EDUCATIONAL COMMENTARY – PLEURAL FLUID ANALYSIS

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LEARNING OBJECTIVES

On completion of this exercise, the participant should be able to:

- define the 2 types of pleural effusions and 1 disease associated with each;
- describe common cells seen in pleural fluid differential cell counts; and
- describe the appearance and the clinical significance of each.

Pleural fluid is normally present in the human body in very small amounts, less than 10 mL. The accumulation of fluid in the pleural cavities surrounding the lungs, known as a pleural effusion, indicates either systemic disease or an acute localized process involving the surfaces of the lungs themselves. It is clinically important to distinguish between the two types of fluid buildup to effectively treat the patient. This exercise will discuss both common and abnormal microscopic findings in a pleural effusion.

Types of Pleural Effusions

A transudate is generally a sign of a serious underlying condition but in itself is considered a benign process. A transudate occurs as fluid passes through a membrane, filtering out cells and protein; it is either caused by increased pressure in the veins and capillaries, or a low level of protein in serum. A transudate can be thought of as a watery filtrate of blood. Pleural transudates are usually clear or straw-colored and can be seen in conditions such as congestive heart failure, nephrotic syndrome, myxedema, postpartum effusion, and peritoneal dialysis.

An exudate is indicative of an acute, localized process requiring active intervention and treatment. Exudate means “to sweat out” and is caused by altered permeability of blood vessels, which permits passage of large molecules and solid matter through their walls. The resulting fluid is rich in protein and cellular matter, as contrasted with the watery appearance of a transudate. Pleural exudates may be pink, white, brown, orange, green, or red, and can appear cloudy, milky, turbid, purulent, clotted, or bloody. Exudates are caused by infection, malignancies, autoimmune disorders, gastrointestinal tract disease, or trauma.
Laboratory testing, including chemistry, cytology, microbiology, and hematology, plays a vital role in
determining the cause of a pleural effusion. This commentary will focus on hematologic differential cell
counts.

**Hematology Cell Counts**
The hematologist will perform a cellular count to determine the total nucleated cells and total red blood
cells present in the sample. The cell count may be performed manually on a hemacytometer chamber
and microscope or with an automated cell counter using flow cytometry or hydrodynamic focusing
detection methods. Normal values for a pleural effusion are not readily defined, as the accumulation of
fluid is by definition abnormal. It is generally accepted that leukocyte counts less than 1000/µL are
suggestive of a transudate and leukocyte counts greater than 1000/µL suggest an exudate, and total red
blood cell count of greater than 10,000/µL may indicate trauma, malignancy, or pulmonary infarction.

In addition to a chamber count, examination of a Wright-Giemsa stained slide is needed to determine if
the patient truly has a high white blood cell count or if many of the cells present in the sample could be
epithelial or lining cells, most commonly mesothelial cells. The appearance and presentation of
nucleated cells found in pleural fluid and whether they are considered common/benign or abnormal is
discussed below.

**Cell Morphology**
Common cells present in pleural fluid include neutrophils, lymphocytes, monocytes, mesothelial cells, and
red blood cells. Less common findings include eosinophils, microorganisms, phagocytizing
macrophages, and tumor cells. Rarely seen are basophils, reactive lymphocytes, plasma cells, lupus
erthematous (LE) cells, and crystals.

Neutrophils, eosinophils, and basophils appear in body fluid differentials as they do in whole blood
differentials; an experienced hematologist should have little difficulty in identifying these cells in pleural
fluid.

Macrophages, also termed histocytes, can be seen engaged in active phagocytosis in pleural fluid. They
are large and often vacuolated. Some may contain giant lipid vacuoles that appear to flatten the nucleus
to one side of the cell. These are called signet ring cells and can mimic carcinoma. Macrophages may
contain red blood cells, neutrophils, hemosiderin granules, bacteria, or other cellular debris.
Mesothelial cells can present a myriad of forms, making their identification more difficult. They can appear reactive, single- or multi-nucleated, and of varying sizes, making it difficult to differentiate them from monocytes, macrophages, lymphocytes, or malignant cells. In fact, because both mesothelial cells and monocytes can convert to macrophages, some laboratories combine all 3 types of cells in the same category on their laboratory reports owing to the difficulty in distinguishing them microscopically.\textsuperscript{2}

Mesothelial cells are generally larger than monocytes and the cytoplasm does not normally contain phagocytized material. Mesothelial cells have more abundant cytoplasm and rounder nuclei than monocytes/macrophages. The nuclei should not be folded, and in a reactive state they may have prominent nucleoli. Mesothelial cells are larger than lymphocytes with less dense nuclear chromatin and more cytoplasm. They may form cell aggregates or have multinucleated forms, unlike normal monocytes/macrophages and lymphocytes. Even a mesothelial clump or a multinucleated form will show distinct “windows” between each cell in the clump and each nucleus in the multinucleated form, with a noncontinuous border around the mass.\textsuperscript{1}
Lymphocytes may be small, medium-sized, or large, and can appear normal, reactive, or immature. Normal lymphocytes in a serous effusion can masquerade as their more sinister counterparts: plasma cells, plasmacytoid lymphocytes, reactive lymphocytes, lymphoma cells, and blasts. Lymphocytes are more susceptible to morphologic changes resulting from cytocentrifugation, often appearing with prominent nucleoli, a clefted or irregular nucleus, or cytoplasmic spreading.1,2

Normal lymphocytes, although variable, typically exhibit a round-oval nucleus with densely packed chromatin and sky blue cytoplasm with or without a few azurophilic granules.1 Plasmacytoid or reactive/viral lymphocytes are generally larger and have more abundant, deeply basophilic cytoplasm and more prominent nucleoli than normal small lymphocytes. Because any condition associated with plasmacytoid or reactive lymphocytes is likely to be benign, it is sometimes considered less clinically significant to differentiate them than to distinguish them from lymphoma or blast cells.1

Plasma cells are small and deep blue with a typical perinuclear clear zone (hof).1 These are usually a nonspecific finding, indicating an inflammatory, infectious, or neoplastic condition and are rarely seen in pleural fluid.

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Lymphoma cells are quite variable in appearance and size, generally with irregular or clefted nuclei, immature nuclear chromatin, slight to moderate amounts of basophilic cytoplasm, and sometimes multiple vacuoles. Blasts can appear very similar in pleural fluid and in whole blood, typically with a high nuclear to cytoplasmic ratio, very immature, finely clumped chromatin, and prominent nucleoli. These are easily confused with mesothelial cells, as discussed above. Flow cytometry is extremely useful in differentiating the origin of a lymphoma or blast cell proliferation, allowing the physician to tailor an appropriate treatment plan.

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Malignant cells can be difficult to identify on a smear alone, and the skilled hematologist will look for several key features to make the identification. Characteristics of malignant cells include high nuclear to cytoplasmic ratio, large cell size, large irregular nuclei with abnormal chromatin, and nucleoli. Tumor cells can be isolated and bizarre or seen in clusters mimicking the appearance of a benign cell clump. However, a tumor cell aggregate begins to lose the individual distinctions seen with benign cells and transforms into a solid mass, often with dark, entire margins. This is a key to distinguishing normal mesothelial cells or clumps from a tumor cell mass. As discussed above, a clump of benign mesothelial cells will have a non-continuous border around the mass, and will have windows between the cells. Correlation of several histochemical stains can aid in the diagnosis; frequently a Wright-Giemsa stain will be used for screening and a Papanicolaou stain for confirmation.

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Lupus erythematosus (LE) cells may occasionally be found in serous fluids. They are not diagnostic for systemic lupus erythematosus, as they may also occur in rheumatoid arthritis and other autoimmune diseases. An LE cell is formed when a neutrophil phagocytizes nuclear material from a dead cell, developing a smooth, homogeneous, pink inclusion that pushes the nucleus to the periphery of the cell. Tart cells appear very similar to LE cells but are not associated with systemic lupus erythematosus or any other autoimmune disease. Tart cells are essentially non-neutrophilic macrophages that have phagocytized the nucleus of another cell, but the ingested nucleus is nonhomogenized and shows some nuclear detail. Images reproduced with permission from PeaceHealth Laboratories.

Summary

The laboratory plays a very important role in determining the composition and clinical significance of a pleural effusion. A complete cell count and differential are two of the most common tests requested by physicians, and the skill of the hematologist in identifying common cells types from the abnormal is critical. These laboratory results may guide the physician to consider an underlying condition causing the effusion, an infectious process, or a malignancy. Confirmatory testing by flow cytometry, Papanicolaou staining, or additional immunoperoxidase staining can provide further accuracy in diagnosing and treating disease.

REFERENCES


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