Comparative study of cytology and immunocytochemistry with trucut biopsy and immunohistochemistry in diagnosis of localized lung lesions: A prospective study

ABSTRACT

Background: Percutaneous lung biopsy is now a common procedure in pulmonary medicine, and several different techniques are in use. The most common has been the use of a fine needle under computed tomography (CT) guidance combined with the trucut needle for histology.

Aim: To evaluate the efficacy of fine needle aspiration cytology (FNAC) and immunocytochemistry in comparison with trucut biopsy and immunohistochemistry in patients with localized intrathoracic lesions suspicious for malignancy.

Materials and Methods: Eighty patients with localized mass lesions in the lung on imaging (chest radiograph/CT) were selected for this study over a period of 1 year. FNAC was carried out by a 22 G spinal needle after localization of the mass in the CT scan followed by guided trucut biopsy. Immunocytochemistry and immunohistochemistry were performed as and when required.

Results: The mean age of our study population was 57.6 years and the M:F ratio was 4.2:1. Majority of the lesions were peripheral and in the right lung. Adenocarcinoma was most prevalent (49%), followed by squamous cell carcinoma and small cell carcinoma. Cyto-histopathological concordance was seen in 60 cases (75%). The highest rate of concordance was seen in small cell carcinoma (83.3%). The overall sensitivity of FNAC in distinguishing malignant lung lesions from benign lesions was 84.2% and the specificity was 100%. The sensitivity of cytology in diagnosing small cell carcinoma was 83.3% and of non-small cell carcinoma was 65.38%. Immunocytochemistry was carried out in 34 cases, all of which were followed by immunohistochemistry. Cyto-histopathological concordance was noted in 31 of these cases (91.2%). We used the standard panel of four markers (cytokeratin-7, thyroid transcription factor-1, p63 and CD56) for all selected cases.

Conclusion: Cytology along with immunocytochemistry is highly effective in diagnosing and categorizing lung neoplasms, producing comparable results to trucut biopsy and immunohistochemistry.

Key words: Immunocytochemistry; immunohistochemistry; lung cancer; trucut biopsy

Introduction

Lung cancer is the leading cause of cancer-related death all over the world, with an average 5-year survival rate of 16.8% despite advancement in chemoradiation. The diagnostic evaluation of patients with suspected lung cancer includes tissue diagnosis and a complete staging work-up, including evaluation of metastases. The modality selected to diagnose a suspected lung cancer is based on the size and location of the primary tumor in the lung, the presence of potential metastatic spread and the anticipated treatment plan (Epidermal Growth Factor Receptor/EGFR and Anaplastic Lymphoma Kinase/Alk mutation study).

Percutaneous lung biopsy is now a common procedure in pulmonary medicine, and several different techniques are
in use. The most common has been the use of a fine needle, yielding an aspirate for cytological evaluation combined with the trucut needle, where material may be obtained for histology. The indications for these techniques overlap with the more recently developed transbronchial needle aspiration (TBNA).

Haaga and Alfidi[2] reported computed tomography (CT)-guided biopsy in the 1970’s, but Menetrier[3] probably performed the first cutting needle biopsy for histological diagnosis.

The diagnostic accuracy has been reported as being >80% for benign disease and >90% for malignant disease.[4]

Cytology with immunocytochemistry is, by its virtue, faster and cost-effective and yields almost as good a result when compared with more invasive trucut biopsy and immunohistochemistry. Therefore, the question arises — Is trucut biopsy loosing importance in diagnostic work-up of lung masses?

For these reasons, we have conducted a prospective study on the efficacy of fine needle aspiration cytology (FNAC) and immunocytochemistry in comparison with trucut biopsy and immunohistochemistry in patients with localized intrathoracic lesions suspicious for malignancy.

Materials and Methods

This institution-based prospective, observational study was conducted over a period of 1 year (January 2013 to January 2014). Eighty patients who were admitted with a radiologically visible localized lesion in the lung (either in chest radiograph or CT), were included in this study.

All cases were thoroughly evaluated radiologically to exclude primary malignancy in any site other than lung.

Informed written consent was obtained from each patient and the lesion was localized on CT scan. Images were obtained of the region of interest by using a section thickness of 3 mm. The slice of interest (entry and depth from the skin surface) was marked with an axial laser beam localizer. FNAC was performed with a 22 G spinal needle for confirmation of the needle track as well as to obtain a cytologic sample. At least two passes were made in each case, with care taken to biopsy the periphery as well as the center of the lesion. Smears were made and were fixed in ethanol for Papanicolaou and dry-fixed to stain in May-Grünwald-Giemsa (MGG). Then, local skin disinfection was carried out and 2% lignocaine was infiltrated along the track. A 2-mm incision was made with a scalpel of size 11. A trucut biopsy needle (BARD, 18” needle with 23 mm throw) was inserted through the cut up to the pre-determined distance and the biopsy material was obtained. Imprint smears were made from the tissue core, which was then transferred to formalin for histopathology. Two to three passes were made in all cases. Local dressing was performed. In each case, the patient was monitored for any complication by erect chest radiograph/repeat CT after 1 h. Immunohistochemistry and immunocytochemistry were carried out as and when required. For immunocytochemistry, we used cold acetone-fixed smear and for immunohistochemistry, a peroxidase-based study was performed in an automated platform.

Results

A total of 80 cases in the age range of 22-82 years were evaluated. The mean age was 57.6 years (57.6 ± 15.3 years) and the M:F ratio was 4.2:1. 93.2% of the cases were above 50 years of age. The earliest age of onset was a 24-year-old male and a 34-year-old female, both with a diagnosis of adenocarcinoma. A total of 52 (65%) patients had a past or present addiction of tobacco. Majority of the cases were in the right lung (right:left = 2.1:1). Majority were peripheral lung lesions (87.8%).

Adenocarcinoma predominated among the cases (49%), followed by squamous cell carcinoma (SCC, 16%) and small cell carcinoma (8%). No malignancy was found in 13% of the patients while the rest comprised of metastatic deposit, lymphoma, plasmacytoma, synovial sarcoma, primitive neuro-ectodermal tumor (PNET), adenoid cystic and langerhan cell histiocytosis (LCH).

Cyto-histopathological concordance was seen in 60 cases (75%) [Table 1]. The highest rate of concordance was in small cell carcinoma (83.3%). 46.6% of SCCs were rightly diagnosed in cytology, whereas 65.78% of adenocarcinoma (including poorly differentiated types) were positively diagnosed in cytology. Plasmacytoma, non Hodgkin lymphoma (NHL), synovial sarcoma and benign hyperplasia were all concordant in cytology.

| Histodiagnosis | Cyto-concordant | Cyto-inconclusive | Cyto NSCLC-NOS | Cyto-histo discordance |
|----------------|----------------|-------------------|---------------|-----------------------|
| Adeno (n=39)  | 14             | 8                 | 10            | 7                     |
| Squamous (n=13)| 6             | 3                 | 4             | 0                     |
| Small cell (n=6)| 5             | 1                 | 1             | 0                     |
| Others (n=12)| 11             | 0                 | 0             | 1                     |
| Benign (n=10) | 10             | 0                 | 0             | 0                     |
Three cases of poorly differentiated squamous cell carcinoma (PD SCC), eight cases of PD adeno and one small cell carcinoma yielded either inadequate or only hemorrhagic/inflammatory cells in cytology. Seven cases of adenocarcinoma were wrongly interpreted as SCC in cytology as the Papanicolaou-stained smear showed orangeophilia.

Histology was taken as the gold standard when we only consider benign and malignancy to be accurately diagnosed by FNAC [Table 2]:

Overall sensitivity: 59/70 = 84.2%
Specificity: 10/10 + 0 = 100% benign compared with malignant
Positive predictive value: 59/59 = 100%
Negative predictive value: 10/21 = 47.62%

When we consider small cell versus non-small cell cancer:

Sensitivity of cytology in diagnosing small cell: 83.3% and non-small cell: 65.38%

Immunocytochemistry was performed in 34 cases. Among them, adenocarcinoma was predominant (n = 23), followed by SCC (n = 4), small cell carcinoma (n = 4) and one case each of spindle cell sarcoma, metastatic adenocarcinoma and non-Hodgkins lymphoma. All of these cases were followed by trucut biopsy and immunohistochemistry. Immunocyto — immunohisto concordance was noted in 31 cases among these (91.2%) [Table 3].

Table 2: Cyto-histo correlation in our study

| Histological diagnosis | Malignant | Non-Malignant | Total |
|------------------------|-----------|--------------|-------|
| Cytology +             | 59        | 0            | 59    |
| Cytology −             | 11        | 10           | 21    |
| Total                  | 70        | 10           | 80    |

Table 3: Immunocytochemistry — immunohistochemistry correlation (n = 34)

| Final diagnosis | Cytology | Immunocyto | Histology | Immunohisto | Discordance |
|-----------------|----------|------------|-----------|-------------|-------------|
| AdenoCA (n=23)  | 19       | 21         | 23        | 23          | 2           |
| Sq cell CA (n=4)| 3        | 4          | 4         | 4           | 0           |
| Small cell CA (n=4)| 3     | 4          | 4         | 4           | 0           |
| Others (n=3)    | 2        | 2          | 3         | 3           | 1           |

One case of small cell carcinoma cytology yielded predominantly necrotic material, but CD56 staining by immunocytochemistry picked up few cells with intense membrane positivity. Trucut biopsy and immunohistochemistry confirmed the case to be small cell carcinoma (TTF-1 +ve, p63 −ve, synaptophysin +ve, CD56 +ve) [Figure 1].

Similarly, in one case of SCC, immunocytochemistry by p63 was helpful to delineate degenerated squamous cells among granulomatous inflammation in cytology smears.

In one case of poorly differentiated adenocarcinoma, the mass was deep seated and close to the mediastinum and we missed the mass in trucut biopsy (yielded only granulation tissue and few scattered atypical cells in the trucut sample). Cytology and immunocytochemistry helped to reach to a conclusive diagnosis in this case (TTF-1 +ve, CK-7 +ve, p63 -ve).

Two cases were diagnosed as SCC in cytology and immunocytochemistry (p63 +ve, TTF-1 −ve, CK-7 +ve) and trucut biopsy revealed adenosquamous carcinoma (CK-7 +ve, Napsin-A +ve, TTF-1 +ve, patchy p63 +ve) [Figure 2].

One case of adenocarcinoma diagnosed by cytology proved to be metastatic in origin, where trucut biopsy showed a villous papillary pattern and mucin secretion (CK-7 +ve, p63 −ve, CDX2 +ve, TTF-1 −ve). Clinical follow-up and endoscopy revealed later mass in the pylorus.

The overall findings suggest that when cytology is combined with immunocytochemistry, it has almost the same precision compared with more invasive trucut biopsy [Table 3].
However, newer prognostic tests like EGFR and ALK mutation studies require tissue samples and cytology samples have not been yet approved for these tests.

Lastly, we evaluated the value of different immunohistochemistry markers in appropriately diagnosing lung neoplasms. Adenocarcinomas expressed napsin-A in 78% and TTF-1 in 62%, whereas small cell carcinoma expressed it in 88%. CD56 positivity and TTF-1 positivity were seen in 50% of the cases. SCC expressed p63 positivity in 100% but TTF-1 positivity was only seen in 25% of the cases. Small cell carcinoma expressed any two of three neuroendocrine markers (CD56, synaptophysin, chromogranin-A) in 100% of the cases. We conclude that, at present, a panel of four markers (CK-7, p63, TTF-1, CD56) should be enough to differentiate or categorize lung neoplasms. Newer markers like napsin-A are more useful in categorizing adenocarcinoma and can replace CK-7 in the near future.

Discussion

The American College of Chest Physicians supports the use of transthoracic needle aspiration biopsy (TNAB) as the procedure of choice in patients in whom the benign nature of the solitary pulmonary nodule cannot be established by clinical criteria and in whom surgery (exploratory thoracotomy or video-assisted thoracoscopic surgery) cannot be undertaken.[5]

Sensitivity of TNAB for specific benign diagnosis was reported to range from 11.7% to 68%.[6,7] We found the mean age of the population of this study to be 57.6 years, with a M:F ratio of 4.2:1 and smoker: Non-smoker ratio of 65%. This is in accordance with Noronha et al.[8]

Non-small cell carcinomas predominated, similar to the experience of other workers.[9,10] We found adenocarcinoma to be the most common malignancy in our study population.

The overall accuracy of diagnosing small cell versus non-small cell lung cancer at cytology in this study was 83.3%. The high accuracy of distinction between small cell and non-small cell lung cancer in our study matched with other studies.[11,12] We found that cytological diagnosis of non-small cell lung cancer is less reliable (misclassification in 12% of the cases) than cytological diagnosis of small cell lung cancer. Mukherjee et al.[1] found the contrary.[11]

Majority of poorly differentiated SCC were misdiagnosed as non-small cell adeno because it failed to demonstrate keratinization in imprint/FNA smears. Three cases were reported as inflammatory/hemorrhagic as the mass was almost entirely necrotic. One case was misdiagnosed as small cell because cytology showed smudging artefacts.

Adenocarcinomas that were misdiagnosed as SCC in cytology were mostly poorly differentiated in nature; three of them showed areas of focal squamous differentiation in histology. One case of bronchioloalveolar carcinoma (minimal deviation adenocarcinoma, lepidic type) was diagnosed as well-differentiated adenocarcinoma, one mucinous adenocarcinoma was diagnosed as SCC as cells showed orangeophilia and three adenocarcinoma yielded inadequate material.

One small cell carcinoma was misdiagnosed as inflammatory in cytology probably due to the needle aspirating necrotic part of the tumor.

The importance of review of the slides has been cited by Tan et al.[12] We found that the review was especially important in the cases diagnosed as negative for malignancy. We also found that the review of slides by a second expert followed by a consensus diagnosis by both increased the accuracy.

Bocking et al.[13] compared fine needle aspiration biopsy (FNAB) and punch biopsy (PB), and found that the overall sensitivities of the biopsy methods were equal. They concluded that FNAB should be the method of choice in
pulmonary and hilar lesions because of the similar diagnostic accuracy. Mediastinal and pleural lesions and presumed mesenchymal tumors should be sampled with PB because the typing accuracy of FNAB is insufficient in these cases. Mondal et al.[14] found FNAC to be superior in categorizing lung neoplasms.

Manhire et al.[15] suggested that percutaneous transthoracic lung biopsy (PTLB) should be considered in the following cases:

- New or enlarging solitary nodule or mass on the chest radiograph that is not amenable to diagnosis by bronchoscopy or CT shows it is unlikely to be accessible by bronchoscopy.
- Multiple nodules in a patient not known to have malignancy or who has had a prolonged remission or more than one primary malignancy.
- Persistent focal infiltrates, either single or multiple, for which no diagnosis has been made by sputum or blood culture, serology or bronchoscopy.
- Hilar mass.

There are relative contraindications to PTLB, and the balance of benefit against risk for the procedure should be assessed at a multidisciplinary meeting.

Pre-operative investigations should include coagulation indices like prothrombin time (PT), activated partial thromboplastin time (APTT) and platelet count. Oral anticoagulants should be stopped before a percutaneous lung biopsy.

They found that an erect chest radiograph should be performed 1 h after the biopsy, and is sufficient to detect the majority of post-biopsy pneumothoraces.[15]

Khan et al.[16] showed that endobronchial ultrasonography-guided transbronchial needle aspiration biopsy (EBUS-TNA) has a very high sensitivity (97%) in the diagnosis of central lung masses related to lung cancer. It could be used as a “one stop” as an early minimally invasive tool in the lung cancer diagnostic pathway to enable accurate diagnosis, and a negative result may warrant other invasive tests.

CT-guided trucut biopsy is generally regarded as a safe procedure with limited morbidity and extremely rare mortality. The pneumothorax remains the most frequent complication of TNAB. Tsukada et al.[16] have shown that the lesion size is a significant factor contributing to diagnostic accuracy and that a lesion size ≥30 mm in diameter has an accuracy as high as 93%. Yeow et al.[17] in a study of 660 consecutive biopsies showed that patients with lesions ≤20 mm have a higher incidence of pneumothorax than those with larger lesions. Wang et al.[18] found a statistically significant correlation between pneumothorax and the factors of smoking (P = 0.015) and position (P < 0.01) and length of the needle in the normal parenchyma (P = 0.111) as well as between hemorrhage and the maximal diameter (P = 0.005) and length of the needle in the normal parenchyma (P < 0.01) and the frequency of needle adjustments (P < 0.01). We encountered only two cases of pneumothorax that resolved by occlusive dressing only.

Bozetti et al.[19] studied the efficacy of cytology and immunocytochemistry to categorize lung neoplasms according to prognostic markers like Ki-67, bcl-2 and p53 and found a concordance rate with histology as high as 95%.

Kulshreshtha et al.[20] used FNA material supplemented by cell block preparation and immunohistochemistry and effectively subcategorized lung neoplasms using IHC markers like CK, chromogranin-A, synaptophysin, TTF-1 and CEA. In our study, we used a panel of TTF-1, CK-7, CD56 and p63 in most of the cases. Several other authors used a combination of markers in cytology specimens and showed efficacy comparable to trucut.[21-24] Newer markers like napsin-A are more specific for lung adenocarcinomas and likely to prove more effective in the near future.[25]

Conclusion

In this study, we evaluated the demographics of lung cancer in our institution and compared cytology, immunocytochemistry, trucut and immunohistochemical parameters of lung cancer. We found adenocarcinoma to be the predominant histological type. We also found that although cytology is highly accurate in distinguishing true negative cases (benign lesions), a needle biopsy is often required to complement the cytology when the material is inadequate or the typing of malignant lesion is critical for treatment. Immunocytochemistry and histochemistry is an invaluable tool to categorize when morphology is equivocal. We found immunocytochemistry to be highly effective in categorizing lung neoplasms, producing comparable results to immunohistochemistry.

References

1. Surveillance, Epidemiology and End Results(SEER) statistics fact sheets: Lung and Bronchus by National Cancer Institute. Available from: http://www.seer.cancer.gov/statfacts/html/lungb.html. [Last accessed on 2014 Feb 10].
2. Haaga JR, Alfidi RJ. Precise biopsy localization by computed tomography. Radiology 1976;118:603-7.
3. Menetrier P. Cancer primitif du poumon. Bull Soc Anat Paris 1886;2:643-7.

4. Tsukada H, Satou T, Iwashima A, Sourn A. Diagnostic accuracy of CT-guided automated needle biopsy of lung nodules. AJR Am J Roentgenol 2000;175:239-43.

5. Sokolowski JW Jr, Burgwer LW, Jones FL, Jr, Patterson JR, Selecky PA. Guidelines for percutaneous transthoracic needle biopsy. This position paper of the American Thoracic Society was adopted by the ATS Board of Directors, June 1988. Am Rev Respir Dis 1989;140:255-6.

6. Ogbole GI, Adeoye PO, Okolo CA, Iseko K. CT-guided percutaneous transthoracic lung biopsy: First experience in Ibadan, Nigeria. Niger J Clin Pract 2013;16:544-7.

7. Lacasse Y, Wong E, Guyatt GH, Cook DJ. Transthoracic needle aspiration biopsy of the lung, hilum and mediastinum. Sensitivity, specificity and typing accuracy. Thorax 1999;54:884-93.

8. Noronha V, Dikshit R, Raut N, Joshi A, Pramesh CS, George K., et al. Changing pattern of bronchogenic carcinoma: A statistical variation or a reality? Lung Cancer 2012;49:74-81.

9. Singh JP, Garg L, Setia V. Computed tomography (CT) guided transthoracic needle aspiration cytology in difficult thoracic mass lesions-not approachable by USG. Ind J Radiol Imag 2004;14:395-400.

10. Shetty CM, Lakhkar BN, Gangadhar VS, Ramachandran NR. Changing pattern of bronchogenic carcinoma: A statistical variation or a reality? Ind J Radiol Imag 2005;15:233-8.

11. Mukherjee S, Bandyopadhyay G, Bhattacharya A, Ghosh R, Barui G, Karmakar R. Computed tomography-guided fine needle aspiration cytology of solitary pulmonary nodules suspected to be bronchogenic carcinoma: Experience of a general hospital. J Cytol 2010;27:8-11.

12. Tan KB, Thamboo TP, Wang SC, Nilsson B, Rajwanshi A, Salto-Tellez M. Audit of transthoracic fine needle aspiration of the lung: Cytological subclassification of bronchogenic carcinomas and diagnosis of tuberculosis. Singapore Med J 2002;43:570-5.

13. Böcking A, Klose KC, Kyll HJ, Hauptmann S. Cytologic versus histologic evaluation of needle biopsy of the lung, hilum and mediastinum. Sensitivity, specificity and typing accuracy. Acta Cytol 1995;39:463-71.

14. Mondal SK, Nag D, Das R, Mandal PK, Biswas PK, Osta M. Computed tomogram guided fine-needle aspiration cytology of lung mass with histological correlation: A study in eastern India. South Asian J Cancer 2013;2:14-8

15. Manhire A, Charig M, Clelland C, Glesson F, Miller R, Moss H, et al. BTS. Guidelines for radiologically guided lung biopsy. Thorax 2003;58:920-36.

16. Khan SL., Haris M, Diver S, Miller B, Munavvar M. P70 can endobronchial ultrasound (EBUS) guided transbronchial needle aspiration (TBNA) be used as a first line investigation in the diagnosis of central lung parenchymal lesions? Thorax 2012;67:A94.

17. Yeow KM, See LC, Lui KW, Lin MC, Tcao TC, Ng KF, et al. Risk Factors for pneumothorax and bleeding after CT guided percutaneous coaxial cutting needle Biopsy of Lung Lesions. J Vasc Interv Radiol 2001;12:1305-12.

18. Wang Y, Li W, He X, Li G, Xu L. Computed tomography-guided coreneedle biopsy of lungenlesions: Diagnostic cyuilder and correlation between factors and complications. Oncol Lett 2014;7:288-94.

19. Bozzetti C, Franciosi V, Crafa P, Carbognani P, Rusca M, Nizzoli R, et al. Biological variables in non-small cell lung cancer: Comparison between immunocytochemical determination on fine needle aspirates from surgical specimens and immunohistochemical determination on tissue sections. Lung Cancer 2000;29:33-41.

20. Kulshreshtha R, Vijayan VK. Immunohistochemical staining on fine needle aspiration biopsy-cell block specimens in the differential diagnosis of lung cancers. Indian J Chest Dis Allied Sci 2009;51:21-5.

21. Tan D, Zander DS. Immunohistochemistry for assessment of pulmonary and pleural neoplasms: A review and update. Int J Clin Exp Pathol 2008;1:19-31.

22. Mandal PK, Mondal SK, Roy S, Adihkari A, Basu N, Sinha SK. Immunocytochemistry: It’s role in diagnosis of undifferentiated neoplasms by fine needle aspiration cytology. J Cytol 2013;30:121-4.

23. Jiang SX, Kameya T, Shoji M, Dobashi Y, Shinada J, Yoshimura H. Large cell neuroendocrine carcinoma of the lung: A histologic and immunohistochemical study of 22 cases. Am J Surg Pathol 1998;22:526-37.

24. Kargi A, Gurel D, Tunas Y. The diagnostic value of TTF-1, CK 5/6, and p63 immunostaining in classification of lung carcinomas. Appl Immunohistochem Mol Morphol 2007;15:415-20.

25. Mukhopadhyay S, Katzenstein AL. Subclassification of non-small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: Utility of an immunohistochemical panel containing TTF-1, napsin A, p63, and CK5/6. Am J Surg Pathol 2011;35:15-25.