COLLEGE of AMERICAN PATHOLOGISTS

Surveys and Anatomic Pathology Education Programs

Virtual Body Fluid
VBF-B 2019

Participant Summary

1.0 Credit of Continuing Education Available

This document is available online in e-LAB Solutions Suite.

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2019 VBF-B
PARTICIPANT SUMMARY

Program Update

Don't Miss Out on this Educational Opportunity!

With your participation in CAP's Surveys programs, *every member of your team* can take part in education activities: earn Continuing Education (CE) credits or receive Self-Reported Training* at no additional charge.

This Survey mailing includes an online education activity to earn **1.0** CE credit. To access the activity, see page 22.

**CAP Self-Reported Training activities do not offer CE credit, but can be used towards fulfilling requirements for certification of maintenance by agencies such as the American Society of Clinical Pathology (ASCP). Please verify with your certifying agency to determine your education requirements.*

Evaluation Criteria

Results for the VBF Survey are **not** formally evaluated; however, statistics will appear in the Participant Summary for your information.

The quantitative data tables provided in the Participant Summary include the mean, SD, median, %CV, and the lowest and highest values reported for each peer group. The low and high values are not the limits of acceptability. The acceptable limits are located on your participant evaluation report.

In the event a result is not graded, a numeric code will appear next to your result. A definition of the code will appear on the first page of your evaluation.

Please see "Actions Laboratories Should Take when a PT Result is Not Graded" on page 20.

To provide a timely evaluation of your results, statistics presented in this Participant Summary reflect participant data received by the due date.

Case History for VBF-13 – VBF-18

This peritoneal fluid cytospin slide is from a 2-month-old girl presenting with peritonitis with renal insufficiency requiring dialysis. Laboratory peritoneal fluid values: TNC = 1,579/µL (1.579 x 10E3/µL) and RBC = > 2,000/µL (> 2.000 x 10E3/µL). Identify the arrowed object(s) on each whole slide image.

(PERITONEAL FLUID, CYTOCENTRIFUGE, WRIGHT-GIEMSA STAIN)

Please click on the hyperlink below to view the DigitalScope images for this case.
http://www.digitalscope.org/LinkHandler.axd?LinkId=1d4ed220-6a85-49f9-aeb4-47e2adb521b5

To access the online Hematology Glossary, please click the hyperlink below:
https://cap.objects.frb.io/documents/2019-hematology-clinical-microscopy-glossary.pdf

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Summary of Participant Survey Results

The following is a statistical summary of all results submitted by participating labs. These are provided to allow participants to see their responses in the context of their peers. These results may identify findings or topics for further education or review. Survey results are not intended to represent the correct or desired responses for proficiency testing purposes and the SD and CV should not be interpreted as acceptable reporting limits. Participants are encouraged to review discrepant results with their medical director.

Total Nucleated Cells Differential – %

		NO. LABS	MEAN	S.D.	C.V.*	Median	Low Value	High Value
VBF-13	Neutrophils, segmented or band	568	42.60	7.96	18.7	41.0	22.0	67.0
	Lymphocytes	520	3.10	2.52	81.3	2.0	0.0	13.0
	Monocyte/macrophage/ mesothelial	568	49.24	10.30	20.9	51.0	16.0	74.0
	Eosinophils	559	3.71	1.87	50.5	3.0	0.0	10.0
	Basophils	289	0.00	0.02	*	0.0	0.0	0.3
	Plasma cells	269	0.01	0.12	*	0.0	0.0	1.0
	Blasts	263	0.05	0.27	*	0.0	0.0	0.3
	Neutrophil, immature (metamyelocyte/myelocyte/ promyelocyte)	257	0.00	0.06	*	0.0	0.0	1.0
	nRBC/100 WBC	269	0.11	0.32	*	0.0	0.0	1.0

***** When low results are reported on an analyte, a high coefficient of variation (CV) may result. When the mean value is very low the C.V. may be exaggerated.

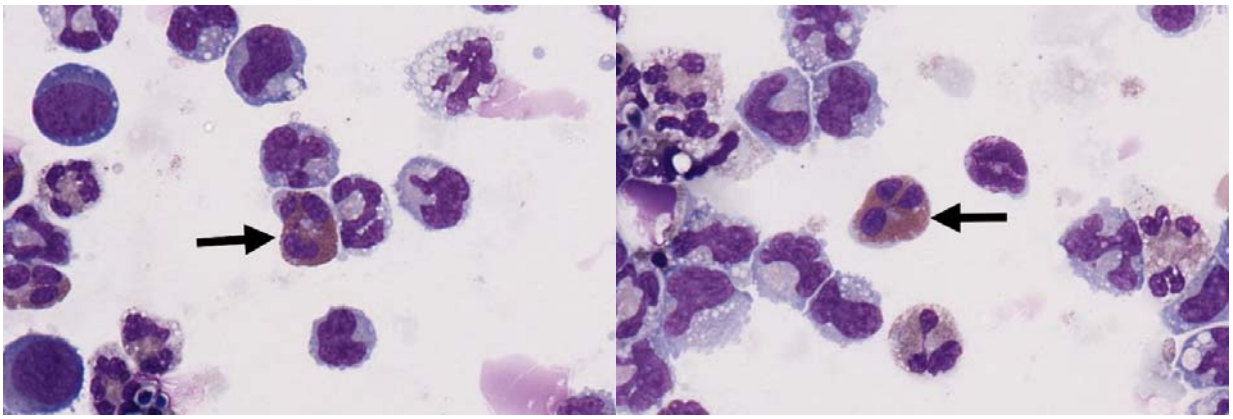
VBF-13	**Other:** Cells not listed and cells not differentiated by your lab	**# participants: (18)**
	Lymphoma cell	3
	Plasma cell, normal/abnormal	2
	Mesothelial/reactive mesothelial cell	2
	Neutrophil/macrophage containing fungi	2
	Abnormal lymphocyte	1
	Immature mononuclear cell	1
	Large mononuclear cell	1
	Malignant cell (non-hematopoietic)	1
	Would refer to pathologist	6

Committee Comments for Online Whole Slide Image

The cytocentrifuge preparation shows a mixed inflammatory population including mesothelial cells, neutrophils, eosinophils, and monocytes/macrophages. No malignant cells are seen.

Cell Identification

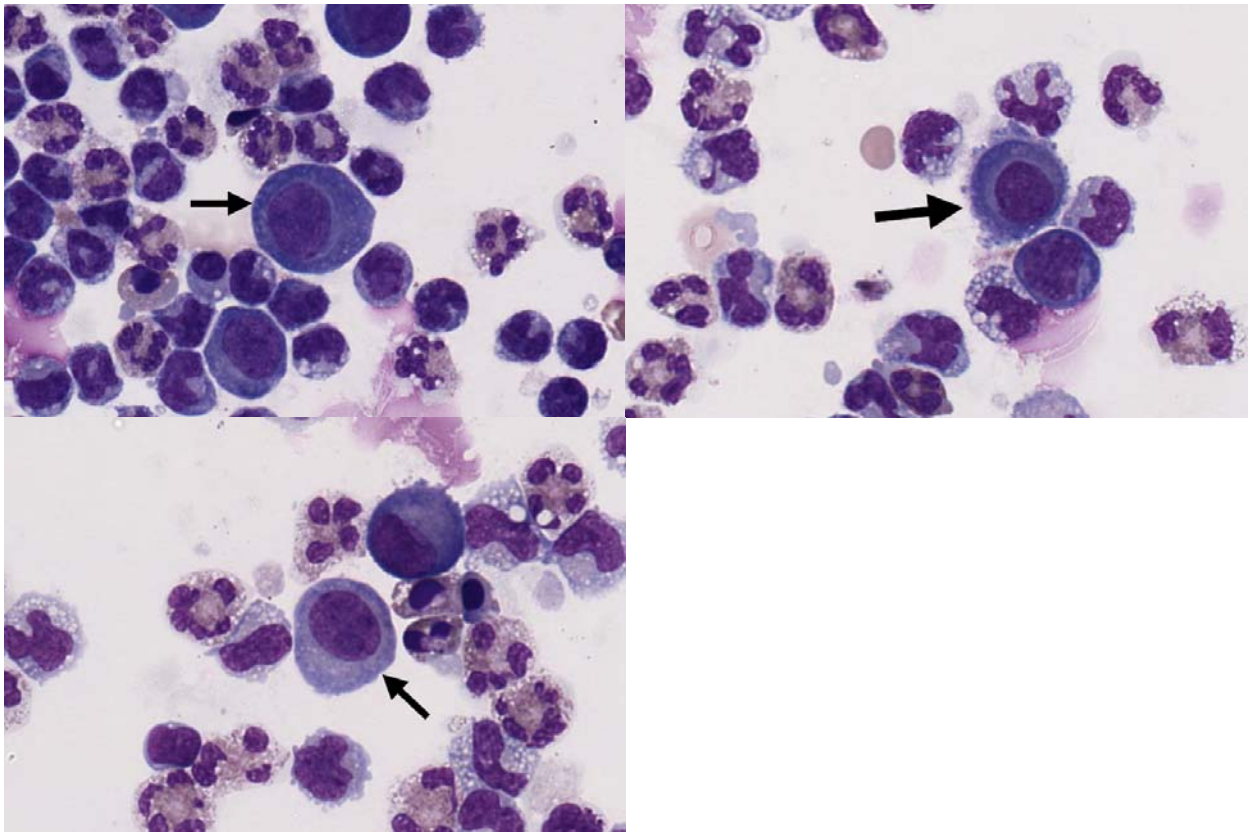
VBF-14



Identification	Participants No.	%	Evaluation
Eosinophil, any stage	590	100.0	Educational

The arrowed objects are eosinophils, as correctly identified by 100% of participants. These cells are recognized by their characteristic round, orange-pink to orange-red granules. These are larger than the primary or secondary granules seen in neutrophils. The arrowed cells are 10 -15 µm, have 2 - 3 nuclear lobes. Particularly large numbers of eosinophils may be seen in foreign body reactions, parasitic infection, and when air is inadvertently introduced into a body cavity.

VBF-15



Identification	Participants No.	%	Evaluation
Mesothelial cell	525	89.0	Educational
Plasma cell, normal/abnormal	23	3.9	Educational
Blast Cell	15	2.5	Educational
Monocyte/macrophage	6	1.0	Educational
Lymphocyte, reactive	5	0.9	Educational
Lymphoma cell	5	0.9	Educational
Malignant cell (non-hematopoietic)	5	0.9	Educational
Immature or abnormal cell, would refer for identification	3	0.5	Educational
Neutrophil, immature (metamyleocyte, myelocyte, promyelocyte)	2	0.3	Educational
Synoviocyte (synovial lining cell)	1	0.2	Educational

The arrowed objects are mesothelial cells, as correctly identified by 89% of participants. The nucleus is round to oval and either centrally or eccentrically located with clumped chromatin. The cytoplasm is blue and may appear granular, sometimes with ruffled borders. Some cells are multinuclated. In adjacent mesothelial cells, a “window” or clear space between cells may be seen.

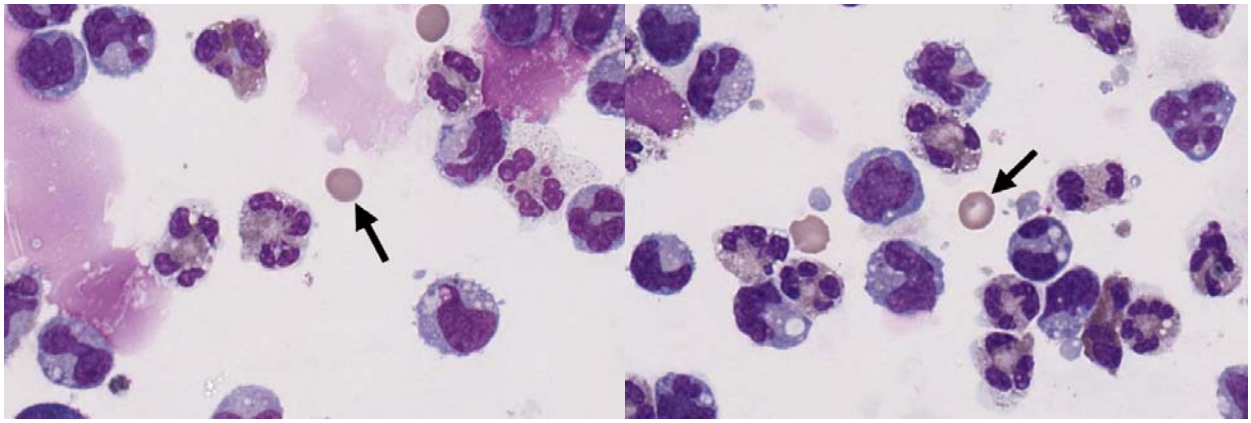
VBF-15 cont

3.9% of participants identified these cells as plasma cells. Plasma cells are typically smaller with a more condensed nucleus and no ruffling of the cell borders.

2.5% of participants identified these images as blasts, however, the nuclear features are not immature and contain an abundance of cytoplasm.

1.0% of participants identified these cells as monocytes/macrophages. Monocyte/macrophages in fluids typically have paler blue cytoplasm, more numerous vacuoles, a more condensed nucleus, and also lack the irregular cell border.

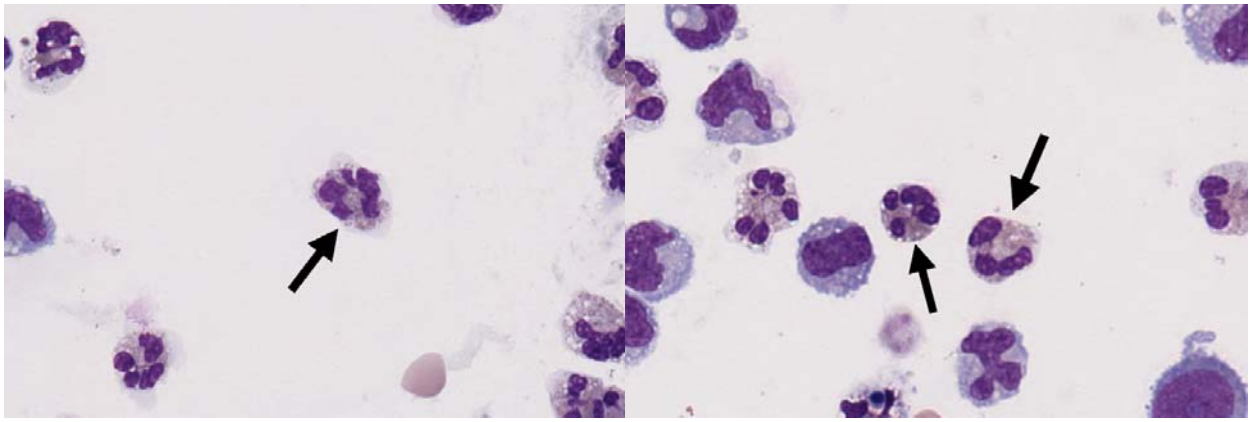
VBF-16



Identification	Participants No.	%	Evaluation
Erythrocyte	590	100.0	Educational

The arrowed objects are erythrocytes, or red blood cells, as correctly indicated by 100% of participants. Erythrocytes should biconcave (6 - 7 µm) with central pallor (1/3 of the cell). The is cytoplasm is hemoglobinized (pink) and mature erythrocytes lack nuclei. In fluid preparations, there may be anisopoikilocytosis that is unreliable for performing an assessment of red cell morphology.

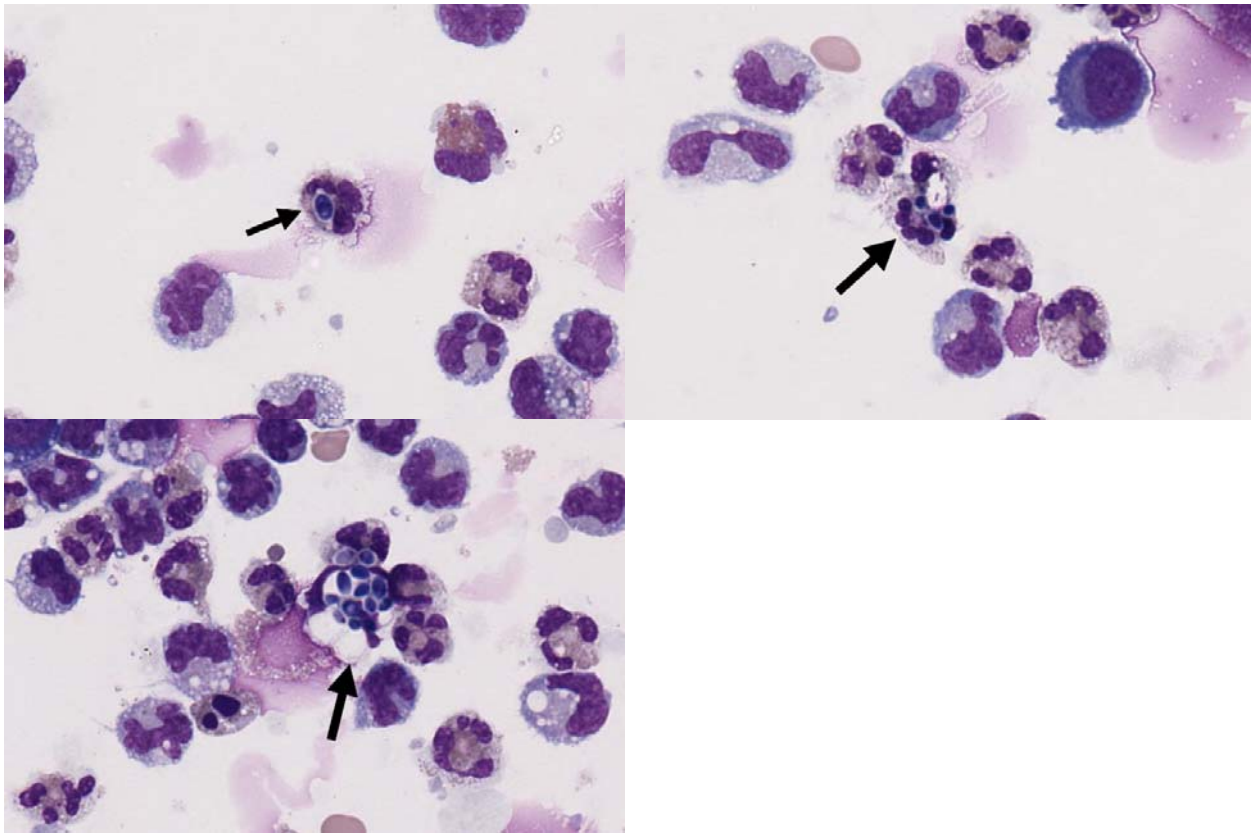
VBF-17



Identification	Participants No.	%	Evaluation
Neutrophil, segmented or banded	582	98.6	Educational
Neutrophil, immature (metamyelocyte, myelocyte, promyelocyte)	6	1.0	Educational
Neutrophil/macrophage containing bacteria	1	0.2	Educational
Eosinophil, any stage	1	0.2	Educational

The arrowed objects are neutrophils, segmented or band, as correctly identified by 98.6% of participants. Neutrophils are 10 - 15 µm in size and contain 3 - 5 nuclear lobes connected with thin filaments. Pink specific granules are present. Darker, larger granules may be seen in the setting of infection (toxic granulation).

VBF-18



Identification	Participants No.	%	Evaluation
Neutrophil/macrophage containing fungi	538	91.2	Educational
Yeast/fungi, extracellular	18	3.0	Educational
Macrophage containing neutrophil(s) (Neutrophage)	10	1.7	Educational
Degenerating cell, NOS	8	1.4	Educational
Neutrophil/macrophage containing bacteria	5	0.9	Educational
Macrophage containing erythrocyte(s) (Erythrophage)	3	0.5	Educational
Ehrlichia/*Anaplasma*	2	0.3	Educational
Macrophage containing hemosiderin (Siderophage)	2	0.3	Educational
Immature or abnormal cell, would refer for identification	1	0.2	Educational
Macrophage containing abundant uniform small lipid vacuole(s)/droplet(s) (Lipophage)	1	0.2	Educational
Mesothelial cell	1	0.2	Educational
Pneumocystis jirovecii	1	0.2	Educational

The objects are neutrophils and monocyte/macrophages containing organisms (fungi), as correctly interpreted by 91.2% of participants. The fungal organisms are round to oval with smooth nuclear contours. In infections, bacteria and fungi may be seen within cells. If they are extracellular, they may represent contamination of the stain rather than true infection. The size and morphology may suggest certain organisms, although cultures are preferable for definitive identification.

Case Presentation:

This peritoneal fluid cytospin slide is from a 2-month-old girl presenting with peritonitis with renal insufficiency requiring dialysis. Laboratory peritoneal fluid values: TNC = 1,579/µL (1.579 x 10E3/µL) and RBC = > 2,000/µL (> 2.000 x 10E3/µL).

(PERITONEAL FLUID, CYTOCENTRIFUGE, WRIGHT-GIEMSA STAIN)

CASE DISCUSSION: Peritonitis

Peritoneal effusions result from accumulation of fluid in abdominal cavity. In a normal person, the amount of fluid in the abdomen should be minimal. Fluid can accumulate abnormally in numerous clinical conditions, including portal hypertension, and in certain cancers such as ovarian adenocarcinoma. Cirrhosis is a common cause of portal hypertension.

Depending on the etiology of the effusion, cytologic preparations may show different types of inflammatory cells and/or malignant cells in ascites fluid. Variable numbers of RBCs will also be seen in some effusions. Peritonitis can be caused by bacteria (commonly) or fungi (rare as in this case). In spontaneous bacterial peritonitis (SBP), a very serious form of infection, many neutrophils are present. The peritoneal fluid is an exudative effusion with a cloudy appearance. It is defined by absolute neutrophil count greater than 250 cells/mmE3, even in the absence of a positive culture. If malignant and/or bloody effusions and systemic lactic acidosis are excluded, SBP can be diagnosed if 2 of 3 of the following criteria are present: 1) WBC (or TNC) is greater than 1000/mmE3 or PMN greater than 500/mmE3, 2) pH less than 7.40, and 3) lactate greater than 25 mmol/L. More recent studies have shown the utility of leukocyte esterase reagent strips for detecting SBP at the bedside for rapid diagnosis.

Spontaneous bacterial peritonitis (SBP) is defined as occurring in the setting of no known surgically repairable intraabdominal source of infection. This is often seen in the setting of ascites, typically in the setting of cirrhosis and portal hypertension. The incidence varies from 7 - 30% in patients with ascites. In addition to numerous neutrophils, intracellular bacteria may be seen in some cases. Cultures should be taken as these are important for confirming bacterial infection and determining the best antimicrobial treatment.

Prompt treatment is required to avoid the high mortality rate (90%) that can be seen in untreated patients. SBP occurs due to the transit of intestinal bacteria to mesenteric lymph nodes and then into the peritoneal fluid. This leads to the generation of cytokines which can lead to septic shock, including renal failture.

The organisms (bacteria) most commonly found in SBP include *E. Coli*, *Klebsiella*, other *Enterococcus* species, *Pseudomonas*, and *Proteus*. Once patients have had one episode of SBP, the recurrence rate is 50 - 70%. Based on this high rate, patients are often placed on prophylactic antibiotics after the first episode.

Other clinical signs of SBP include renal impairment, confusion, and peripheral blood leukocytosis. Paracentesis is often performed on admission in order to anticipate and treat these infections promptly.

The present case is due to fungal peritonitis, a condition that is rarer than SBP, but commonly occurs after SBP, often in the setting of peritoneal dialysis. In cases of fungal peritonitis, bacterial cultures will be negative, but fungal cultures, if performed, may be positive. In both bacterial and fungal peritonitis, organisms may be seen within neutrophils or macrophages and may provide a presumptive etiology. Cultures should always be performed for definitive identification.

Lauren B. Smith, MD
Hematology and Clinical Microscopy Resource Committee

References

1. Prasad N, Gupta A. Fungal peritonitis in peritoneal dialysis patients. *Perit Dial Int.* 2005;25:207-22.

2. Yang C-Y, Liaw Y-F, Chu C-M, Sheen I-S. White count, pH, and lactate in the ascites in the diagnosis of spontaneous bacterial peritonitis. *Hepatology.* 1985:5;85-90.

Case History for VBF-19– VBF-24

This pleural fluid cytocentrifuge slide is from a 75-year-old woman with a history of non-small cell lung carcinoma who now presents with shortness of breath and large pleural effusion with compression atelectasis. Laboratory pleural fluid values include: TNC = 27,910/µL (27.910 x 10E3/µL); and RBC = 26,720/µL (26.720 x 10E3/µL). Identify the arrowed object(s) on each whole slide image.

(PLEURAL FLUID, CYTOCENTRIFUGE, WRIGHT-GIEMSA STAIN)

Please click on the hyperlink below to view the DigitalScope images for this case.
http://www.digitalscope.org/LinkHandler.axd?LinkId=373dff0d-28e9-4dff-8901-0f1a755ae17e

To access the online Hematology Glossary, please click the hyperlink below:
https://cap.objects.frb.io/documents/2019-hematology-clinical-microscopy-glossary.pdf

Summary of Participant Survey Results

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Total Nucleated Cells Differential – %

		NO. LABS	MEAN	S.D.	C.V.*	Median	Low Value	High Value
VBF-19	Neutrophils, segmented or band	565	7.94	4.30	54.1	7.0	0.0	24.0
	Lymphocytes	534	6.46	4.97	76.9	5.0	0.0	25.0
	Monocyte/macrophage/ mesothelial	573	64.73	18.84	29.1	69.0	6.0	94.0
	Eosinophils	570	7.39	3.83	51.9	7.0	0.0	20.0
	Basophils	277	0.00	0.00	0.0	0.0	0.0	0.0
	Plasma cells	269	0.01	0.09	*	0.0	0.0	1.0
	Blasts	262	0.04	0.36	*	0.0	0.0	5.0
	Neutrophil, immature (metamyelocyte/myelocyte/ promyelocyte)	269	0.16	1.08	*	0.0	0.0	12.0
	nRBC/100 WBC	250	0.00	0.00	0.0	0.0	0.0	0.0

***** When low results are reported on an analyte, a high coefficient of variation (CV) may result. When the mean value is very low the C.V. may be exaggerated.

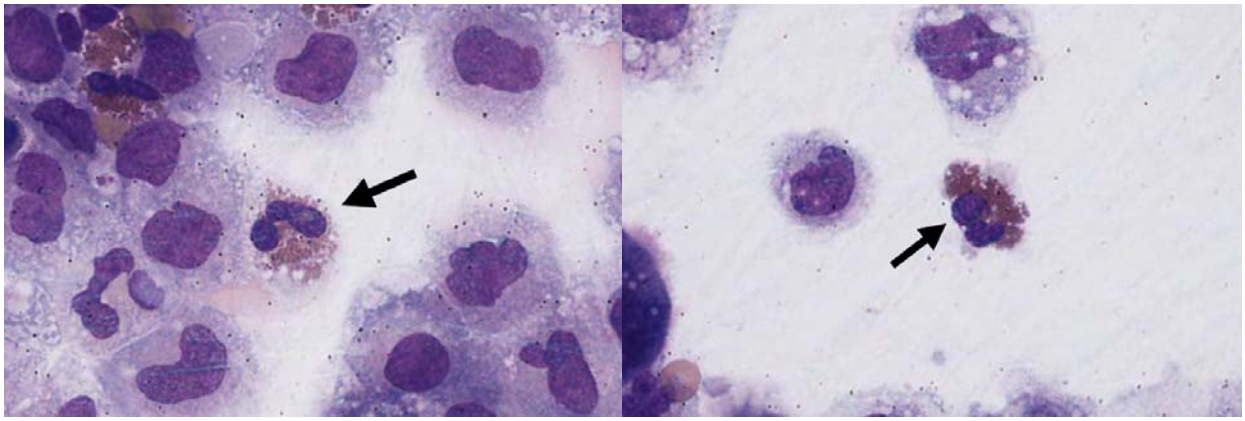
VBF-19	**Other:** Cells not listed and cells not differentiated by your lab	**# participants: (174)**
	Malignant cell (non-hematopoietic)	107
	Abnormal atypical mononuclear cell	7
	Atypical/reactive mesothelial cell	4
	Tumor cell	2
	Lymphoma cell	1
	Would refer to pathologist	53

Committee Comments for Online Whole Slide Image

The cytospin preparation shows a very high count of a mixed population of nucleated cells, including abundant neoplastic cells. These are composed of large and giant cells (with occasional malignant cells exceeding 10-times the size of a normal segmented neutrophil), with round/oval to irregular nuclear contours, coarse chromatin and prominent nucleoli. Several malignant cells demonstrate multinucleation or form dense clusters with distinct nuclear molding. A subset of the neoplastic cells shows prominent cytoplasmic vacuolization and/or signet ring-like morphology. Also present are numerous monocytes/macrophages, as well as neutrophils and eosinophils. Mesothelial cells, typically encountered in pleural effusions, are difficult to identify. Frequent red blood cells are also present.

Cell Identification

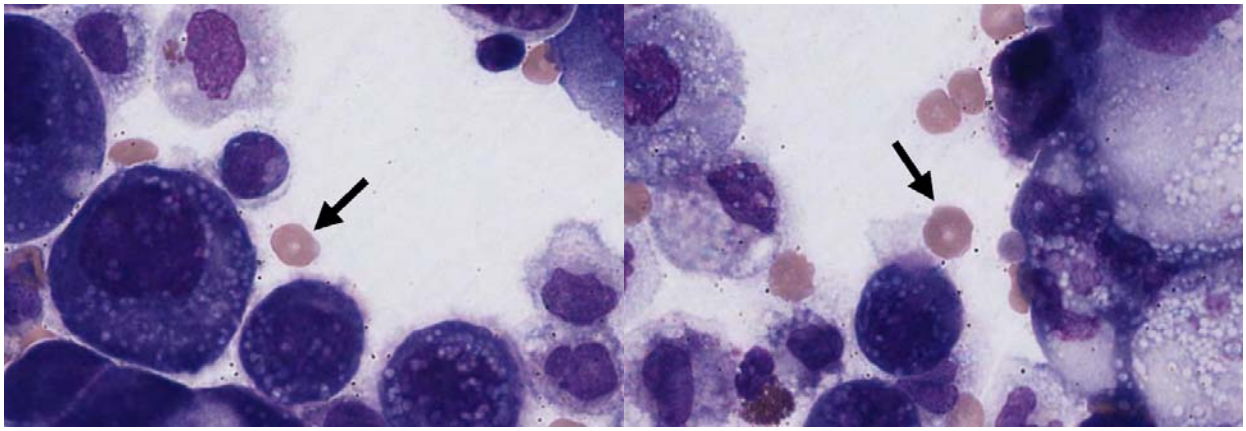
VBF-20



Identification	Participants No.	%	Evaluation
Eosinophil, any stage	589	100.0	Educational

The arrowed objects are eosinophils, as correctly identified by 100% of participants. These cells are recognized by their characteristic round, orange-pink to orange-red granules. These are larger than the primary or secondary granules seen in neutrophils. Particularly large numbers of eosinophils may be seen in foreign body reactions, parasitic infection, and when air is inadvertently introduced into a body cavity.

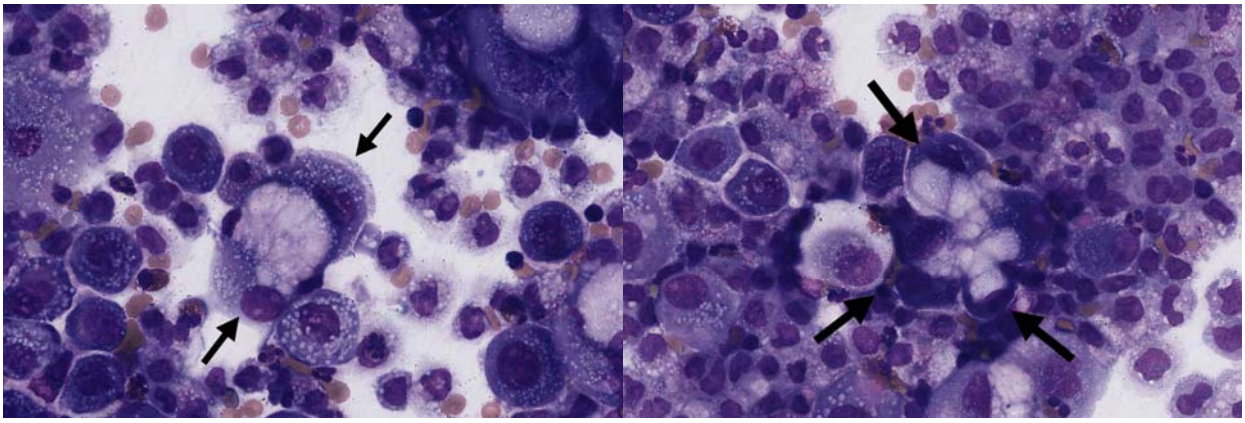
VBF-21



Identification	Participants No.	%	Evaluation
Erythrocyte	588	99.8	Educational
Erythrocyte, nucleated	1	0.2	Educational

The arrowed objects are erythrocytes, as correctly identified by 99.8% of participants. Erythrocytes within body fluid samples are typically without nuclei and similar to those present in the peripheral blood. They are not typically found in normal body fluid samples and reflect hemorrhage or traumatic contamination. They may also be seen in association with other disease states, such as malignancy.

VBF-22



Identification	Participants No.	%	Evaluation
Malignant cell (non-hematopoietic)	478	81.2	Educational
Mesothelial cell	52	8.8	Educational
Macrophage containing abundant uniform small lipid vacuole(s)/droplet(s) (Lipophage)	23	3.9	Educational
Immature or abnormal cell, would refer for identification	15	2.5	Educational
Monocyte/macrophage	11	1.9	Educational
Macrophage containing neutrophil(s) (Neutrophage)	3	0.5	Educational
Degenerating cell, NOS	2	0.3	Educational
Megakaryocyte	2	0.3	Educational
Bronchial lining cell	1	0.2	Educational
Lupus erythematosus (LE) cell	1	0.2	Educational
Macrophage containing erythrocyte(s) (Erythrophage)	1	0.2	Educational

The arrowed objects are malignant cells (non-hematopoietic), as correctly identified by 81.2% of participants. A variety of neoplastic cells may be found in body fluids, and their morphology is dependent on that of the primary underlying malignancy. Malignant cells may be numerous and clustered (as in the current specimen) or appear as rare single cells. Cytologic features of malignant cells on cytocentrifuge preparations include high nuclear-to-cytoplasmic ratio, increased cell and nuclear size, irregularly shaped nuclei, atypical chromatin patterns, large nucleoli, and a tendency to form large clusters, frequently with nuclear molding. In the present case, there are abundant large neoplastic cells (with occasional cells exceeding 10-times the size of a normal segmented neutrophil), with round/oval to irregular nuclear contours, coarse chromatin and prominent nucleoli. Several malignant cells demonstrate multinucleation or form clusters. A subset of the neoplastic cells shows prominent cytoplasmic vacuolization and/or signet ring-like morphology.

VBF-22 cont

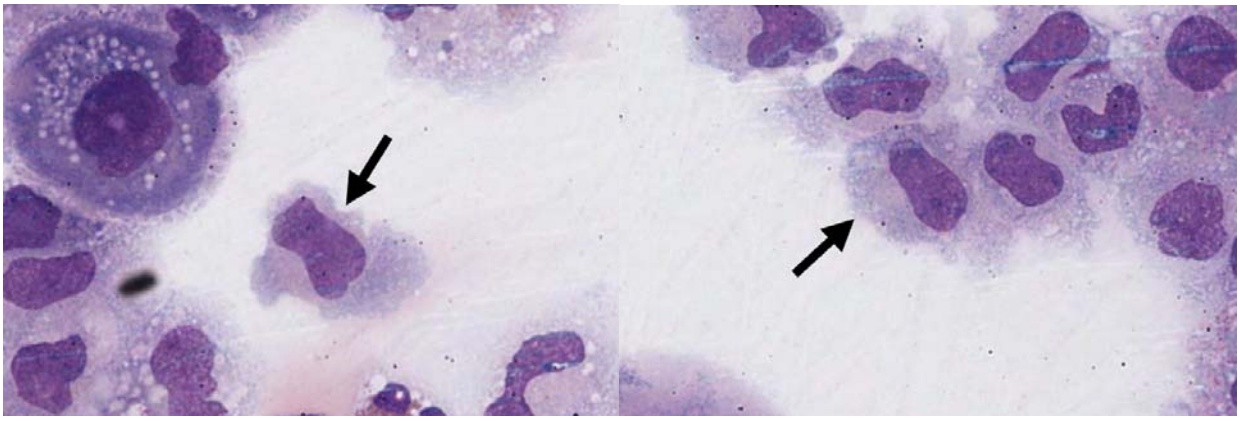
These cells were incorrectly identified as mesothelial cells by 8.8% of participants. Mesothelial cells (20 to 50 µm) normally lines pleural surfaces. These cells can be shed individually or in clusters. When found in pairs or clusters, mesothelial cells have articulated or coupled cell borders with a discontinuous outer border (clear spaces or “windows”) between many of the cells. The nucleus is round to oval in shape with a definitive nuclear membrane and regular contour. Chromatin varies from dense to fine, but it is evenly distributed. Multiple nuclei may occur and the nuclei may overlap; however, the nuclei remain of approximately equal size and shape. One or more nucleoli may be present. With degeneration, additional small vacuoles may occur throughout the cell. In chronic effusions or during inflammatory processes, mesothelial cells proliferate and become very large. Mitotic figures occasionally are seen within mesothelial cells. The chromatin is less condensed and nucleoli may be prominent; however, the nucleus still retains a definitive, smooth, nuclear membrane. In comparison, metastatic carcinoma cells found in this malignant effusion have irregular nuclear contours, coarse, unevenly distributed chromatin, variable nuclear size and shape, and abundant cytoplasmic vacuoles.

These cells were also incorrectly identified as macrophage (lipophage) by 3.9% of participants. The lipophage is a macrophage containing uniform, small lipid vacuoles that completely fill the cytoplasm. They may be present in pleural fluid associated with chylothorax or with extensive cell membrane destruction.

2.5% of participants identified these cells incorrectly as immature/abnormal cells. The nuclear and cytoplasmic features do not support an immature (blast) morphology.

1.9% of participants identified these cells incorrectly as monocyte/macrophage. Please see discussion in the next paragraph on monocyte/macrophage cytology and morphologic differential diagnosis.

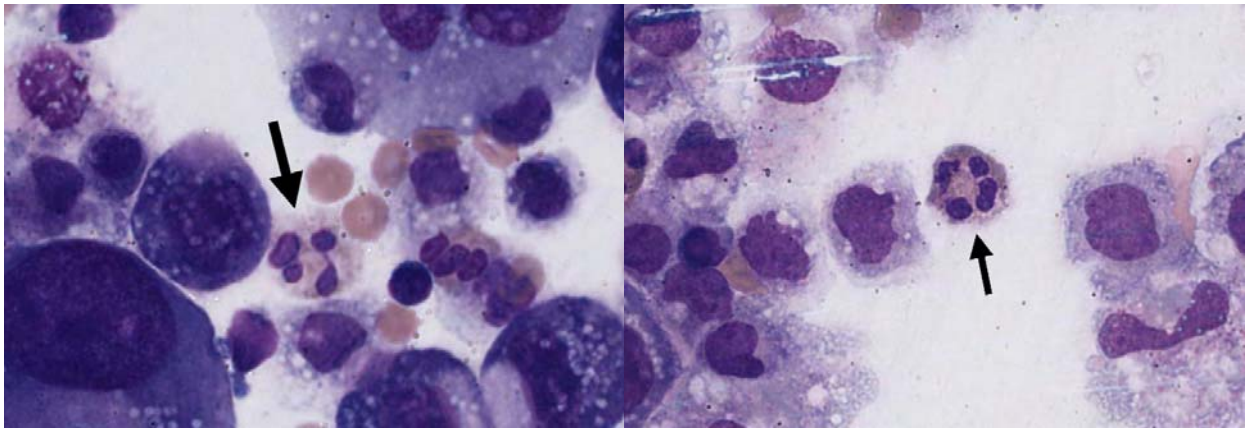
VBF-23



Identification	Participants No.	%	Evaluation
Monocyte/macrophage	562	95.4	Educational
Neutrophil, immature (metamyleocyte, myelocyte, promyelocyte)	10	1.7	Educational
Mesothelial cell	7	1.2	Educational
Degenerating cell, NOS	2	0.3	Educational
Macrophage containing hemosiderin (Siderophage)	2	0.3	Educational
Malignant cell (non-hematopoietic)	2	0.3	Educational
Endothel cell/Capillary	1	0.2	Educational
Lymphocyte	1	0.2	Educational
Lymphoma cell	1	0.2	Educational
Ventricular lining cell (ependymal or choroid cell)	1	0.2	Educational

The arrowed objects are monocytes/macrophages, as correctly interpreted by 95.4% of participants. Monocytes are bone marrow-derived cells that circulate in the blood. Macrophages arise from monocytes that migrate into tissues and evolve morphologically. Monocyte/macrophage morphology in fluids is quite variable, ranging from the typical monocyte of the peripheral blood to a vacuolated, activated stage with the morphology of a typical macrophage. Monocytes are usually large (12 to 20 µm) with abundant blue-gray cytoplasm and often containing sparse azurophilic granules. The nucleus is round to oval and may show indentation, giving it a kidney bean or horseshoe shape. The chromatin is lacy and small nucleoli may be apparent. Macrophages are larger cells (15 to 80 µm) with abundant cytoplasm showing evidence of active phagocytosis. One or more round to oval nuclei are present and occasionally prominent nucleoli may be seen. Macrophages can at times be difficult to differentiate from mesothelial cells. Mesothelial cells are usually larger than monocytes/macrophages and usually show a biphasic staining cytoplasm and surface microvilli.

VBF-24



Identification	Participants No.	%	Evaluation
Neutrophil, segmented or band	585	99.3	Educational
Degenerating cell, NOS	2	0.3	Educational
Neutrophil, immature (metamyleocyte, myelocyte, promyelocyte)	1	0.2	Educational
Neutrophil/macrophage containing fungi	1	0.2	Educational

The arrowed objects are segmented neutrophils, as correctly identified by 99.3% of participants. Neutrophils are 10 - 15 µm in size, with pink granulated cytoplasm and three to five nuclear lobes. In cytocentrifuge preparations, the nuclear lobes may become slightly eccentric, and degenerative changes leading to pyknosis and nuclear fragmentation may complicate identification of these cells in this setting.

Actions Laboratories Should Take when a PT Result is Not Graded

The CAP uses exception reason codes that signify the proficiency testing (PT) for an analyte has not been graded. The exception reason code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all analytes with an exception reason code, review, and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions laboratories should take include, but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
11	Unable to analyze	Document why the specimens were not analyzed (eg, instrument not functioning or reagents not available). Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
20	Response was not formally graded due to insufficient peer group data. Please see the participant summary for additional information.	Applies to a response that is not formally evaluated when a peer group is not established due to fewer than 10 laboratories reporting. Document that the laboratory performed a self-evaluation using the data presented in the participant summary and compared its results to a similar method, all method, all participant statistics, or data tables for groups of 3-9 laboratories, if provided. Perform and document the corrective action of any unacceptable results. If self-evaluation is not possible, it is up to the laboratory director/designee to determine an alternative performance assessment.
21	Specimen problem	Document that the laboratory has reviewed the proper statistics supplied in the participant summary. Perform and document alternative assessment for the period that commercial PT was not tested to the same level and extent that would have been tested. Credit is not awarded in these cases.
22	Result is outside the method/ instrument reportable range	Document the comparison of results to the proper statistics supplied in the participant summary. Verify detection limits. Perform and document the corrective action of any unacceptable results.
24	Incorrect response due to failure to provide a valid response code	Document the laboratory's self-evaluation against the proper statistics and evaluation criteria supplied in the participant summary. Perform and document the corrective action of any unacceptable results. Document corrective action to prevent future failures.
25	Inappropriate use of antimicrobial	Document the investigation of the results as if they were unacceptable and review the proper reference documents to gain knowledge of the reason your response is not appropriate.
26	Educational challenge	Review participant summary for comparative results and document performance accordingly. Evaluation criteria are not established for educational challenges. Laboratories should determine their own evaluation criteria approved by their laboratory director for self-evaluation. Response to the CAP is not required.
27,31	Lack of participant or referee consensus	Document that the laboratory performed a self-evaluation and compared its results to the intended response when provided in the participant summary. If comparison is not available, perform and document alternative assessment (ie, split samples) for the period that commercial PT reached non-consensus to the same level and extent that would have been tested.
28	Response qualified with a greater than or less than sign; unable to quantitate	Applies to a response that is not formally evaluated when a less than or greater than sign is reported. Document that the laboratory performed a self-evaluation and compared its results to the proper statistics supplied in the participant summary. Verify detection limits. Perform and document the corrective action of any unacceptable results.
30	Scientific committee decision	Applies to a response that is not penalized based on scientific committee decision. Document that the laboratory has reviewed the proper statistics supplied in the participant summary.

Actions Laboratories Should Take when a PT Result is Not Graded

The CAP uses exception reason codes that signify the proficiency testing (PT) for an analyte has not been graded. The exception reason code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all analytes with an exception reason code, review and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions laboratories should take include but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
33	Specimen determined to be unsatisfactory after contacting the CAP	Document that the laboratory has contacted the CAP and no replacements specimens were available. Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
40 41	Results for this kit were not received. Results for this kit were received past the evaluation cut-off date.	Document why results were not received, corrective action to prevent recurrence and the laboratory's self-evaluation of the results by comparing results to the proper statistics and evaluation criteria supplied in the participant summary. If PT specimens were not analyzed, perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
42	No credit assigned due to absence of response	The participant summary indicates which tests are graded (see evaluation criteria) and which tests are not evaluated/educational. Updates to grading will also be noted. If a test is educational, the laboratory is not penalized for leaving a result(s) blank. If a test is graded (regulated and non-regulated analytes) and your laboratory performs that test, results cannot be left blank. The laboratory is required to submit results for **all** challenges within that test or use an appropriate exception code or indicate test not performed/not applicable/not indicated. Exceptions may be noted in the kit instructions and/or the result form. Document corrective actions to prevent future failures.
44	This drug is not included in our test menu. Use of this code counts as a correct response.	Verify that the drug is not tested on patient samples and document to ensure proper future reporting.
45	Antimicrobial agent is likely ineffective for this organism or site of infection	Document that the laboratory performed a self-evaluation of written protocols and practices for routine reporting of antimicrobial susceptibility reports to patient medical records. Document that routine reporting of this result to clinicians for patient care is compliant with specific recommendations of relevant medical staff and committees (eg, infectious diseases, pharmacy and therapeutics, infection control). Response to the CAP is not required.
77	Improper use of the exception code for this mailing	Document the identification of the correct code to use for future mailings.
91	There was an insufficient number of contributing challenges to establish a composite grade.	Document the investigation of the result as if it were an unacceptable result. Perform and document the corrective action if required.
35, 43, 46, 88, 92	Various codes	No action required.

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Disclosure Statement

The following authors/planners have no financial relationships to disclose:
Horatiu Olteanu, MD, PhD; Stephanie A. Salansky, MEd, MS, MT(ASCP)

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None

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Learning Objectives

Upon completing the reading and answering the learning assessment questions, you should be able to:

1. Identify the morphologic features of benign and malignant cells in pleural fluid.
2. Understand general clinical and diagnostic findings in lung cancer.
3. Identify normal and abnormal laboratory findings in pleural fluid.
4. Understand general therapeutic strategies and prognostic parameters in lung cancer.

VBF-B 2019: Metastatic Adenocarcinoma of Lung and Pleura

Case Presentation
This pleural fluid cytocentrifuge slide is from a 75-year-old woman with a history of non-small cell lung carcinoma who now presents with shortness of breath and large pleural effusion. Laboratory pleural fluid values include: TNC = 27,910/µL (27.910 x 10E3/µL); and RBC = 26,720/µL (26.720 x 10E3/µL).

(PLEURAL FLUID, CYTOCENTRIFUGE, WRIGHT-GIEMSA STAIN)
Note: Slide image can be found in the VBF-B 2019 Participant Summary

INTRODUCTION

Lung cancer is the leading cause of cancer deaths worldwide in both men and women. Non-small cell lung cancer (NSCLC) accounts for the vast majority of lung cancers (approximately 85%), with the remainder of cases comprised of mostly small cell lung cancer (SCLC). The worldwide incidence of lung cancer in 2018 was approximately 2.1 million cases. In the United States, there are approximately 230,000 new patients diagnosed with lung cancer every year, and over 140,000 deaths result annually. When analyzing prevalence and mortality data over the last 60 years, lung cancer-related deaths appear to be declining in both men and women, presumably due to a decrease in smoking.

The term lung cancer refers to malignancies that originate in the airways or pulmonary parenchyma. Approximately 95% of all cases are classified as either SCLC or NSCLC. This morphologic distinction is required for appropriate staging, treatment, and prognosis. Other histologic types comprise the remaining 5% of malignancies arising in the lung.

CLINICAL PRESENTATION

The majority of patients who present with clinical signs and symptoms due to lung cancer have advanced disease. The most common presenting manifestations are cough (50% - 75% of cases); hemoptysis (25% - 50%); dyspnea or shortness of breath (25%); and chest pain (20%). Less common manifestations include signs, symptoms, and/or laboratory findings, related to distant metastases or paraneoplastic syndromes. Lung cancer should always be suspected in a current or former smoker with new onset of cough or hemoptysis. Both NSCLC and SCLC can present with similar symptoms, and few clinical or laboratory features can reliably distinguish between them. For example, features suggestive of SCLC include rapidly progressive symptoms, the presence of paraneoplastic syndromes (such as syndrome of inappropriate antidiuretic hormone secretion), bulky mediastinal lymph node metastases, superior vena cava syndrome, and bone and brain metastases. In contrast, hypercalcemia is more frequently encountered in NSCLC.

DIAGNOSIS

Asymptomatic patients may come to clinical attention during screening or following the incidental detection of imaging abnormalities. Patients with symptoms suggestive of primary or metastatic lung cancer should undergo initial imaging with a chest radiograph. Findings suggestive of cancer or cancer-related complications on a chest X-ray should be further evaluated with a contrast-enhanced chest computed tomography (CT) scan. CT findings suggestive of malignancy in a patient with a solitary pulmonary nodule include large lesion size (> 15 mm), irregular or spiculated borders, upper lobe location, thick-walled cavity, presence of a solid component with a

"ground glass" appearance, and detection of growth by follow-up imaging. In contrast, the finding of multiple nodules in a patient with a known or suspected extrathoracic malignancy strongly suggests pulmonary metastases. The probability of lung cancer may be estimated by using clinical data and/or radiographic features of the lung nodules. If lung cancer is suspected based upon symptoms, CT findings, or probability calculations, formal CT staging to assess the primary tumor (**T**-factor in the **T**umor **N**ode **M**etastasis staging) and lymph nodes (**N**) should be obtained. Imaging is also helpful in detecting pleural disease in lung cancer. Two common clinical presentations of pleural disease are metastases associated with pleural effusion or multiple pleural based nodules or direct extension of the primary tumor to the pleura or chest wall. Complete evaluation of pleural disease may require multiple imaging modalities (ie, positron emission tomography [PET], CT, ultrasound, and/or magnetic resonance imaging [MRI]) as well as invasive testing (ie, thoracentesis, thoracoscopy, or pleural biopsy).

A diagnosis of lung cancer should not be made without definitive pathology confirmation. At a minimum, this involves selecting a biopsy site and obtaining an adequate sample for microscopic examination. Additional consideration needs to be given to obtaining a large enough sample for supplemental immunohistochemistry (IHC) and genetic analysis. A pathologic diagnosis may be made on cytology or histology samples. In general, if both types of specimens can be obtained with similar feasibility and risks, a tissue biopsy is preferable to a cytologic specimen, based upon the ability to differentiate with great accuracy adenocarcinoma from squamous cell carcinoma, as well as the importance of obtaining sufficient material for ancillary testing (IHC and genetic analysis). The common genetic mutations with known targeted therapies include mutations in epidermal growth factor receptor (*EGFR*) and rearrangements of the anaplastic lymphoma kinase (*ALK*) gene.

As mentioned previously, pleural involvement can manifest as pleural thickening without pleural effusion or as malignant pleural effusion. Patients with malignant effusions are considered incurable and managed palliatively. Although malignant pleural effusions can cause dyspnea and cough, approximately 25% of patients who have lung cancer and pleural metastases are asymptomatic. Although a malignant pleural effusion precludes curative tumor resection, not all pleural effusions in patients with lung cancer are malignant. A benign pleural effusion may occur in a patient with a resectable lung cancer due to lymphatic obstruction, post-obstructive pneumonitis, or atelectasis. Malignant effusions are typically exudates and may be serous, serosanguinous, or grossly bloody. The yield of pleural fluid cytology after a single thoracentesis in patients with documented pleural involvement by lung cancer is approximately 60%; the yield increases to approximately 75% with a second thoracentesis, beyond which there is little incremental gain with additional procedures. In a patient with a suspected malignancy, repeat pleural fluid cytology—with or without pleural biopsy—is appropriate if the initial study is negative. During the course of their disease, approximately 10% - 15% of patients who have lung cancer will have malignant pleural effusions.

ANCILLARY STUDIES

The following discussion addresses some of the laboratory workup and findings in patients with malignant pleural effusions as it relates to the current case presentation. Normal pleural fluid is clear and yellow, comprises 1 - 10 mL, has a pH of 7.6, and a protein level of 1 - 2 g/dL. In addition, glucose levels are similar to plasma; lactate dehydrogenase (LDH) levels are < 50% of those in plasma; and sodium, potassium, and calcium levels are similar to those of interstitial fluid. The total nucleated cell (TNC) count is < 1000/µL, consisting almost exclusively of mononuclear cells and rare mesothelial cells, admixed with few red blood cells. For the purpose of clinical diagnosis and patient management, serous effusions are classified as transudates or exudates. In general,

transudates are benign processes, while exudates constitute evidence of a serious local process, such as malignancy. Richard W. Light, MD, first proposed the objective criteria for differentiating transudates from exudates, referred to as “Light’s criteria,” in 1972. These criteria still remain the gold standard and correctly identify virtually 100% of exudates but misclassify 10% - 30% of transudates as exudates. Light’s criteria for exudates include: pleural fluid/serum protein ratio > 0.5, pleural fluid/serum LDH ratio > 0.6, and pleural fluid LDH > 200 IU. Because body cavity lining cells and macrophages may appear similar to nonhematopoietic malignant cells, identification of malignant cells in Wright-Giemsa stained cytocentrifuge preparations can be difficult. Key morphologic features that help to separate benign and malignant cells include nuclear contour and nuclear membranes, nuclear texture, nucleoli, nuclear to cytoplasmic ratio, multinuclearity, mitotic cells, nuclear molding, cannibalism, cytoplasmic granules, signet ring cells, unusual homogeneous population, cell clusters, and cytoplasmic vacuoles. For example, malignant cells may show a high nuclear to cytoplasmic ratio, with irregular nuclear contours, coarsely clumped chromatin, with prominent (large), single or multiple nucleoli, multinucleation with dissimilar nuclear size and shape, and nuclear molding (or indentation of the nucleus in one cell by the nucleus of a separate cell). The cytoplasm may show large, mucin-containing vacuoles. Frequent cell clusters of malignant cells may show the appearance of tight, morula-like or three-dimensional (3D) clusters, such that it may be difficult to tell where the cytoplasm of one cell stops and another begins. When mucin vacuoles are large and compress a flattened nucleus to the periphery, a signet ring morphology results, often occurring in tight clusters. In contrast, normal lining cells, such as mesothelial cells, tend to have a low to intermediate nuclear to cytoplasmic ratio (< 1:2), round/oval nuclear contours, and uniformly dispersed chromatin, with only small or medium-sized nucleoli (when present). Also, while multinucleation may be seen in mesothelial cells, the nuclear size and shape is similar. Furthermore, if body fluid slides are properly prepared, benign cells rarely show nuclear molding on cytocentrifuge slides, and contact between two nuclei is seen only in phagocytosis. Similarly, in well-prepared cytocentrifuge slides (using an appropriate dilution to obtain a monolayer of cells), clusters of benign mesothelial cells look like they are adjacent to each other, with demarcation lines or thin spaces (“windows” between neighboring cells), and do not exhibit the tight 3D architecture of malignant cell clusters. Finally, while phagocytic macrophages may occasionally appear as signet ring cells, they tend to occur singly or in mixed clusters with other benign cells, and overall the sample typically contains an increased number of phagocytic macrophages.

PROGNOSIS AND THERAPY

Advances in the molecular pathogenesis of NSCLC have shown that this entity is actually comprised of a heterogeneous group of diseases. Because of this understanding, although the initial treatment of localized disease is the same, the molecular characterization of tumor tissue in patients with NSCLC will guide the treatment both in those who present with metastatic disease, as well as those with relapses after the primary therapy. Currently defined NSCLC subsets that benefit from specific targeted therapies, include those with mutations in *EGFR* and *BRAF* proto-oncogenes, *ALK* fusions; and *ROS1* oncogene fusions, respectively. For patients without driver mutations, in whom a high level of PD-L1 expression is present, immunotherapy with so-called immune checkpoint inhibitors may be used as first-line treatment.

In general, for patients with NSCLC, initial management is largely determined by the stage of disease. The TNM stage at presentation in patients with NSCLC is the prognostic factor with the greatest impact, as survival decreases progressively with more advanced disease. For patients with early stage disease, surgical resection offers the best opportunity for long-term survival and cure, while concurrent chemoradiation therapy is preferred

for those with more extensive intrathoracic disease. In contrast, patients with advance disease are managed palliatively with systemic therapy and/or local palliative modalities.

For patients with SCLC, systemic chemotherapy is an important component of treatment, because SCLC is disseminated at presentation in almost all patients. For those with limited-stage disease, thoracic radiation therapy is used in combination with chemotherapy.

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Horatiu Olteanu, MD, PhD, FCAP, is Professor of Laboratory Medicine and Pathology at Mayo Clinic in Rochester, MN. He is also medical director for the Cell Kinetics Laboratory and a senior associate consultant in the Division of Hematopathology at Mayo Clinic. Dr. Olteanu's clinical and research interests are focused on the applications of flow cytometric immunophenotyping in the diagnosis of prognosis of hematologic malignancies. He has authored more than 130 peer-reviewed papers, book chapters, and abstracts on topics in hematopathology and flow cytometry, and lectured at national and international pathology meetings. In addition, Dr. Olteanu has a strong interest in continuing and professional development, having served as medical director for continuing and professional education at the Medical College of Wisconsin, as chair of the Pathologist Recertification Individualized Self-Assessment Examination (PRISE) Committee of the American Society for Clinical Pathology (ASCP), and as member of the Education Committee and editor of the Case of the Quarter for the Society for Hematopathology. Currently, he is an associate editor for Hematopathology for Laboratory Medicine and serves as a member of both the College of American Pathologists (CAP) Hematology and Clinical Microscopy Committee and the International Clinical Cytometry Society (ICCS) Education Committee.

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This concludes the report.

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