Updates in Effusion Cytology

Christin M. Lepus, MD, PhD, Marina Vivero, MD*

KEYWORDS

• Effusion • Cytology • Molecular • Immunohistochemistry • Mesothelioma

Key points

- Serous effusions are most commonly benign and, with the exception of connective tissue disease, usually present nonspecific cytologic findings.
- Effusion cytology yields an overall sensitivity and specificity of up to 80% and 98%, respectively, for malignant disease. These both improve with ancillary diagnostic testing.
- Immunohistochemistry, cytogenetic, and molecular analysis can all be done on cell blocks, smears, and liquid-based preparations of effusion fluids for diagnostic and predictive purposes.
- Immunohistochemical loss of BAP1 protein expression, seen in 57% to 67% of mesotheliomas, can be useful to distinguish between benign and malignant mesothelial proliferations.
- Immunohistochemical staining of effusion samples is helpful in the diagnosis of rare mimics of more common epithelial malignancies.

ABSTRACT

Effusion cytology plays multiple roles in the management of benign and malignant disease, from primary diagnosis to tissue allocation for ancillary diagnostic studies and biomarker testing of therapeutic targets. This article summarizes recent advances in pleural effusion cytology, with a focus on the practical application of immunohistochemical markers, cytogenetic techniques, flow cytometry, and molecular techniques for the diagnosis and management of primary and secondary neoplasms of the pleura.

OVERVIEW

Cytologic examination of serous effusions provides a unique opportunity to obtain clinically impactful information with minimal discomfort and risk to patients. Reported rates of malignancy in effusions range from 15% to 50%,[1,2] of which 15% to 42% may represent the first manifestation of disease.[1–3] Interobserver concordance and sensitivity varies based on experience, fluid type, diagnosis (benign vs malignant), number of specimens examined, and preparation type.[2,4,5] Overall, sensitivity and specificity of effusion cytology for the detection of malignancy in effusions varies from 40% to 80% and 89% to 98%, respectively.[2,5] Significant cytomorphologic overlap exists in effusion specimens between benign and malignant mesothelial cells and adenocarcinomas, as well as between carcinomas of different primary origins.[4,6,7] Ancillary techniques are frequently used to resolve these diagnostic challenges and avoid diagnostic pitfalls, as well as to increase the sensitivity and specificity of effusion cytology to 94% and 100%, respectively.[2,5]

ANCILLARY TESTING OF EFFUSIONS

IMMUNOHISTOCHEMISTRY

Immunohistochemistry is widely adopted in cytologic diagnosis of effusions and allows for

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Department of Pathology, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA
* Corresponding author.
E-mail address: mvivero@bwh.harvard.edu

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accurate classification of most tumors. Cell block preparations are most commonly used owing to the ease of morphologic interpretation, existing validation of immunohistochemical stains on formalin-fixed paraffin-embedded material, minimal background staining, and the ability to evaluate numerous antigens on a single preparation.[8] Immunohistochemical stains also provide a cost-effective way to evaluate predictive biomarkers, such as quantification of hormone receptors in metastatic breast carcinoma and anaplastic lymphoma kinase (ALK), ROS proto oncogene 1 (ROS1), and programmed cell death-ligand 1 (PD-L1) staining in lung cancer.[9–11] Although inherent intratumor heterogeneity may limit interpretation of immunohistochemistry on targeted needle and excisional biopsies owing to sampling bias,[12] intertumor heterogeneity may be a more significant factor in effusions because metastatic disease and treated tumor may show differing immunophenotypes compared with the primary source.[9,13,14]

FLOW CYTOMETRY

Flow cytometric immunophenotyping of effusion specimens has emerged as another sensitive, reproducible, and low-cost quantitative ancillary diagnostic technique with a quick turnaround time, particularly for the detection and classification of hematolymphoid neoplasms.[15] Lymphocyte-rich effusions often present a diagnostic challenge for cytopathologists. Low-grade lymphomas can look deceptively bland, whereas reactive lymphocytes can show marked atypia, requiring flow cytometry to differentiate between them. Although not routine practice, protein expression can also be assessed by flow cytometry in nonhematologic malignant effusions to detect metastatic disease or monitor treatment response, and is highly sensitive and specific.[16]

CYTOGENETIC ANALYSIS

Because mechanical and enzymatic tissue disaggregation is unnecessary, cytology specimens are appealing substrates for cytogenetic studies. Both karyotype and fluorescence in situ hybridization (FISH) studies can be performed, and a variety of preparations, including fresh cell suspensions, cytocentrifugation preparations, thin-layer slides, and cell blocks, can be used.[17] Cytogenetic analysis can be used for diagnostic purposes, particularly for mesothelioma, lymphomas, and sarcomas.[18–21] Or it can be used for predictive purposes, such as assessment of *ALK* and *ROS1* gene rearrangements in non-small cell lung carcinoma.[22,23]

MOLECULAR TESTING

In some patients, effusions may be the only available specimen for molecular profiling of genetic alterations that are increasingly important in diagnosis and prediction of drug responsiveness, metastatic potential, and likelihood of recurrence.[24] The development of improved DNA extraction protocols and next-generation sequencing (NGS) platforms requiring less DNA input has enabled use of cytologic specimens for high-throughput mutational analysis,[25,26] allowing multiple target genes to be analyzed in a single assay. A variety of routine cytologic preparations, including cell blocks, smears, and liquid-based preparations, all of which can be prepared from effusion fluid, can be used for molecular testing.[27–44] A subset of studies evaluating molecular analysis of cytology specimens have focused on effusion fluids using different methods, with occasional comparison to surgical specimens (**Table 1**).

Among other preanalytic factors, including preparation type, tumor fraction, and tumor quantity,[26–29] the success of gene sequencing, particularly NGS techniques that show superiority compared with traditional Sanger sequencing,[29,35] is strongly affected by DNA yield as opposed to tumor cell content alone.[27] In 1 study, *EGFR* mutations matching those detected in surgical samples were seen in 42% of cytologic specimens containing no tumor cells,[35] raising the possibility that NGS may be sufficiently sensitive to detect cell-free DNA in effusion specimens. Moreover, some studies have reported the detection of mutations in cytology specimens that were not detected in corresponding surgical biopsies despite adequate sequence coverage, possibly reflecting the potential of cytologic specimens to more accurately sample tumor heterogeneity during tumor progression and metastasis.[42]

DIAGNOSIS OF BENIGN SEROUS EFFUSIONS

Benign effusions, defined as effusions lacking microscopic evidence of malignant cells, account for up to 74% of all effusions and are typically associated with nonspecific cytologic findngs.[45] Benign effusions occur due to several causes, including inflammatory conditions, hemodynamic disturbances, malignancy (as a secondary result of lymphatic obstruction), obstructive pneumonia, or other mechanical issues.

INFECTION

Infection, either via direct involvement or parapneumonic involvement, is the most common