Comparison of Conventional Smear and Liquid-based Cytology Preparation in Diagnosis of Lung Cancer by Bronchial Wash and Transbronchial Needle Aspiration

Aasma Nalwa, Ritika Walia, Varsha Singh, Karan Madan, Sandeep Mathur, Venkateshwaran Iyer, Deepali Jain
Departments of Pathology, Pulmonary Medicine and Sleep Disorders, All India Institute of Medical Sciences, New Delhi, India

Abstract

Introduction: Liquid-based cytology (LBC), initially developed for screening gynecologic specimens, is now being used in nongynecologic aspiration and exfoliative specimens. In this study, the diagnostic yield and utility of thin-prep (TP) was compared with conventional preparations to ascertain its utility in improving the diagnosis of respiratory lesions. Materials and Methods: Bronchial washings (BW) and transbronchial needle aspirates (TBNA) (bronchoscopy/endobronchial ultrasound-guided) from 70 consecutive patients of mediastinal masses and endo/peribronchial growths were included. The diagnostic yields of both conventional smears and thin-prep were compared. Immunocytochemistry (ICC) was performed on direct/cytospin smears of TBNA/BW and TP slides when the tumor could not be subtyped by morphology. Histopathologic correlation was done. Results: Although well-preserved morphological features and cleaner background in TP allowed accurate diagnosis of malignancies, diagnostic yield was comparable to conventional preparations. Immunocytochemistry was successfully employed on TP smears which helped in accurate subtyping of the tumors. Few shortcomings of TP smears were uneven distribution of cells, thick cell clusters, and inadequate cellularity. Conclusion: Liquid-based TP preparation is an effective diagnostic tool for respiratory tract cytology, however, results are comparable to conventional smears.

Keywords: Cytology, respiratory tract, thin prep

Introduction

The role of transbronchial needle aspiration (TBNA) and bronchial washings (BW) is well established for the diagnosis of lung lesions. However, conventional smear interpretation is at times limited by the presence of air-drying artefacts, mucous, blood, and cellular overlap. Liquid-based cytology (LBC), initially introduced for cervical screening, has been increasingly used for exfoliative and nongynecologic cytology in the past decade. The main advantages of LBC are clearer background, uniform cell thickness, and removal of air-drying artefacts.

In the present study, our main aim was to compare the diagnostic yield of thin-prep (TP) preparation against the matched conventional preparations of BW (cytospin) and TBNA (aspirate smears) to ascertain its utility in improving the diagnosis of lung cancer. Final diagnosis on the paired biopsy was considered the gold standard. Our aim was not to compare the diagnostic yield of BW and TBNA as indications and sites of both techniques are different with different sensitivity and specificity.

Materials and Methods

BW and TBNA (bronchoscopy/endobronchial ultrasound-guided) from 70 consecutive patients (57 males and 13 females; 53.3 ± 13.5 years) of mediastinal masses and endo/peribronchial growths were included over a period of 7 months from March 2015 to September 2015. Ethical clearance was obtained from the Institute’s ethics committee (IEC/NP-349/2013).

Results:

Although well-preserved morphological features and cleaner background in TP allowed accurate diagnosis of malignancies, diagnostic yield was comparable to conventional preparations. Immunocytochemistry was successfully employed on TP smears which helped in accurate subtyping of the tumors. Few shortcomings of TP smears were uneven distribution of cells, thick cell clusters, and inadequate cellularity.

Conclusion:

Liquid-based TP preparation is an effective diagnostic tool for respiratory tract cytology, however, results are comparable to conventional smears.

Address for correspondence: Dr. Deepali Jain, Department of Pathology, All India Institute of Medical Sciences, New Delhi - 110 029, India.
E-mail: deepalijain76@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Nalwa A, Walia R, Singh V, 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.
Overall, TBNA and BW were done in 29 and 22 cases, respectively. Both BW and TBNA were performed in 19 cases.

TBNA was done for the diagnosis of mediastinal masses or lymphadenopathy and peribronchial lesions. A total of 4 direct smears were prepared comprising two air-dried and two alcohol-fixed slides. On average, two direct smears were examined.

BW was done in cases of endobronchial lesions or luminal narrowing due to peribronchial obstruction. Two cytospin smears were prepared per case, one was stained with May–Grunwald–Giemsa (MGG) and the other with Papanicolaou (PAP) stain. Although the indications of TBNA and BW are different, the two techniques were clubbed together in the study to examine the diagnostic yield on TP irrespective of the method used for obtaining the material from the respiratory tract for various lesions.

TP was prepared in 57 cases. It was prepared for all patients presenting with lesions in lung and hilar and/or mediastinal lymph nodes who underwent TBNA. TP from BW was done only when a clinical diagnosis of malignancy was present. Only those cases were included in the study whose corresponding biopsy was available. Cases where matching biopsies were not available were excluded from the study.

One out of three passes of TBNA was taken for TP in a random manner. The sample (BW/TBNA) was transferred to a fixative (CytoLyt, Hologic Inc., MA, USA). The sample was centrifuged at 600 g for 10 min. The supernatant was discarded and vortexed to resuspend the cell pellet and evaluated for appearance. If bloody, additional CytoLyt washes were given and the material was transferred to a vial containing cytopreservative solution (PreservCyt; Cytac, Co), which fixed the cells, and slides were prepared with the help of ThinPrep 2000 automated slide processor and stained with PAP stain. Direct aspirate smears of TBNA were processed by fixing in 95% alcohol for PAP and air-dried for MGG staining.

Cytospin smears of BW, direct smears of TBNA aspirate, and TP were independently evaluated for adequacy, cellularity, and diagnostic yield (benign, suspicious of malignancy, and malignant) and categorized as unsatisfactory, negative, inconclusive, or positive for malignancy. The diagnostic yields of both conventional preparations and TP were compared. World health organization (WHO) Classification of the Tumours of the lung, Pleura, Thymus and Heart, 2015 was used for establishing the diagnosis, and the tumors were subtyped as small cell carcinoma and nonsmall cell carcinoma that were further subtyped into adenocarcinoma and squamous cell carcinoma based on their cytomorphological features.

Immunocytochemistry (ICC) for Napsin-A (Clone EP205,1:50 dilution, BioSB, USA), Thyroid Transcription Factor-1 (TTF-1; Clone 8G7G3/1, 1:40 dilution, BioSB, USA), p40 (Clone ZR8, 1:100 dilution, BioSB, USA), chromogranin (Clone SP12,1:200 dilution, Spring Biosciences, USA), and synaptophysin (Syn; CloneSP11,1:200 dilution, Spring Biosciences, USA) was performed on TP slides when the tumor could not be subtyped by morphology alone. Briefly, PAP-stained TP slides or direct smears were dipped in xylene for 12 h, followed by removal of the coverslip by immersion in xylene again overnight. Smears were de-stained by immersing in methanol for 15 min and subjected to ICC. Immunocytochemical staining was performed with a monoclonal antibody using a routine streptavidin-biotin horseradish peroxidase detection system with diaminobenzidine as the chromogen. Antigen retrieval was achieved by boiling slides immersed in citrate buffer (10 mmol/l) at pH 6.0 in a microwave oven for 30 min. To diminish endogenous peroxidase activity, slides were treated with 4% hydrogen peroxide in methanol for 30 min.

**Results**

Only 57 cases were analyzed where paired biopsy (endobronchial/image-guided lung biopsy) of TP was available [Table 1]. Diagnostic yield of direct smears was 68.4% (39/57) and TP was 57.8% (33/57).

Direct smears (TBNA)/Cytospin (BW): In 6 cases, smear preparation helped in establishing diagnosis, however, TP was unsatisfactory. These include two cases of small cell carcinoma (SCC), two cases of adenocarcinoma, and one case each of nonsmall cell carcinoma (NSCC) and granulomatous inflammation. ICC was done in direct aspirate smears which was positive for chromogranin, synaptophysin (small cell carcinoma), and TTF-1 (adenocarcinoma).

Thin-Prep: On TP, four cases of small cell carcinoma [Figure 1a-d], 5 adenocarcinomas and 7 squamous cell carcinomas were noted. Fifteen cases could not be subtyped on morphology alone and were labelled as NSCC. Two cases showed epithelioid cell granulomas on TP. ICC was performed on 12 NSCC cases, of which 4 were immunopositive for Napsin-A, and TTF-1 and were diagnosed as adenocarcinomas [Figure 1e-h]. Similar results were seen on conventional direct or cytospin smears also with or without hemorrhagic background and air-drying artefacts [Figure 2a-c]. P40 was immunopositive in 4 cases of NSCC and were diagnosed as squamous cell carcinomas [Figure 2d-f]. ICC was negative in 4 cases, of

**Table 1: Correlation of diagnostic accuracy of TP and smear preparation in 57 cases**

| Smear preparation (cytospin + aspirate smear) | TP |
|---------------------------------------------|----|
| Diagnostic yield                           | 68.4% (39/57) | 57.8% (33/57) |
| Sensitivity                                 | 79.5% (39/49) | 76.7% (33/43) |
| Specificity                                 | 100% (5/5)    | 100%           |
| PPV                                         | 100%          | 100%           |
| NPV                                         | 23%           | 23%            |

PPV: Positive predictive value, NPV: Negative predictive value
which 1 was a metastatic carcinoma of colon on biopsy and showed immunopositivity for CK20 and was negative for CK7 and TTF-1; biopsy diagnosis was not available in 1 case and 2 showed blood clot and tumor necrosis. In 3 cases of NSCC no ICC was performed.

Histopathology was considered the gold standard. Two cases of BW were negative whereas biopsy and TBNA (smear and TP) were positive for tumor. However, biopsies were CT-guided and sampled lung mass directly. TBNA for staging were done from the subcarinal lymph node which were positive for adenocarcinoma.

Of 18 falsely negative cases, three cases of squamous cell carcinomas and one case of SCC showed scant necrosis and cellular debris on smear and TP both. Four cases of NSCC and adenocarcinoma showed only few clusters of atypical/reactive cells which were insufficient to establish the final diagnosis and were inconclusive. Remaining 10 cases (8 NSCC and 2 SCC) showed only bronchial epithelial cells. No false positive case was seen.

**Discussion**

In the past decade, role of endobronchial ultrasound guided transbronchial needle aspirate (EBUS-TBNA) and conventional TBNA has been well established in diagnosing lesions of the lung and mediastinum.[6-10] The procedure is minimally invasive and provides accurate cytology and histology samples for evaluation of lung cancer, its subtyping, as well as predictive marker analysis.[11,12] Proper sampling along with accurate diagnosis is essential as the treatment is diagnosis dependent.

Direct smear and cytopsin preparations from aspirations and BW or brushings have been the mainstay for cytological evaluation in respiratory tract lesions. However, with the advent of LBC and its success in improving the diagnostic efficacy of cervical samples, the present study was designed to compare the accuracy of conventional smears (cytospin and aspirate) and TP.

In comparison to cytopsin and conventional aspirate smears, TP did not add to the final diagnosis. Excessive hemorrhage,
necrosis, and air-drying were some of the problems that were encountered while evaluating the direct smears of TBNA.

Although there was not much difference in the tumor cell appearance by the two methods, TP eliminated blood and mucus providing a clean and uniform background.

TP showed uneven spread of cell population and thick cell clusters in which it was difficult to see nuclear details. However, for ICC, TP smears offered a clean background without hemorrhage and mucus, hence better interpretation of the results. Nuclear stains (p40 and TTF-1) were not affected by alcohol fixation in TP or conventional preparations. ICC in our study was done in only those cases which were difficult to subtype on morphology alone on TP.

Studies have been done to compare the utility of TP over other preparations in respiratory tract cytology with superior results with the LBC. We did not find increased diagnostic yield of TP in comparison to conventional smear preparations [Table 1].

The present study observed that it was better to interpret ICC on TP smears due to clean background. Dabbs et al. reported a clean background and consumption of less antibodies with LBC, and additional smears can be prepared if needed. Another study used a panel of antibodies on LBC and found it suitable for different antigens. In addition, monolayer preparation of TP as well as removal of unnecessary background staining caused by blood and proteinaceous debris enabled easy interpretation of the staining. However, in our experience, nuclear stains are easier to interpret on conventional smears also.

We have observed diagnostic challenges in TP in the form of thickened cellular clusters, disruption of architectural pattern, and nonuniformly spread cells. Subtle nuclear moulding of SCC with loss of manually-induced nuclear smearing was another difficulty experienced during the study. Loss of background material such as necrosis and mucus also posed diagnostic dilemma in confirmation of the malignant nature of the lesion. Moreover, the cost per case for TP consumables is higher than that required for direct smears, along with the requirement of trained technical staff for running the machine and trained pathologists who are aware of the morphological changes in the appearance of cells on TP smears are needed.

Overall, there is a learning curve for interpretation of LBC. At our centre we routinely perform rapid on-site evaluation in EBUS-TBNA, however, with LBC it cannot be performed.

To conclude, use of TP has not increased diagnostic yield in our series of patients, however, it was helpful in performing ancillary studies and establishing the final diagnosis of lung cancer. The application of both methods may be helpful. TP may be added in respiratory tract cytology as a complimentary method, however, not as a substitute of conventional cytology preparations.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
There are no conflicts of interest.

1. Qiu T, Zhu H, Cai M, Han Q, Shi J, Wang K. Liquid-Based Cytology Preparation Can Improve Cytological Assessment of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration. Acta Cytol 2015;59:139-43.
2. Ramieri MT, Marandino F, Visca P, Salvitti T, Gallo E, Casini B, et al. Usefulness of conventional transbronchial needle aspiration in the diagnosis, staging and molecular characterization of pulmonary neoplasias by thin-prep based cytology: Experience of a single oncological institute. J Thorac Dis 2016;8:2128-37.
3. Choi YD, Han CW, Kim JH, Oh JJ, Lee JS, Nam JH, et al. Effectiveness of sputum cytology using ThinPrep method for evaluation of lung cancer. Diagn Cytopathol 2008;36:167-71.
4. Imura J, Abe K, Uchida Y, Shibata M, Tsunematsu K, Sathoh M, et al. Introduction and utility of liquid-based cytology on aspiration biopsy of peripheral nodular lesions of the lung. Oncol Lett 2014;7:669-73.
5. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG, editors. WHO Classification of Tumours of Lung, Pleura, Thymus and Heart (4th edition). IARC: Lyon; 2015.
6. Madan NK, Madan K, Jain D, Walia R, Mohan A, Hadda V, et al. Utility of conventional transbronchial needle aspiration with rapid on-site evaluation (c-TBNA-ROSE) at a tertiary care center with endobronchial ultrasound (EBUS) facility. J Cytol 2016;33:22-6.
7. Walia R, Madan K, Mohan A, Jain D, Hadda V, Khilnani GC, et al. Diagnostic utility of conventional transbronchial needle aspiration without rapid on-site evaluation in patients with lung cancer. Lung India 2014;31:208-11.
8. Madan K, Mohan A, Ayub II, Jain D, Hadda V, Khilnani GC, et al. Initial experience with endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) from a tuberculosis endemic population. J Bronchology Interv Pulmonol 2014;21:208-14.
9. Madan K, Dhugana A, Mohan A, Hadda V, Jain D, Arava S, et al. Conventional Transbronchial Needle Aspiration Versus Endobronchial Ultrasound-guided Transbronchial Needle Aspiration, With or Without Rapid On-Site Evaluation, for the Diagnosis of Sarcomoidosis: A Randomized Controlled Trial. J Bronchology Interv Pulmonol 2017;24:48-58.
10. Ayub II, Mohan A, Madan K, Hadda V, Jain D, Khilnani GC, Guleria R. Identification of specific EBUS sonographic characteristics for predicting benign mediastinal lymph nodes. Clin Respir J 2016 [Epub ahead of print].
11. Schmid-Bindert G, Wang Y, Jiang H, Sun H, Henzler T, Wang H, et al. EBUS-TBNA provides highest RNA yield for multiple biomarker testing from routinely obtained small biopsies in non-small cell lung cancer patients—a comparative study of three different minimal invasive sampling methods. PLoS One 2013;8:e77948.
12. Navani N, Brown JM, Nankivell M, Woolhouse I, Harrison RN, Jeebun V, et al. Suitability of endobronchial ultrasound-guided transbronchial needle aspiration specimens for subtyping and genotyping of non–small cell lung cancer: A multicenter study of 774 patients. Am J Respir Crit Care Med 2012;185:1316-22.
13. Elsheikh TM, Kirkpatrick JL, Wu HH. Comparison of ThinPrep and cytoospin preparations in the evaluation of exfoliative cytology specimens. Cancer 2006;108:144-9.
14. Liu C, Wen Z, Li Y, Peng L. Application of ThinPrep bronchial brushing cytology in the early diagnosis of lung cancer: A retrospective study. PLoS One 2014;9:e90163.
15. Konofaos P, Tomas P, Malagaris K, Karakatsani A, Pavlopoulos D, Lachanas E, et al. The role of ThinPrep cytology in the investigation of lung tumors. Surg Oncol 2006;15:173-8.
16. Michael CW, Bedrossian CC. The implementation of liquid-based cytology for lung and pleural-based diseases. Acta Cytol 2014;58:563-73.
17. Zardawi IM, Blight A, Ling S, Braye SG. Liquid-based vs. conventional cytology on respiratory material. Acta Cytol 2009;53:481-3.
18. Wallace WA, Monaghan HM, Salter DM, Gibbons MA, Skwarski KM.
Endobronchial ultrasound-guided fine-needle aspiration and liquid-based thin-layer cytology. J Clin Pathol 2007;60:388-91.

19. Dubbs DJ, Abendroth CS, Grenko RT, Wang X, Radcliffe GE. Immunocytochemistry on the Thinprep processor. Diagn Cytopathol 1997;17:388-92.

20. Leung SW, Bedard YC. Immunocytochemical staining on ThinPrep processed smears. Mod Pathol 1996;9:304-6.

21. Jain D, Mathur SR, Guleria R, Iyer VK. Utility and pattern of positivity of 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.

22. Jain D, Mathur SR, Sharma MC, Iyer VK. Cytomorphology of sebaceous carcinoma with analysis of p40 antibody expression. Diagn Cytopathol 2015;43:456-61.

23. Roy M, Jain D, Yadav R, Mathur SR, Iyer VK. TTF-1 and Napsin-A Are Not Markers for Biliary Phenotype: An Immunohistochemical Study of Gallbladder Adenocarcinomas. Am J Surg Pathol 2015;39:1742-4.

24. Yadav R, Jain D, Mathur SR, Iyer VK. Cytomorphology of neuroendocrine tumours of the gallbladder. Cytopathology 2016;27:97-102.

25. Vallonthaiel AG, Jain D, Singh V, Kaur K, Madan K, Kumar V, et al. c-Myb Overexpression in Cytology Smears of Tracheobronchial and Pulmonary Adenoid Cystic Carcinomas. Acta Cytol 2017;61:77-83.

26. Rana DN, O'Donnell M, Malkin A, Griffin M. A comparative study: Conventional preparation and ThinPrep 2000 in respiratory cytology. Cytopathology 2001;12:390-8.

27. Michael CW, Bedrossian CC. The implementation of liquid-based cytology for lung and pleural-based diseases. Acta Cytol 2014;58:563-73.

Author Help: Reference checking facility

The manuscript system (www.journalonweb.com) allows the authors to check and verify the accuracy and style of references. The tool checks the references with PubMed as per a predefined style. Authors are encouraged to use this facility, before submitting articles to the journal.

- The style as well as bibliographic elements should be 100% accurate, to help get the references verified from the system. Even a single spelling error or addition of issue number/month of publication will lead to an error when verifying the reference.
- Example of a correct style
  Sheahan P, O’leary G, Lee G, Fitzgibbon J. Cystic cervical metastases: Incidence and diagnosis using fine needle aspiration biopsy. Otolaryngol Head Neck Surg 2002;127:294-8.
- Only the references from journals indexed in PubMed will be checked.
- Enter each reference in new line, without a serial number.
- Add up to a maximum of 15 references at a time.
- If the reference is correct for its bibliographic elements and punctuations, it will be shown as CORRECT and a link to the correct article in PubMed will be given.
- If any of the bibliographic elements are missing, incorrect or extra (such as issue number), it will be shown as INCORRECT and link to possible articles in PubMed will be given.