Abstract

Primary lung epithelial malignancies are the most common neoplasms among all pulmonary tumors. Lung cancer (LC) is the leading cause of cancer-related mortality for which a histologic or cytologic confirmation of malignancy is required before treatment. Specimen management is an important task for pathologists in the field of LC. Biopsy and fine needle aspiration are comparable. It is desirable to have both for diagnosis and mutation testing to maximize their use for patient care.

Keywords: Biopsy, fine-needle aspiration cytology, lung cancer

INTRODUCTION

Primary lung epithelial malignancies are the most common neoplasms among all pulmonary tumors. Lung cancer (LC) is the leading cause of cancer-related mortality for which a histologic or cytologic confirmation of malignancy is required before treatment. Most patients with LC present in clinically advanced stage of the disease and are not candidates for surgery. In the era of personalized medicine, small biopsies or cytological material obtained from fine-needle aspiration cytology (FNAC) may be the only available specimen for diagnosis of LC, subtyping of non-small cell carcinoma (NSCC) into adenocarcinoma and squamous cell carcinoma, and for further mutation testing.

Types of thoracic FNA and biopsies

FNA can be of the transbronchial (TBNA) or transthoracic (TTNA) type. Transbronchial FNA can be done either blindly or under endobronchial ultrasound guidance (EBUS). TTNA can be done under fluoroscopy or computerized tomography (CT)/ultrasonography (USG) guidance. Types of biopsies range from endobronchial biopsies (EB), transbronchial lung biopsies (TBLB), image-guided transthoracic, and cryobiopsies. EB and TBLB can be done blindly or under fluoroscopic guidance or can be performed under the guidance of EBUS. Clot core biopsies can be obtained from the needle used for transbronchial needle aspiration. Cell blocks from TBNA and TTNA can be made and used as histology sections.

Needle size for TBNA is usually of 21–22 gauge. Conventional TBNA can be used for endobronchial and subcarinal lesions, whereas EBUS TBNA can approach smaller and peripheral lesions. Core needle biopsies can be obtained through cutting needle or automated core biopsy needle of 18–25 gauge. TTNA or biopsies are typically performed for evaluation of an indeterminate peripherally placed pulmonary nodule or mass. Radial EBUS has emerged for acquisition of diagnostic material in peripherally situated parenchymal masses.

Acquisition of tissue by FNA and biopsy

With the paradigm shift of first line therapy in LC management, pathologists are placed into a crucial position, in patient care of LC, by contributing to accurate histologic typing, testing for epidermal growth factor receptor (EGFR)/Anaplastic lymphoma kinase (ALK)/ROS1 alterations and programmed death-ligand 1 (PD-L1) assessment.

Cytology or histology samples can be triaged for their maximum utilization. Aspiration specimens can be used for preparing direct smears or processed through liquid-based cytology (LBC). Needle rinses of aspiration needle can used for preparing cell blocks or cytospin smears. Rapid on-site evaluation (ROSE) has emerged as a powerful tool in triaging thoracic FNA specimens. It is used not only for adequacy

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judgement but also for establishing preliminary diagnosis of cancer versus non-cancer. According to preliminary evaluation, sample can be utilized for mutation testing or microbiologic examination or for flow cytometric assessment if there is a suspicion of cancer or infective pathology or lymphoproliferative disorders respectively.[14]

ROSE can be applied in tru-cut biopsies by making imprint smears or touch preparations. Imprint smears can be assessed on-site for adequacy and diagnostic evaluation. However, vigorous touch preparations have shown to result into loss of cellularity in subsequent biopsies, hence, loss of DNA content for mutation testing.[13]

A systematic review compared FNA versus core needle biopsies in LC based on the data available in the literature from 1990 to 2009. A comparable sensitivity (81.3%–90.8% and 85.7–97.4%), specificity (75.4%–100% and 88.6%–100%) and diagnostic accuracy (79.7%–91.8% and 89.0%–96.9%) was observed between FNA and biopsy, respectively. There was no difference between two procedures in developing post-procedural complications such as pneumothorax or hemoptysis. There was not much data available by this time to compare results for molecular testing between the two procedures.[16]

Molecular testing guidelines for LC patients published in year 2013 preferred cell block specimens as they match histology specimens in terms of processing and fixation.[17]

**Role of FNA and biopsy in lung cancer diagnosis and typing**

Both FNA and biopsies can accurately diagnose common categories of LC such as nonsmall cell and small cell carcinoma.

NSCLC can be further subtyped into adenocarcinoma, based on glandular differentiation and/or presence of mucin, and squamous cell carcinoma based on presence of keratin pearls. Small cell carcinoma can also be diagnosed easily on cytology by identifying their characteristic features such as nuclear molding, hyperchromasia, and crushed proliferating tumor cells.

FNA is fairly accurate in the pathological typing of NSCLC. The positive predictive value of FNAC in typing NSCLC was 92% for adenocarcinoma and 82% for squamous cell carcinoma.[18]

Ancillary studies such as mucin stain and immunohistochemistry (IHC) for TTF-1 and p40 are helpful in further characterization of NSCLC into adenocarcinoma and squamous cell carcinoma respectively and in reducing diagnosis of NSCLC-NOS.[19] They can be performed on cytology smears and cell blocks.[20]

Paired biopsy and FNA specimens maximize the number of definitive diagnoses and decrease the rate of unclassified diagnoses (NSCLC-NOS).[21,22]

Further pattern analysis of adenocarcinoma is not easy to assess on cytology smears however due to heterogeneity of most of pulmonary adenocarcinomas, it is not useful to do pattern subtyping on biopsy and cytology specimens.[23,24]

There was no statistically significant difference on comparison of FNA, core biopsy or both in obtaining sufficient tissue for a specific diagnosis and performing molecular testing of primary lung adenocarcinoma.[25]

**Role of FNA and biopsy in molecular testing**

The national comprehensive cancer network (NCCN) 2017 guidelines recommend EGFR and ALK as category 1 biomarkers for testing in NSCLC.[26] Methods for biomarker analysis include immunocytochemistry/IHC, Fluorescence in-situ Hybridization (FISH) and mutation analysis by PCR-based methods/sequencing.

FNA specimens are suitable for any DNA based analysis whether they are direct smears, liquid-based cytology specimens or cell blocks. Both alcohol fixed Papanicolaou stained and air-dried Giemsa stained smears can be used for mutation testing. Tumor enrichment should be done in both cytology or histology material either by laser capture micro-dissection or scraping of tumor from the glass slide directed under the bright field microscope.

**Specimen requirement for molecular testing**

At least 5 forceps biopsy samples of EB, at least 7–10 forceps biopsy samples of TBLB, at least 2 tru-cut needle biopsies and at least 4 passes per station of lymph node or lesion are sufficient requirements for molecular testing.[27]

**EGFR mutation**

Sanger sequencing is considered less sensitive, however, PCR-based methods and next generation sequencing (NGS) are sensitive methods to detect EGFR mutation. That is why updated molecular testing guidelines for LC patients have recommended using a detection platform which has a sensitivity of 20% in contrast to earlier guidelines where the limit of detection was kept at 50%.[28]

A review of 22 studies using 4999 cytology samples showed higher mutation detection and lower insufficient cases in pleural effusions and EBUS-TBNA specimens compared to biopsies. Average success rate for the assays that used cytologic specimens was 95.87% (range, 85.2%–100%). Formalin fixation of histology specimens yields DNA fragmentation and creates sequencing and PCR artefacts.[29]

**ALK, ROS-1, and PD-L1 testing**

Since LC present in advanced stage of the disease, only cytology specimens are available in approximately 40% cases. After EGFR mutation testing, ALK, ROS-1, and PD-L1 are recommended tests in NSCLC. IHC has been recommended for ALK testing in LC for selection of patients for targeted therapy. However, for ROS-1 testing, IHC can only be used for screening. In case of positive IHC for ROS-1, results have to be confirmed by FISH due to nonspecific IHC staining. Although cell blocks can be used for IHC, these are not routinely performed in many centers. Cytology smears are...
valuated and can be used for FISH and immunocytochemistry for ALK and ROS-1 testing. Even alcohol fixed Papanicolaou stained and air-dried Diff-Quik/Giemsa stained smears can be recycled for FISH and ICC.[16,31]

Several studies, to date, have shown the suitableness of FFPE biopsy samples for PD-L1 testing. However, there are only few reports on performance, utility, and satisfactory results of cytology specimens for PD-L1 testing. Studies have shown satisfactory concordance of PD-L1 testing on cell block and cytology smears with FFPE histology blocks.[12]

Because of equal suitability of cytology specimens for mutation testing, updated molecular testing guidelines recommend any cytology sample with adequate cellularity and preservation can be used for mutation testing.[28] Earlier guidelines preferred cell blocks.[17]

**CONCLUSION**

To conclude, both FNA and biopsies are comparable. In situations where we have both the specimens, FNA smears can be utilized for identifying morphologic features such as keratin pears, mucin and glands for accurate histotyping of NSCLC. Biopsy or FFPE CB can be used for IHC, if needed, for the diagnosis of adenocarcinoma/squamous cell carcinoma in morphologically undifferentiated cases as most of the IHC is validated on FFPE material. FNA material can be used for DNA based assays to avoid formalin artifacts. If biopsy or CB is available, predictive IHC or FISH can be performed on them.

FNA and biopsies both are precious patient’s material and should be used judiciously and fully to obtain maximum information for patient care that is our ultimate goal.

Biopsy and FNA achieve comparable rates of definitive and accurate LC diagnosis and subtyping of NSCLC. Optimal results are attained when the two modalities are considered jointly. Whenever clinically feasible, obtaining parallel biopsy and cytology specimens is encouraged to ensure the greatest diagnostic accuracy. Specimen management is an important and a new task for pathologists in the field of LC. Hence, a tight collaboration is necessary between cytopathologists and surgical pathologists.

Remaining challenges for cytology specimens are related to predictive biomarker assays which are primarily designed for biopsies. As each laboratory has different protocols for specimen fixation, processing, and CB preparation, it is essential to validate tests with histology specimens before practicing in routine care.[31] Expertise in cytology is mandatory for correct diagnosis. External quality assessment programs should be available for maintaining quality assurance.

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**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017;67:7-30.
2. Ramalingam S, Belani C. Systemic chemotherapy for advanced non-small cell lung cancer: Recent advances and future directions. Oncologist 2008;13(Suppl 1):5-13.
3. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011;6:244-85.
4. DiBardino DM, Yarmus LB, Semaan RW. Transthoracic needle biopsy of the lung. J Thorac Dis 2015;7(Suppl 4):S304-16.
5. Manhire A, Charig M, Clelland C, Gleseson F, Miller R, Moss H, et al; BTS. Guidelines for radiologically guided lung biopsy. Thorax 2003;58:920-36.
6. Jain D, Roy-Chowdhuri S. Molecular Pathology of Lung Cancer Cytology Specimens: A Concise Review. Arch Pathol Lab Med 2018 [Epub ahead of print].
7. Hibare KR, Goyal R, Nemani C, Avinash R, Ram B, Ullas B. Radial endobronchial ultrasound for the diagnosis of bronchoscopically invisible lesions: First case series from India. Lung India 2017;34:43-6.
8. Kohler J, Schuler M. Aftatinib, erlotinib and gefitinib in the first-line therapy of EGFR mutation-positive lung adenocarcinoma: A review. Onkologie 2013;36:510-9.
9. Park JH, Choi CM, Kim H, Jang SJ, Choe G, Kim DK, et al. Clinicopathologic implication of ALK rearrangement in surgically resected lung cancer: A proposal of diagnostic algorithm for ALK-rearranged adenocarcinoma. Lung Cancer 2012;76:403-9.
10. Wu S, Wang J, Zhou L, Su D, Liu Y, Liang X, et al. Clinicopathological characteristics and outcomes of ROS1-rearranged patients with lung adenocarcinoma without EGFR, KRAS mutations and ALK rearrangements. Thorac Cancer 2015;6:413-20.
11. Weinstock C, Khozin S, Suzman D, Zhang L, Tang S, Wahby S, et al. U.S. Food and Drug Administration Approval summary: Atezolizumab for metastatic non-small cell lung cancer. Clin Cancer Res 2017;23:4534-9.
12. Boussiotis VA. Molecular and biochemical aspects of the PD-1-checkpoint pathway. N Engl J Med 2016;375:1767-78.
13. Natwa A, Walia R, Singh Y, Madan K, Mathur S, Iyer V, et al. Comparison of Conventional Smear and Liquid-based Cytology Preparation in Diagnosis of Lung Cancer by Bronchial Wash and Transbronchial Needle Aspiration. J Cytol 2018;35:94-8.
14. Jain D, Allen TC, Asner DL, Beasley MB, Cagle PT, Capelozzi VL, et al. A Perspective From Members of the Pulmonary Pathology Society. Arch Pathol Lab Med 2018;142:253-62.
15. Rekhtman N, Kazi S, Yao J, Dogan S, Yannes A, Lin O, et al. Depletion of Core Needle Biopsy Cellularity and DNA Content as a Result of Vigorous Touch Preparations. Arch Pathol Lab Med 2015;139:907-12.
16. Yao X, Gomes MM, Tsao MS, Allen CJ, Geddie W, Sekhon H. Fine-needle aspiration biopsy versus core-needle biopsy in diagnosing lung cancer: A systematic review. Curr Oncol 2012;19:e16-27.
17. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol 2013;8:823-59.
18. Nizzoli R, Tiseo M, Gelsomino F, Bartolotti M, Majori M, Ferrari L, et al. Accuracy of fine needle aspiration cytology in the pathological typing of non-small cell lung cancer. J Thorac Oncol 2011;6:489-93.
19. Walia R, Jain D, Madan K, Sharma MC, Mathur SR, Mohan A, et al. p40 & thyroid transcription factor-1 immunohistochemistry: A useful panel to characterize non-small cell lung carcinoma-not otherwise specified (NSCLC-NOS) category. Indian J Med Res 2017;146:315-20.
20. Jain D, Mathur SR, Guleria R, Iyer VK. Utility and pattern of positivity for p40 in the diagnosis of squamous cell carcinoma of the lung by cytology: The first study on fine needle aspiration smears. Cytopathology 2014;25:330-5.
21. Aviram G, Greif J, Man A, Schwarz Y, Marmor S, Graif M, et al.
Diagnosis of intrathoracic lesions: are sequential fine-needle aspiration (FNA) and core needle biopsy (CNB) combined better than either investigation alone? Clin Radiol 2007;62:221-6.

22. Yamagami T, Iida S, Kato T, Tanaka O, Nishimura T. Combining fine-needle aspiration and core biopsy under CT fluoroscopy guidance: A better way to treat patients with lung nodules? AJR Am J Roentgenol 2003;180:811-5.

23. Nambirajan A, Kaur H, Jangra K, Kaur K, Madan K, Mathur SR, et al. Adenocarcinoma predominant pattern subtyping and nuclear grading in cytology: Is there a role in prognostication of advanced pulmonary adenocarcinomas? Cytopathology 2018;29:163-71.

24. Moreira AL. Subtyping of pulmonary adenocarcinoma in cytologic specimens: The next challenge. Cancer Cytopathol 2013;121:601-4.

25. Coley SM, Crapanzano JP, Saqi A. FNA, core biopsy, or both for the diagnosis of lung carcinoma: Obtaining sufficient tissue for a specific diagnosis and molecular testing. Cancer Cytopathol 2015;123:318-26.

26. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Non-Small Cell Lung Cancer. Version 8. https://www.nccn.org/. [Accessed on September 2017].

27. Thunnissen E, Kerr KM, Herth FJ, Lantuejoul S, Papotti M, Rintoul RC, et al. The challenge of NSCLC diagnosis and predictive analysis on small samples. Practical approach of a working group. Lung Cancer 2012;76:1-18.

28. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. Arch Pathol Lab Med 2018;142:321-46.

29. da Cunha Santos G, Saieg MA. Preanalytic parameters in epidermal growth factor receptor mutation testing for non-small cell lung carcinoma: A review of cytologic series. Cancer Cytopathol 2015;123:633-43.

30. Savic S, Bode B, Diebold J, Tosoni I, Barascud A, Baschiera B, et al. Detection of ALK-positive non-small-cell lung cancers on cytological specimens: High accuracy of immunocytochemistry with the 5A4 clone. J Thorac Oncol 2013;8:1004-11.

31. Vlajnic T, Savic S, Barascud A, Baschiera B, Bihl M, Grilli B, et al. Detection of ROS1-positive non-small cell lung cancer on cytological specimens using immunocytochemistry. Cancer Cytopathol 2018 [Epub ahead of print].

32. Jain D, Supraja KS, Mohan A, Iyer VK. PD-L1 immunoexpression in matched biopsy and liquid based cytology samples of advanced stage non-small cell lung carcinomas. Cytopathology. 2018 Jun 25. doi: 10.1111/cyt.12605.

33. Jain D, Mathur SR, Iyer VK. Cell blocks in cytopathology: A review of preparative methods, utility in diagnosis and role in ancillary studies. Cytopathology 2014;25:356-71.