Evaluation of cytology in lung cancer diagnosis based on EBUS-TBNA

ABSTRACT

Aims: Endobronchial ultrasound (EBUS) is a relatively new modality that can be used to guide transbronchial needle aspiration (TBNA) of mediastinal lymph nodes. At present, researches on the sensitivity and specificity of cytopathology based on the EBUS-TBNA are deficient; therefore, we want to evaluate the value of cytology based on the EBUS-TBNA in this article.

Materials and Methods: We reviewed the 379 cases that underwent the EBUS-TBNA in Shanghai Pulmonary Hospital from April 2010 to May 2011. Discarding the 139 cases with insufficient cells, we analyzed the remaining 240 cases that had enough cells on the smears.

Statistical Analysis Used: The Statistical Package for the Social Sciences version 15.0 (SPSS Inc., Chicago, IL) was used for data analysis. A P value of <0.05 was considered significant.

Results: We found that the cytologic diagnosis of sensitivity and specificity reached 94.52% and 95.12%, respectively. The sensitivity of squamous cell carcinoma, adenocarcinoma, and small cell carcinoma was up to 88.24%, 100.00%, and 96.00%, respectively. The specificity of squamous cell carcinoma, adenocarcinoma, and small cell carcinoma reached to 100.00%, 100.00%, and 99.25%, respectively.

Conclusion: Here, we report that the cytological examination of EBUS-TBNA should be acknowledged as a simple, fast, and safe procedure that provides a reasonable sensitivity and specificity of diagnosis in lung cancer.

Key words: Cell block; cytopathology; differential diagnosis; EBUS-TBNA; lung cancer

Introduction

Malignant thoracic diseases, such as lung cancer, has increased significantly.[1,2] Lung cancer can be mainly classified into squamous cell carcinoma, adenocarcinoma, small cell carcinoma and the other subtypes.[2] It is very important to diagnose the subtype of lung cancer fast and accurately as the therapy is different.[2] Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), a new clinical technology, has been applied in the clinical diagnosis of advanced cancer and tuberculosis. Because of direct smears from needle aspiration and brushing specimens, EBUS-TBNA can be used for on-site evaluation.[3-7] However, few studies have investigated the sensitivity and specificity of cytology compared to histology using EBUS-TBNA. However, few researches are focused on sensitivity and specificity of the cytopathology based on the EBUS-TBNA. Through a retrospective study of EBUS-TBNA cases, we provided the sensitivity and specificity of lung cancer subtype based on the EBUS-TBNA and found that EBUS-TBNA was a simple,
fast, safe and effective technology which can be routinely applied in the clinical practice.

Materials and Methods

Case selection
The cytological specimens were collected using an ultrasound bronchofiber videoscope with a 22-gauge needle and was followed by cytology and histological diagnosis. An immediate assessment was given to the clinician after each puncture. In many patients, multiple sites were sampled. The average number of slides of each case were 2–4, with a range of 1–7.⁸,⁹

All cases diagnosed as thoracic disease at Shanghai Pulmonary Hospital from April 2010 to May 2011 were reviewed. During this period, 379 cytological samples were obtained by EBUS-TBNA during this period are originally diagnosed as inadequate, suspicious, benign or malignant. Of all 379 cases, we analyzed 240 cases with enough cells on the smears. 159 histological-control-paired cases underwent a bronchofiber biopsy, percutaneous transthoracic biopsy, or postsurgical pathology. Immunocytochemistry (ICC) was requested on the cytological material if there were enough cells left. Cytology samples were processed by cytopsin or cell block technique. Retrospective analyses of all of the pathology cases were available for review. Based on the diagnosis by two cytopathologists, we classify the cases into the positive group and the negative group. The positive group referred to the cases of malignant diagnosis such as squamous cell carcinoma, adenocarcinoma, small cell carcinoma and adenosquamous carcinoma etc. And the negative group contained the cases diagnosed as benign or no evidence of malignancy.¹⁰

Cell blocks production and immunocytochemistry
Samples obtained by EBUS-TBNA were centrifuged for 5 minutes in the 50ml centrifuge tube at speed of 2000r/s. Supernatant was collected in the centrifuge tube and the cleaning solution was added (TCT). When the sediment contained more blood cells, 10% acetic acid wash solution was used after shaking centrifuge (at 2000 r/s, 5 min). 95% ethanol was added into the supernatant along the wall (gentle movements) and fixed for 1 h. The amount of alcohol added into the precipitate was at least five times that of the supernatant volume. After discarding the alcohol, 10% neutral formalin was added along the wall to separate the natural sediment from the wall and fixed for 1 h. The sediment was poured from the centrifuge tube with a gauze wrapping and pressed down gently from the side into a color-embedded basket. The baskets were placed into a dryer for automatic dehydration.

IHC was performed on 4-μm thick formalin-fixed and paraffin-embedded (FFPE) sections. Slides were deparaffinized and pretreated with heat-mediated antigen retrieval solution in a microwave oven. Further steps were done at room temperature in a hydrated chamber. Slides were preincubated in 20% normal goat serum. P63 (1:50, DAKO), CK5/6 (1:50, DAKO), TTF-1 (1:100, DAKO), CK7 (1:100, DAKO), CEA (1:100, DAKO), and CD56 (1:100, DAKO), SYN (1:100 DAKO) were applied. The slides were then washed in Tris-HCl and detected with horseradish peroxidase-conjugated anti-rabbit EnVision+kit (DAKO). All slides were counterstained with hematoxylin.

Statistical analysis
Sensitivity and Specificity of the cytological diagnosis is calculated as below equation shows: % Sensitivity = True Positive/(True Positive + False Negative) × 100%. % Specificity = True Positive/(True Negative + False Positive) × 100%. Correlations between groups were performed using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL). A P-value < 0.05 was considered significant.

Results

Of the all 379 cases, 277 (73%) patients were males and 102 (26.9%) were females. The median age was 59 years (range: 15–83 years). In this study, there was no association between the histological subtype of thoracic disease and neither patient’s age (χ² = 2.7, P = 0.412) or sex (χ² = 0.687, P = 0.812). The number of slides in these cases (excluding ICC slides) ranged from 2 to 6 slides (median: 4 slides). the rest 240 cases that had enough cytological materials on the smear comprised the objects for this study. Among 240 cases, 135(56.25%) cases were negative while 105 (43.75%) were positive. Of the whole 240 cases, 159 cases did have histological material preparations performed. Further, the 37.92% (91/240) of the adequate cytological sample had been made the cell block [Table 1].

To compare the differences in sensitivity and specificity between the two methods, we analyzed the 159 cases which We analyze 159 cases which were diagnosed by cytology and histology at the same time. The sensitivity rate of the cytology reached to 94.52% and the specificity rate was as high as 95.12% [Table 2].

There are 69 cases diagnosed as positive by both cytology and histology. To further analysis these cases, the subtype diagnosis by cytology was inconsistent with histology. When adding cell block into cytology diagnosis, it did not improve the consistency of diagnosis by cytology and histology [Table 3].
ICC was dedicated to the cases which were hard to distinguish between benign and malignant or hard to subtype malignant diseases based on the smear. In our research, the following ICC biomarkers were routinely used to differentiate between benign cells (such as lymphocytes) and malignant cells: TTF-1, Ck7, and CEA for adenocarcinoma [Figure 1a and b]; P63 and CK5/6 for squamous cell carcinoma [Figure 1c]; and TTF-1, CD56, and SYN for small cell carcinoma [Table 4, Figure 1d]. There was an association between histological subtypes of EBUS-TBNA and the sensitivity of the cytological diagnosis was showed in Table 3. Further, the ICC helped to improve the sensitivity rate of the cytology based on the EBUS. P63 positive was significant (squamous cell carcinoma vs. adenocarcinoma or SCLC, \( P < 0.001 \)). TTF-1 positive of adenocarcinoma was similar with P63 for squamous cell carcinoma (adenocarcinoma vs. squamous cell carcinoma, \( P < 0.001 \)). However, TTF-1 positive between adenocarcinoma and small cell carcinoma was no difference (adenocarcinoma vs. small cell carcinoma, \( \chi^2 = 0.003, P > 0.05 \)). CD56 and NSE positive of small cell lung carcinoma was showed significantly (small cell carcinoma vs. squamous cell carcinoma or adenocarcinoma, \( P < 0.05 \) [Table 4].

**Discussion**

Mediastinal lymph node induced by malignant diseases is a common clinical symptom. They always appear in lung cancer, as well as in tuberculosis, sarcoidosis, etc. Lung cancer is the second most common cancer and is the leading cause of cancer mortality worldwide for both men and women. Most patients present with incurable disease and face a 5-year relative survival rate of 17% with current standard therapies. The treatment of lung cancer depends on the tumor type, clinical stage, and the patients’ overall health condition. Earlier effective treatment based on histology is much important for patient to extend their life expectancy. Because the advanced patients have lost the chance of surgery, it is difficult to get pathological samples from them, except body fluid, HYPERLINK “http://dict.baidu.com/s?wd=sputum”	”_blank” sputum, bronchial brushing cells and bronchoalveolar lavage fluid. EBUS-TBNA, a new way to obtain the samples of the lung cancer patients, is helpful for medical treatment. EBUS-TBNA is proved to be a safe, sensitive, and inexpensive diagnostic procedure.\(^1\,^2\) Clinical studies have shown that EBUS-TBNA has the sensitivity, specificity, and positive and negative predictive values of 84–100%, 100%, and 100% and 67–97%, respectively.\(^6\,^8\) The rate of diagnosis of thoracic disease is improved by applying

| Pathology Subtype                  | Cytology (Cell block) | Histology |
|-----------------------------------|-----------------------|-----------|
| Cancer                            |                       |           |
| Squamous cell carcinoma            | 19(12)                | 15        |
| Adenocarcinoma                     | 35(23)                | 27        |
| Small cell carcinoma               | 16(13)                | 15        |
| Adenosquamous carcinoma            | 3(0)                  | 0         |
| Non-small cell lung cancer         | 15(2)                 | 3         |
| Carcinoid                         | 2(0)                  | 1         |
| Large cell carcinoma               | 6(1)                  | 3         |
| Malignant lymphoma                 | 2(1)                  | 1         |
| Sarcomatoid carcinoma              | 2(0)                  | 0         |
| Other*                             | 5(3)                  | 3         |
| Benign                             |                       |           |
| Tuberculosis                       | 22(14)                | 18        |
| Sarcoildsis                        | 4(2)                  | 24        |
| Reactive hyperplasia of lymph node | 83(15)                | 20        |
| Other*                             | 26(5)                 | 22        |
| Total                              | 240(91)               | 159       |

*Other subtypes are not shown

Table 2: Correlation between cytologic outcome (original report) and the final histologic diagnosis of lung cancer \( (\chi^2=0.213, P=0.645) \)

| Cytology         | Positive | Negative |
|------------------|----------|----------|
| Histology        |          |          |
| Positive         | 69       | 4        |
| Negative         | 0        | 78       |

The sensitivity rate of ICC diagnosis was 88.24% in squamous cell carcinoma, 100.00% in adenocarcinoma, 96.00% in small cell carcinoma compared to the conventional cytology of 82.35% in squamous cell carcinoma, 81.48% in adenocarcinoma, 72.00% in small cell carcinoma, and the specificity rate of ICC diagnosis was 100.00% in squamous cell carcinoma, 100.00% in adenocarcinoma, 99.25% in small cell carcinoma compared to the cytology of 98.59% in squamous cell carcinoma, 98.51% in adenocarcinoma, 72.00% in small cell carcinoma. It suggested that ICC diagnosis had advantage in the diagnosis of adenocarcinoma.

| Pathology Subtype                  | Smear | Smear + Cell block | Histology |
|-----------------------------------|-------|--------------------|-----------|
| Squamous cell carcinoma            | 12    | 14*                | 15        |
| Adenocarcinoma                     | 22    | 26*                | 27        |
| Small cell lung carcinoma          | 12    | 13*                | 15        |
| Others*                            | 33    | 16                 | 12        |

*Group “Smear + Cell block” Compared to the group “Smear” \( P>0.05 \)

| Pathology Subtype                  | P63 | TTF-1 | CEA | SYN |
|-----------------------------------|-----|-------|-----|-----|
| Squamous cell carcinoma \( n=10 \) | 9   | 0     | 2   | 3   |
| Adenocarcinoma \( n=21 \)        | 2   | 18    | 1   | 5   |
| Small cell lung carcinoma \( n=22 \) | 3   | 20    | 20  | 18  |
EBUS-TBNA applied, while patients get little hurt during the diagnosis.\[10,11,12]\] It also reduces the cost of examination. Therefore, cytological diagnosis in thoracic disease is important for the detection and management to improve patients’ survival. However, the sensitivity of cytological examination for its diagnosis varies widely in literature. In this study, we have reviewed a large series of histological diagnostics combining EBUS-TBNA technology to provide an estimate of the sensitivity and specificity of the cytological diagnosis. We also examined whether this sensitivity and specificity could be improved.

Our results showed that cytological diagnosis provided a definite or suspected diagnosis of EBUS-TBNA in 63.32% of all the cases (240/379). The sensitivity and specificity of the squamous cell carcinoma, adenocarcinoma, and small cell carcinoma were high based on the characteristic of the cell. These data were not improved on revision of the cytological preparations, which were carried out by two experienced cytopathologists. The data was obtained either in the presence of a relevant clinical history with accompanying positive endobronchial biopsy or with a background knowledge of the reviewing.\[13-17]\]

The rest of the 139 cases had insufficient diagnostic value cells on the smears. If the lymph node phagocytic cell had been seen, we considered it was a valid puncture. Otherwise the smear was not proper. In this research, we only used the effective smears as research samples.

The sensitivity and specificity was improved in small cell carcinoma when the cell block was applied in the cytological diagnosis. The size of the small cell carcinoma is as large as that of the lymphocyte when the cell on the smear was swelling. It is hard to distinguish the tumor cells from the lymphocyte. The ICC would label the tumor cells of the small cell carcinoma, SYN and CD56 was showed in the cell plasma, while TTF-1 was stained in the nucleus.

The sensitivity of the squamous cell carcinoma was not high because the probability that squamous cell carcinoma metastasizes to lymph nodes was less than adenocarcinoma and small cell carcinoma. Because the transbronchial puncture would bring the bronchial epithelium onto the smear, we could not differ it from tumor cells when squamous metaplasia occurred and the CK5/6 and P63 were both expressed in the bronchial epithelium and squamous cell carcinoma.

The adenocarcinoma had the highest sensitivity and specificity. Owing to the adenoid structure, we could identify the adenocarcinoma easily. However, it would be more difficult to do this in case of the solid adenocarcinoma, which was without any glandular cavity structure. We should use cell block and ICC in the diagnosis of solid adenocarcinoma. Both the specificity and the sensitivity reached 100% when cell block and ICC were used in the diagnosis.

We found that 4 cases were identified as false negative. The primary reason was that no tumor cells were taken from the puncture; in other words, the puncture just got a sample of lymphocyte. Second, cytopathologists did not recognize the tumor cells on the smear. Therefore, the technique of the operating doctor and cytopathologist is very important and influences the results of the EBUS-TBNA.

Here, we reported that cytological diagnosis had a high sensitivity and specificity of thoracic disease detection based on sampling from EBUS-TBNA. The data would be more satisfactory if we make the cytology diagnosis according to the results of ICC.

**Conclusion**

In conclusion, combined with newly developed EBUS-TBNA, cytological diagnosis is sensitive. The value of cytological examination of EBUS-TBNA should be acknowledged as a simple, fast, and safe procedure that provides a reasonable degree of sensitivity and specificity. In case of the high skilled puncture doctors and cytopathologists, the EBUS-TBNA will replace the mediastinoscope as a gold standard.\[18\] It will give the patient a choice for diagnosis, which is painless and inexpensive.
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Conflicts of interest
There are no conflicts of interest.

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