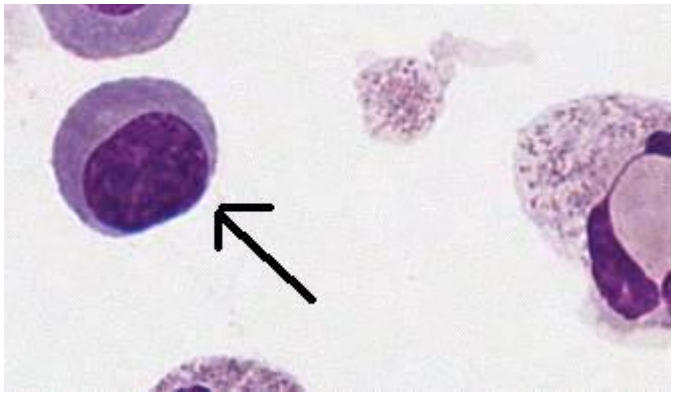
Cell Identification

VBF-14

Identification	Participants No.	Participants %	Evaluation
Neutrophil/macrophage with phagocytized bacteria	372	93.2	Educational
Neutrophil, segmented or band	22	5.5	Educational
Neutrophil/macrophage with phagocytized fungi	4	1.0	Educational
Erythrocyte, mature	1	0.3	Educational

The arrowed cell is a segmented neutrophil with phagocytized bacteria as correctly identified by 93.2% of participants. The segmented neutrophil is the mature cell of the myeloid series and is usually 10-15 µm in size, has 2-5 lobes of condensed nuclear chromatin which are connected by thin filaments. When bacteria are present within neutrophils, they can be difficult to distinguish from toxic granulation. Toxic granulation tends to involve nearly all of the cytoplasm of the neutrophil, whereas engulfed bacteria tend to be few in number. Bacteria are larger than toxic granules. In this case, the bacteria demonstrate a clear zone between the bacteria and the cytoplasm representing the cytoplasmic vacuole. Cytoplasmic vacuoles are not seen surrounding toxic granulations.

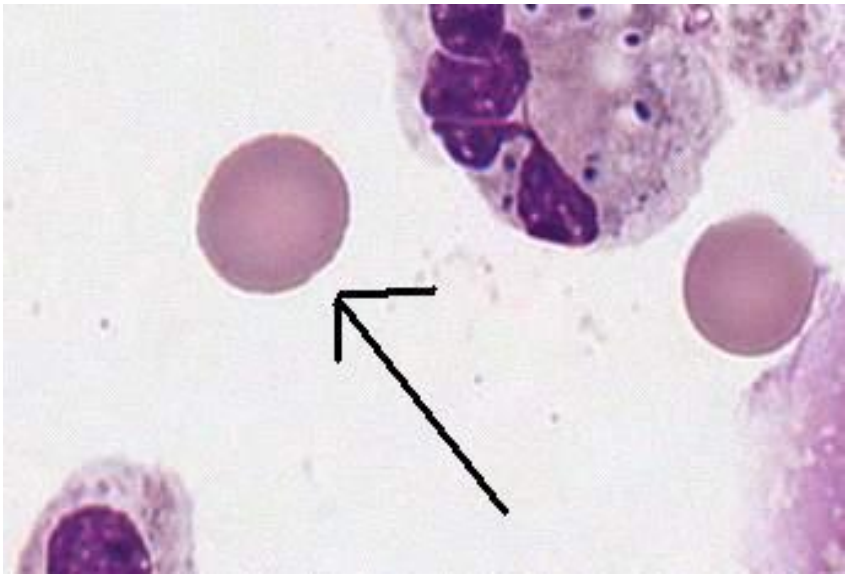
VBF-15



Identification	Participants No.	Participants %	Evaluation
Lymphocyte	372	93.2	Educational
Lymphocyte, reactive (atypical)	10	2.5	Educational
Plasma Cell	11	2.8	Educational
Erythrocyte, nucleated	2	0.5	Educational
Neutrophil, immature (metamyelocyte, myelocyte, promyelocyte)	2	0.5	Educational
Neutrophil, segmented or band	1	0.3	Educational
Yeast/fungi, extracellular	1	0.3	Educational

The arrowed cell is a lymphocyte/reactive lymphocyte as correctly identified by 95.7% of participants. The lymphocyte nucleus has a diffusely dense chromatin pattern and a small amount of agranular moderately blue cytoplasm. The platelet present to the right of the neutrophil is granular by comparison. Reactive lymphocytes are distinguished by their wide range of nuclear sizes, shapes, and chromatin patterns. The most common type of reactive lymphocyte resembles a large granular lymphocyte with moderately condensed chromatin and abundant pale, gray-blue cytoplasm. Granules, if present, are usually small and few in number. Reactive lymphocytes may also exhibit immunoblastic and plasmacytoid features.

VBF-16



Identification	Participants No.	%	Evaluation
Erythrocyte, mature	397	99.5	Educational
Monocyte/macrophage	1	0.3	Educational
Neutrophil/macrophage with phagocytized bacteria	1	0.3	Educational

The arrowed cell is an erythrocyte as correctly identified by 99.5% of participants. An erythrocyte is a mature, non-nucleated red cell of fairly uniform size (7µm) and round shape. It contains hemoglobin and has a zone of central pallor due to the biconcavity of the cell which occupies approximately one third of the cell diameter. Reticulocytes and erythrocytes share some similarities but reticulocytes distinctively have a bluish color to their cytoplasm. A supravital stain is often used to better identify and enumerate reticulocytes. Part of a neutrophil with ingested bacteria is seen to the right of the erythrocyte.

VBF-17

Identification	Participants No.	%	Evaluation
Neutrophil, segmented or band	398	99.8	Educational
Malignant cell (non-hematopoietic)	1	0.9	Educational

The arrowed cell is a neutrophil as correctly identified by 99.8% of participants. This neutrophil has two lobes which are connected by a thin filament. The chromatin is condensed and the cytoplasm is uniform without readily identified granules.

VBF-18

Identification	Participants No.	%	Evaluation
Monocyte/macrophage	383	96.0	Educational
Lymphocyte, reactive	5	1.3	Educational
Neutrophil, immature (metamyelocyte, myelocyte, promyelocyte)	4	1.0	Educational
Bacteria - extracellular	1	0.3	Educational
Immature or abnormal call, would refer for identification	1	0.3	Educational
Lymphocyte	1	0.3	Educational
Megakaryocyte	1	0.3	Educational
Mitotic figure	1	0.3	Educational
Ventricular lining cell (ependymal or choroid cell)	1	0.3	Educational
Plasma cell	1	0.3	Educational

The arrowed cell is a Monocyte/macrophage as correctly identified by 96.0% of participants. Monocytes are leukocytes that are slightly larger than neutrophils, 12-20 µm in diameter. Most monocytes have round cytoplasmic borders with smooth edges, but some have pseudopod-like cytoplasmic extensions. The cytoplasm is abundant and gray to gray-blue. Fine, evenly distributed, azurophilic granules or vacuoles may be present. Monocytes have round/oval to indented nuclear contours with condensed chromatin that is less dense than that of a neutrophil or lymphocyte. Mature monocytes lack nucleoli.

Case Discussion

This patient has *Streptococcus pneumoniae* meningitis. This spinal fluid cytospin preparation demonstrates large numbers of neutrophils, many of which have ingested bacteria that are lancet shaped cocci seen in pairs and singly with a clear zone around the bacteria. *Streptococcus pneumoniae* is a gram positive, alpha hemolytic bacterium with a polysaccharide capsule. There are over 90 surface capsular polysaccharides responsible for individual serotypes, though 5 serotypes are the most prevalent and account for 60-80% of infections. Pneumococci adhere to epithelial cells of the nasopharynx and colonize the upper respiratory tract and thus are considered normal flora. In the normal host, bacteria reaching the lung are usually cleared rapidly by alveolar macrophages or migrating neutrophils. If there is absence of type-specific humoral immunity, prior viral infection, or if the serotype is invasive, then *Streptococcus pneumoniae* can infect any organ, and is a common cause of otitis media, pneumonia, sepsis, meningitis,sinusitis, and conjunctivitis.

The diagnosis of pneumococcal infection can be made with certainty only by isolation of the organism from culture of a specimen of blood or other body fluid which is usually sterile. Gram stain of infected body fluids should be performed because the finding of gram-positive diplococci suggests pneumococcal infection. However, this finding provides only presumptive evidence of pneumococcal disease, as other streptococci may have a similar microscopic appearance. Bile solubility,optochin sensitivity and hemolysis on blood agar also help to identify *Streptococcus pneumoniae* from other species of Strep. Rapid antigen tests for pneumoccal capsular polysaccharide, latex agglutination and enzyme immunosorbent assays are available.

Vaccines for *Streptococcus pneumoniae* are routinely recommended for children less than 24 months old. Vaccination is also recommended for older individuals who are at high risk for pneumococcal disease and include those with asplenia, immunosuppression, immunodeficiency, and chronic illnesses. *Streptococcus pneumoniae* has been traditionally treated with penicillin, but strains with resistance to penicillin are increasing worldwide, so alternative therapies must be utilized.

References:

1. Long S, Pickering L, Prober C, eds, *Principles and Practice of Pediatric Infectious Diseases*. 2nd ed. New York, NY: Churchill Livingstone: 2003.
2. Murray P, Baron E, Jorgensen J, Landry M, Pfaller M, eds, *Manual of Clinical Microbiology*. 9th ed. Washington, DC: ASM Press: 2007.

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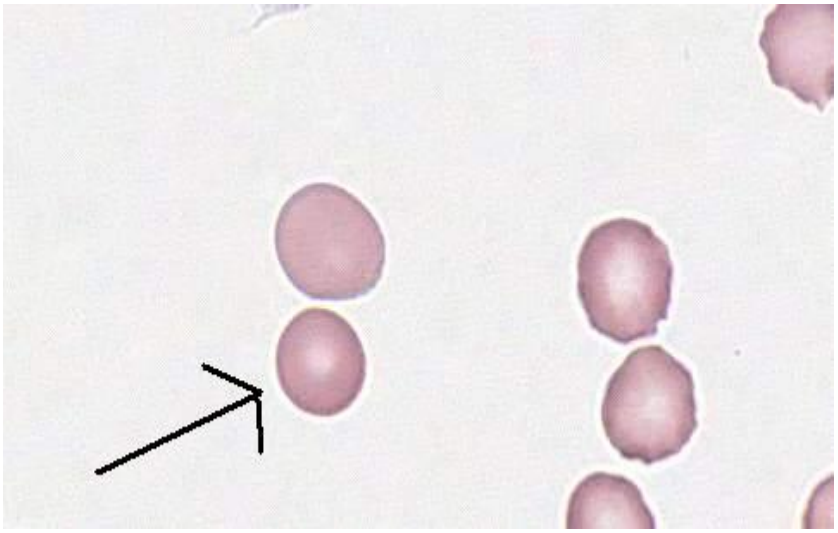
Hematology and Clinical Microscopy Resource Committee

Cell Identification

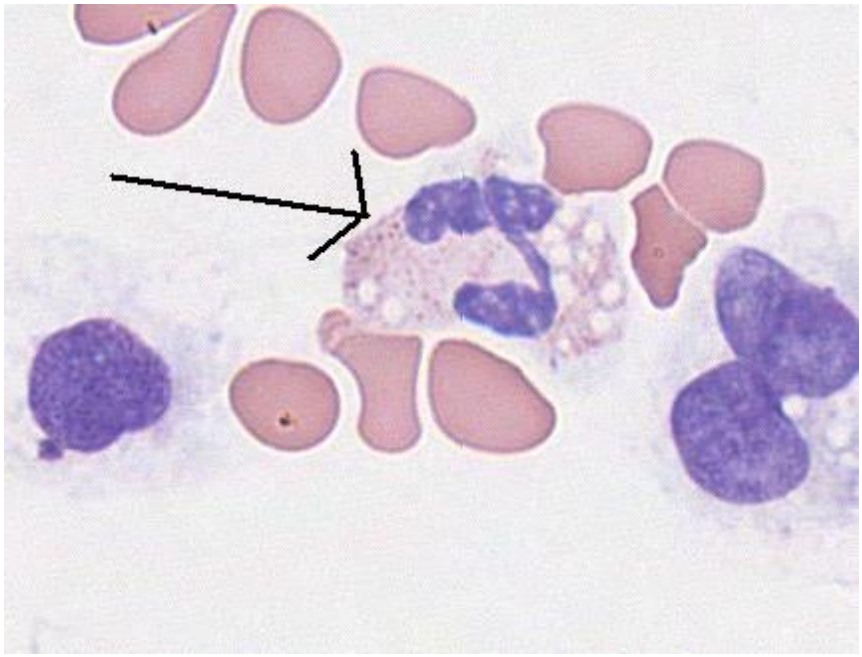
VBF-20



Identification	Participants No.	%	Evaluation
Erythrocyte, mature	394	99.2	Educational
Neutrophil/macrophage with phagocytized bacteria	2	0.5	Educational
Erythrocyte, nucleated	1	0.3	Educational

The arrowed object is a red blood cell, as correctly identified by 99.2% of participants. This cell is well preserved and has retained central pallor. Two of the adjacent red blood cells appear somewhat crenated. The presence of red blood cells in fluids may be related to blood contamination during the procedure. Counts of 5,000 to 10,000 RBC/µL will produce a blood-tinged fluid. RBC counts higher than 100,000/µL are suggestive of trauma or neoplasm.

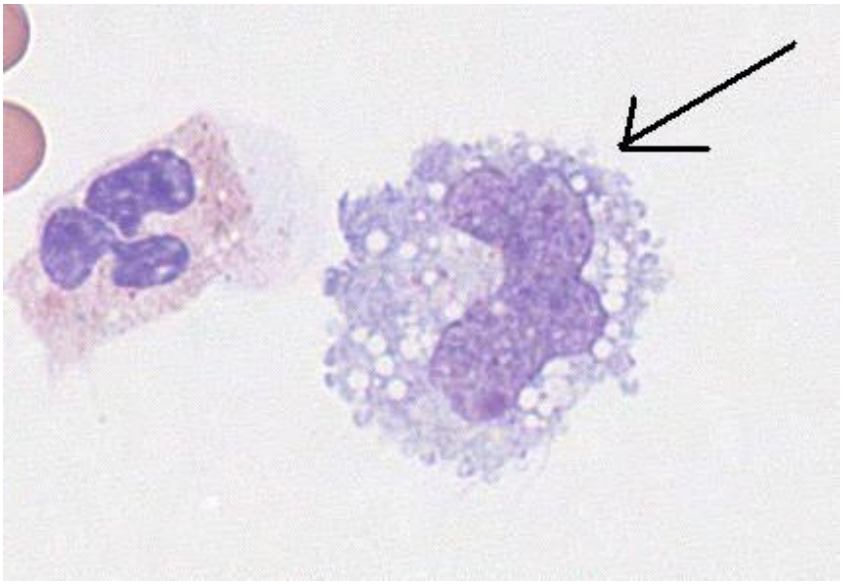
VBF-21



Identification	Participants No.	Participants %	Evaluation
Neutrophil, segmented or band	389	98.0	Educational
Neutrophil, immature (metamyelocyte, myelocyte, promyelocyte)	3	0.8	Educational
Eosinophil	2	0.5	Educational
Degenerating cell, NOS	1	0.3	Educational
Lymphocyte	1	0.3	Educational
Macrophage containing erythrocyte(s) (Erythrophage)	1	0.3	Educational

The arrowed object is a neutrophil, as correctly identified by 98.0% of participants. Neutrophils in serous fluids may appear identical to those in the blood or may be difficult to identify, depending upon the age of the effusion and the degree of degeneration. Over time, the cytoplasmic granules may be decreased or lost. The nucleus may become densely stained and the segments may form spherical fragments suggestive of nucleated RBCs. This cell shows cytoplasmic vacuoles; more extensive cytoplasmic vacuolation can be seen in infected effusions. A predominance of neutrophils in an effusion is suggestive of bacterial infection.

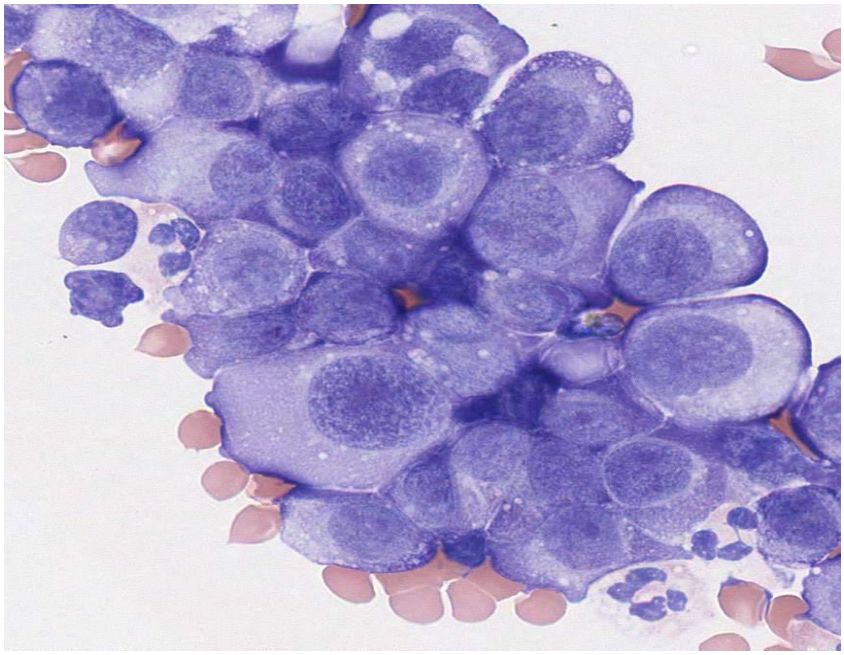
VBF-22



Identification	Participants No.	Participants %	Evaluation
Monocyte/macrophage	322	81.1	Educational
Macrophage containing abundant small lipid vacuoles/droplets (Lipophage)	71	17.9	Educational
Erythrocyte, mature	1	0.3	Educational
Lymphocyte	1	0.3	Educational
Mesothelial cell	1	0.3	Educational
Neutrophil, segmented or band	1	0.3	Educational

The arrowed object is a monocyte/macrophage, as correctly identified by 81.1% of participants. Monocytes and macrophages (also called histiocytes) are commonly present in pericardial, pleural and peritoneal effusions. They form a spectrum ranging from the typical blood monocyte to the vacuolated, activated stage of a macrophage, and ranging in size from 12 to 80 µm. They have pale grey cytoplasm, which is frequently vacuolated and may contain cellular components or pigments. The nuclei are bean shaped, round, oval, lobular or multinucleated. Mesothelial cells can also have vacuolated cytoplasm and may be hard to differentiate from macrophages. Macrophages with a single large vacuole can resemble “signet-ring” cells seen in some adenocarcinomas.

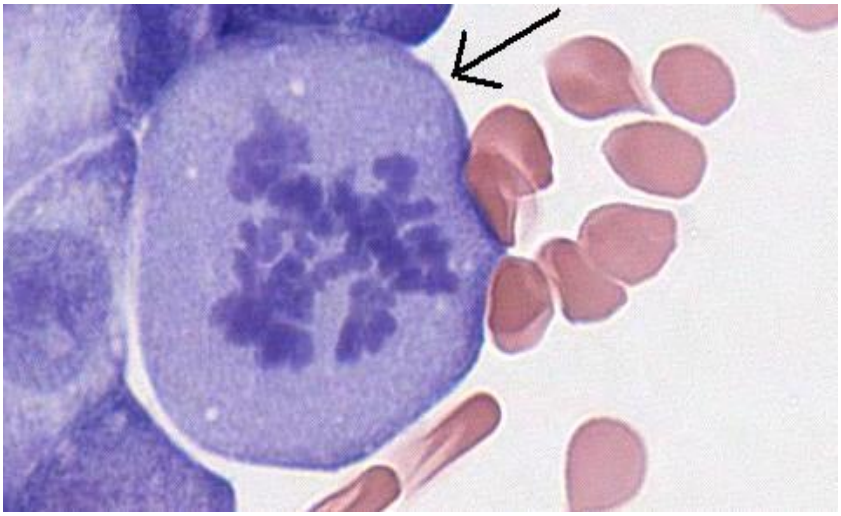
VBF-23



Identification	Participants No.	%	Evaluation
Malignant cell (non-hematopoietic)	302	76.1	Educational
Mesothelial cell	67	16.9	Educational
Immature or abnormal call, would refer for identification	24	6.1	Educational
Lymphoma cell	1	0.3	Educational
Macrophage containing abundant small lipid vacuoles/droplets (Lipophage)	1	0.3	Educational
Neutrophil, segmented or band	1	0.3	Educational
Ventricular lining cell (ependymal or choroid cell)	1	0.3	Educational

The arrowed object is a cluster of malignant cells (non-hematopoietic) as correctly identified by 76.1% of participants. Note the large size of these cells compared to the red cells and neutrophils in the field. The nuclear chromatin is coarse and nucleoli can be detected in some of the malignant cells. Some of the cells appear tightly aggregated and lack intercellular spaces or “windows” typical of mesothelial cells. Flattening of adjacent tumor cells’ nuclei, termed molding, is another characteristic of malignant epithelial cells and helps to distinguish them from reactive mesothelial cells, which can also show cellular enlargement and nucleoli. Differentiating reactive mesothelial cells from malignant cells can be very difficult, especially when the tumor cells are relatively small and uniform in size, as is the case in many breast carcinomas.

VBF-24



Identification	Participants No.	%	Evaluation
Mitotic figure	389	98.0	Educational
Degenerating cell, NOS	3	0.8	Educational
Immature or abnormal call, would refer for identification	3	0.8	Educational
Malignant cell (non-hematopoietic)	1	0.3	Educational
Monocyte/macrophage	1	0.3	Educational

The arrowed object is a mitotic figure, as correctly identified by 98.0% of participants. The condensed chromosomes in the nucleus are just beginning to separate (metaphase to anaphase) prior to cell division. Effusion fluids contain nutrients, oxygen and carbon dioxide and are at body temperature with a pH that provides a good culture medium. Benign and malignant cells exfoliated into effusions can survive, divide and proliferate. Therefore, mitotic figures are not synonymous with malignancy. In this case, the cell is quite large and likely represents a tumor cell. Benign and malignant dividing cells in effusions tend to round up, become plump, show nuclear enlargement and nucleoli and form three dimensional cell clusters.

Case Discussion

This is an example of metastatic breast carcinoma involving the pericardium with resulting pericardial effusion. An effusion is an abnormal accumulation of fluid in body spaces including the pericardium, pleura and abdominal cavity, or peritoneum. These spaces usually contain only a small amount of fluid. Disease states may result in increased fluid in these spaces, which may cause clinical symptoms necessitating medical or surgical treatment.

The causes of pericardial effusions are many including infections, (viral, bacterial, fungal), postoperative following cardiac surgery or cardiac transplant, drugs, hypothyroidism, renal failure, myocardial infarction, congestive heart failure, fluid overload, hypoproteinemia, autoimmune diseases such as rheumatoid arthritis, and cancer. Patients with HIV may experience pericardial effusion as a result of other infections, malignancies, capillary leakage or due to unknown causes. The most common cause of pericardial effusion is viral infection, with enterovirus the most common virus.

Most pericardial effusions are asymptomatic. However, due to the limited space for expansion, accumulation of fluid in the pericardium may compromise cardiac function by interfering with the ability of the heart to pump, called cardiac tamponade. Small, asymptomatic pericardial effusions are not usually removed and examined in the laboratory. Larger effusions, those of unknown etiology and those causing cardiac tamponade will be removed by sterile needle aspiration, called pericardiocentesis or through surgical opening of the pericardium, called pericardiotomy.

The volume of the pericardial fluid is documented at the bedside or in the operating room before being sent to the laboratory for testing. The fluid should be evaluated for color and clarity. Normal pericardial fluid is clear and pale yellow. Effusions due to uremia and congestive heart failure are usually clear. Turbid or hemorrhagic fluid is seen in infections and malignancies. Hemorrhagic fluids occur in trauma or rupture of a large blood vessel and may need to be distinguished from inadvertent aspiration of intracardiac blood. In the latter instance, the specimen will have roughly the same hematocrit and blood gases as in the peripheral blood and the specimen will clot. Hemorrhagic effusions have lower hematocrits, lower pO2 and higher pCO2 and usually do not clot. Milky effusions are observed in chylous and pseudo chylous effusions.

Chemical tests performed on pericardial fluids include pH, total protein, lactate dehydrogenase (LDH) and cholesterol. These tests help to classify the fluid as a transudate or exudate. Exudates have higher fluid to serum protein ratio, higher fluid to serum LDH ratio and an LDH level greater than two thirds of the serum upper limit of normal. Cholesterol levels are higher in exudates. Exudates are usually observed with infectious etiology and in malignancy. Gram stain, acid fast stain and cultures, cell count and cytology should also be performed. In many cases of viral pericarditis, the culture will be negative and acute and convalescent serum antibodies must be used to confirm the diagnosis.

Cellular elements in pericardial effusion fluids are neutrophils, eosinophils, basophils, lymphocytes, plasma cells, monocytes, histiocytes and macrophages, mesothelial cells and malignant cells. While the appearance of these cells is similar to those observed in other fluids, variation in size and appearance and degree of preservation can make cell identification challenging. With degeneration, mesothelial cells may acquire small cytoplasmic vacuoles. In chronic effusions, mesothelial cells can be phagocytic, resembling macrophages. In inflammatory effusions and in chronic effusions, mesothelial cells proliferate and become very large. They may be shed as single cells or pairs and clusters. Mesothelial cells in pericardial fluids can show striking reactive changes, possibly due to the pumping action of the beating heart. The degree of reactive atypia can mimic carcinoma. Large papillary clusters may form suggestive of tumor cell clusters. Cytologic evaluation with immunohistochemical stains on cell blocks are very helpful in correctly classifying these cells.

Lung, breast and esophageal carcinomas are the most common tumors that metastasize to the pericardium. When this occurs, the tumor has usually spread to other organs, as well. Lymphoma, sarcoma and melanoma are other relatively common primary tumors. Flow cytometry may be appropriate in patients with lymphoma. Malignant melanoma cells may be identifiable by cytoplasmic melanin pigment. Breast cancer can be very difficult to diagnose in fluids as the cells may be relatively small and form few cell clusters, thereby mimicking mesothelial cells.

References:

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