdominal Fluids; Not All Indications Must Be Present, but All Should Be Considered and Evaluated

<table>
<thead>
<tr>
<th>Ancillary tests</th>
<th>Indication</th>
<th>Expected result</th>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>Creatinine/Potassium</td>
<td>Azotemia, history of trauma, and loss of serosal detail radiocraphically</td>
<td>Fluid creatinine &gt;2 times serum, fluid protein &gt;1.4 times serum</td>
<td>Uroabdomen</td>
</tr>
<tr>
<td>Lipase/Amylase</td>
<td>Suspicion of pancreateitis-increased amylase/lipase on biochemical profile, vomiting, supportive ultrasonographic changes of pancreas, fever, leukocytosis</td>
<td>Lipase and amylase values greater in abdominal fluid than serum</td>
<td>Pancreatitis</td>
</tr>
<tr>
<td>Triglyceride (TG)/Cholesterol</td>
<td>Milky fluid, primarily lymphocytes (especially if acute, may be mixed inflammatory if chronic)</td>
<td>Fluid TG &gt;3 times serum fluid cholesterol &lt; fluid TG</td>
<td>Chylous effusion</td>
</tr>
<tr>
<td>Albumin/Globulin (A:G)</td>
<td>Increased serum globulin, abdominal fluid viscous</td>
<td>A:G ratio of fluid &lt;0.8 very suggestive</td>
<td>Feline infectious peritonitis</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>History of trauma; bile pigment, or bilirubin crystals on cytology</td>
<td>Fluid greater than serum value</td>
<td>Bile peritonitis</td>
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<tr>
<td>Bacterial culture</td>
<td>Intracellular bacteria in degenerate neutrophils, any exudate</td>
<td>Positive bacterial culture</td>
<td>Septic peritonitis</td>
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<tr>
<td>Bacterial culture</td>
<td>Exudate without intracellular bacteria or significant number of degenerate neutrophils</td>
<td>Negative bacterial culture</td>
<td>Nonseptic peritonitis</td>
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</table>
TABLE 2. Possible Causes of Septic Versus Nonseptic Peritonitis

<table>
<thead>
<tr>
<th>Septic peritonitis</th>
<th>Nonseptic peritonitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penetration/Rupture of visceral organ</td>
<td>Feline infectious peritonitis</td>
</tr>
<tr>
<td>Hematogenous spread</td>
<td>Steatitis</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>Foreign body reaction</td>
</tr>
<tr>
<td>Abscessation of liver or prostate</td>
<td>Immune-mediated disease</td>
</tr>
<tr>
<td>Surgical entry</td>
<td>Parasite migration</td>
</tr>
<tr>
<td>Chronic bile peritonitis</td>
<td>Hemorrhage</td>
</tr>
<tr>
<td></td>
<td>Chronic urethra-abdomen</td>
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<tr>
<td></td>
<td>Acute bile peritonitis</td>
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</tbody>
</table>

Cytologic Evaluation

After the smear has been stained and dried, it should first be evaluated at low power using the 4× and/or 10× objective. This degree of magnification allows the clinician to assess the adequacy of staining and to identify areas of high cellularity or areas with unique staining features. In addition, larger objects, including some crystals, parasites, and plant debris, may be seen while scanning at low magnification. Magnification can then be increased to the 10× or 20× objective. At this degree of magnification, the clinician can gain an impression of the overall cellularity and cellular composition. When an area of increased or unique cellularity is identified, the smear is viewed with either the 40× or 50× objective. To improve resolution when using the 40× objective, one can place a drop of oil on the smear and cover it with a coverslip. At this magnification, individual cells are examined and compared with other cells, and most microscopic organisms can be seen. A differential cell count can also be performed at 40× to 50× magnification. Finally, the smear should be evaluated using the 100× (oil immersion) objective, so one can definitively identify organisms and cellular inclusions, and can examine cellular morphology.

Effusions Associated with Specific Diseases

Septic Peritonitis

Septic effusions are characterized by an accumulation of exudative fluid secondary to presence of bacterial agents. There are numerous possible causes of a septic effusion (Table 2), including penetration or rupture of a visceral organ, hematogenous spread, gastroenteritis, internal abscessation involving liver or prostate, or surgical entry. Characteristically, septic fluid contains an increased number of neutrophils and macrophages, with free or phagocytedized bacteria (Fig 1). It is important to note that there is not a correlation between the total white blood cell (WBC) count and the severity of disease. Bacteria are generally phagocytized by the neutrophils, as opposed to fungal organisms, which are more commonly seen within macrophages. Neutrophil nuclear degeneration may be seen in the absence of bacteria. The degree of neutrophil degeneration depends on the amount and virulence of toxins present within the exudate. If nuclear degeneration is noted, but bacteria cannot be found, further investigation and bacterial culture are warranted. Aging neutrophils have a different appearance than degenerating neutrophils. Aging neutrophils display features of karyopyknosis and karyorrhexis. This differs from the nuclear swelling and chromatolysis that is characteristic of neutrophilic degeneration secondary to bacteria, or less frequently, chemical toxins. Toxic changes that are frequently reported in neutrophils of peripheral blood may also be seen in the neutrophils from effusions; caution should be exercised when interpreting this change, as it has limited specificity. Additionally, vacuolation may be noted in the cytoplasm of neutrophils from an effusion due to EDTA exposure or as an age-associated change. Macrophages, lymphocytes, and plasma cells may all be present in varying amounts in both acute and chronic abdominal effusions. Plasma cells are generally seen as a response to chronic antigenic stimulation.

Nonseptic Peritonitis

Nonseptic exudates may result from feline infectious peritonitis, steatitis, foreign body reactions, immune-mediated diseases, parasite migration, or hemorrhage (Table 2). Effusions are generally described based on the predominant WBC present. “Suppurative” or “purulent” may be used for a neutrophilic exudate (Fig 2), “mononuclear” if the effusion is primarily composed of macrophages or lymphocytes, and “eosinophilic” if eosinophils are the predominant cell type. In many...
cases, the effusion is described as "mixed inflammation" because several cell lines are present in high numbers. Nonseptic effusions may also be classified based on the concentration of neutrophils present. An effusion may be considered "acute" if greater than 70% of the cells are neutrophils, "chronic active" if 50% to 70% of the cells are neutrophils, and "chronic" if less than 30% of the cells are neutrophils. Recent abdominal surgery can result in an elevated WBC count, but it is usually less than 10,000 cells/μL. There should be few, if any, degenerate neutrophils or intracellular bacteria, depending on the surgery that was performed.

Hemorrhage into the abdomen may occur secondary to trauma, neoplasia, splenic rupture, hemorrhagic defects, or heartworm disease, or, rarely, due to rupture of an aneurysm. A packed cell volume of diagnostic peritoneal lavage fluid that is 5% or greater is suggestive of significant hemorrhage. Cytologically, it may be difficult to differentiate acute hemorrhage from iatrogenically induced hemorrhage such as an inadvertent paracentesis into the spleen or liver. Signalment of the patient, history, and physical examination findings should also help the clinician determine which is more likely. Comparison of the packed cell volume of the effusion with that of peripheral blood may also be helpful. Platelets quickly aggregate, degranulate, and disappear within an effusion, so their presence may be suggestive of iatrogenically induced hemorrhage or peracute hemorrhage into the abdominal cavity. Within 1 day of the onset of hemorrhage, macrophages become activated and begin to phagocytose erythrocytes within the abdominal cavity. Therefore, the presence of erythrophagocytosis is suggestive of chronic hemorrhage and would rule out iatrogenically induced hemorrhage (Fig 3).

Uroabdomen

In terms of composition, fluid from a patient with uroabdomen can range from a transudate to modified transudate to exudate, depending on the chronicity and severity of the disease. If uroabdomen is suspected, the most useful abdominal fluid chemistry evaluation is creatinine. A creatinine level of the abdominal effusion twice that of the serum is highly suggestive of uroabdomen. Another useful test is measurement of the potassium concentration of the effusion and comparing that with the serum concentration; a value greater than 1.4:1 is suggestive of uroabdomen. If uroabdomen is diagnosed, other diagnostic tests should be pursued to determine the location of the rupture before surgical intervention.

Pancreatitis

Lipase and amylase are very useful in the diagnosis of pancreatitis. Values that are significantly higher in the effusion than in the serum are strongly suggestive of pancreatitis. Cytologic examination should demonstrate a nonseptic suppurative effusion.

Bile Peritonitis

Biliary rupture will create a modified transudate early that progresses to an exudate later in the disease course. Both protein and cellularity increase with time due to the release of bile into the abdominal cavity, resulting in chemical peritonitis. Bile peritonitis stimulates extravasation of phagocytic cells, particularly neutrophils and macrophages. A concomitant bacterial peritonitis may also be noted. Grossly, the effusion typically appears greenish to yellow-orange in color. On cytologic eval-
Chylous Effusion

Chylous effusions are uncommon in the abdomen. Chyle is a combination of lymph and chylomicrons. If present, a chylous effusion is characterized as a modified transudate or exudate, depending on the chronicity of the process. The cellular content of the fluid changes over time from mostly small lymphocytes early in the disease process to a mixed population of inflammatory cells, including neutrophils and vacuolated macrophages over time. Potential causes for development of chylous fluid in the abdomen include, but are not limited to, abdominal neoplasia, steatitis, biliary cirrhosis, lymphatic rupture or leakage, postoperative accumulation following ligation of the thoracic duct, and congenital lymphatic abnormalities.

Neoplasia

Effusions may be secondary to exfoliation of tumor cells, most frequently from epithelial tumors (Fig 5) or round cell tumors, and rarely from stromal tumors. Because of variability in protein concentration and nucleated cell counts, neoplastic effusions do not fit as readily into the transudate/exudate classification system. The tumor cells may be accompanied by varying degrees of inflammation, from mild to marked, depending on the location of the tumor and amount of tissue invasion and exfoliation or necrosis. Mesothelial cells may also be seen. A distinction must be made between reactive mesothelial cells,
exfoliating epithelial cells of neoplastic origin, and the rare case of mesothelioma. Even if the cell type of origin cannot be determined, if a monomorphic population of cells is noted that are not hematopoietic or mesothelial in origin, they are most likely clinically significant, regardless of whether or not criteria of malignancy are present. If the cells are adequately differentiated, cytoplasmic characteristics may be most helpful in determining the cell type of origin. For example, if there is evidence of secretory activity within the cytoplasm of the cells, an adenocarcinoma should be suspected.

Transudates/Modified Transudates

Transudates and modified transudates are not usually associated with acute abdominal pain. However, if an animal presents on emergency and abdominocentesis yields a transudate, congestive heart failure, liver failure, and hypoproteinemia should be considered as differential diagnoses. Modified transudates may result from chronic transudation of fluid. Although the total cell count is generally low, macrophages or mesothelial cells are the predominant cell type present.

Result Interpretation

Fluid with a WBC count greater than 1000 cells/μL in a patient that has not recently had an exploratory laparotomy and that contains many degenerative neutrophils is suggestive of peritoneal inflammation or suppuration with possible sepsis; an exploratory laparotomy is indicated. Other indications for surgical exploration of the abdomen include the presence of intracellular bacteria, a finding that is suggestive of bacterial peritonitis, and vegetable fibers that indicate visceral perforation with leakage of bowel contents. Patients with chemical peritonitis secondary to biliary rupture or uroabdomen will often benefit from medical stabilization. Surgery can be performed later when the patient's cardiovascular status is more stable, rather than on an emergency basis.

References