


The Morphological Analysis of Cells in the Bronchoscopic Brushing and TBNA of Patients with Lung Adenocarcinoma

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Abstract

Biopsy, brushing, and transbronchial needle aspiration (TBNA) are the most common methods for diagnosis of lung adenocarcinoma and are taken during the same diagnostic bronchoscopic procedure. However, it is not clear what the morphological diagnostic criteria of cytology by brushing or TBNA are. A retrospective analysis was performed on 136 patients who underwent video bronchoscopy examination for diagnostic purposes. All the subjects were performed brushing or TBNA and confirmed as lung adenocarcinoma by biopsy or postoperative pathology. An additional 140 randomly selected patients with benign lung diseases were included in the study and used as a control group. The benign cells usually confused with adenocarcinoma cells were ciliated columnar cells, mucous columnar cells, ciliated cuboid cells, and reactive ciliated cells, respectively. The number of cases diagnosed as adenocarcinoma cells, carcinoma cells, suspicious cancer cells, and atypical proliferative cells by cytology was 101, 11, 20, and 4, respectively. The main basis for the interpretation of adenocarcinoma cells is the enlargement of individual nucleus, the arrangements of multistage papillary, and the general enlargement of nuclei, while the main clue for the interpretation of suspicious cancer cells and dysplasia cells comes from escape cells. The results suggested that the degree of nuclear enlargement, multiple papillary arrangement, and escape cells or escape trend cells are important clues for the interpretation of lung adenocarcinoma cells, while the atypical proliferative cells were similar to escape cells or escape trend cells, which were essentially benign cells beside the cancer.

Keywords

bronchial brushing, transbronchial needle aspiration, lung cancer, cytological diagnosis, adenocarcinoma cell

Introduction

Lung adenocarcinoma is one of the leading causes of cancer death and accounted for nearly 40% of all lung cancers¹. In recent years, the incidence of lung adenocarcinoma is on the rise in many countries². Accurate diagnoses and discrimination between lung adenocarcinoma and reactive bronchial epithelium for lung adenocarcinoma are of vital importance for optimal therapeutic decision-making and improved prediction of patients' response to treatment³. Forceps biopsy, bronchial brushing, and transbronchial needle aspiration (TBNA) are the most common methods for diagnosis of lung cancer and are taken during the same diagnostic bronchoscopic procedure. The biopsy is limited by the status of certain lesions, such as the lesions of the distal bronchus or severe surface ulceration, and in order to avoid the bleeding, the biopsies often obtains paracancerous tissues, resulting in false negative results. Brushing and TBNA are the less invasive diagnostic technique and suitable for all lesions, so it is

important for the complements forceps biopsy in the diagnostic work-up of lung cancer⁴. A combination of bronchial brushing and forceps biopsy is thought to be an essential step for the diagnosis of bronchoscopically visible lung cancer^{5,6},

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TBNA or E-bus may increase the diagnostic yield of lung adenocarcinoma^{7,8}.

Although most of the lung adenocarcinoma occurred in peripheral type, the appearance of ultrasound bronchoscope and E-bus brought new dawn for the acquisition of abundant lung adenocarcinoma cells. However, the cytological diagnostic criteria and the common cell arrangement types of adenocarcinoma cells as well as cell types easily confused with adenocarcinoma cells are not clear. The main purpose of this study was to evaluate both the accuracy of cytological diagnosis and the main cause of false negative for the diagnoses of lung adenocarcinoma by bronchoscopy. In particular, we aimed to evaluate the common arrangements types and heteromorphic characteristics of lung adenocarcinoma cells.

Materials and Methods

This study was a retrospective analysis of cytological diagnoses of lung adenocarcinoma patients confirmed by histology, and this study was approved by the Ethics Committee of the First Hospital of China Medical University and informed consent was obtained from each patient. The pathological diagnosis of both histology and cytology from 136 patients who attended the department of pathology at the First Hospital of China Medical University during the period August 2018–December 2019 were included in the study. The study group comprised 74 men and 62 women, ranging in age from 25 to 80 years. An additional 140 randomly selected patients with benign lung diseases were included in the study and used as a control group.

All bronchoscopies were performed by two experienced bronchoscopists using a standard video bronchoscope (1T260, Olympus, Tokyo, Japan), a flexible long biopsy forceps (FB21C-1, Olympus), and straight brushes (BC-202D-2010, Olympus). Both forceps biopsies and brushings were obtained from all subjects except some special lesions. TBNA method is used for some special lesions, such as hilar or mediastinal masses, lesions outside the bronchus, and enlarged lymph nodes. To obtain sufficient numbers of cells from the brushings, the brushings were performed before the forceps biopsies, and this point had been confirmed in our previous experiments⁹. The area of suspected malignancy was brushed 2 or 3 times; the smears were fixed in 95% alcohol and stained by Papanicolau's method. Next, the forceps biopsy specimens were taken for histological examination from the same area of brushing and stained with hematoxylin and eosin (H&E) and/or immunohistochemistry after being processed. TBNA samples were used for both histological wax and cytological smear. The histology and cytology specimens were processed separately in different laboratories, and the findings were reviewed and reported separately by 2 different pathologists at different times daily.

Based on the histological diagnoses of forceps biopsy, TBNA, and resection specimens and the cytological diagnoses of brushing and TBNA, the diagnosis results were

Table 1. The Benign Cell Characteristics Easily Confused with Adenocarcinoma Cells in the Bronchial Brush or Needle Aspiration Smears.

Cell types	Cell arrangement	Morphological characteristics
Ciliated columnar cells	Sheets	There are cilia at the top of cells and terminal bars at the base of cilia. In some cases, cilia and terminal bars may not be obvious, and they can only be found on one side of the cell mass by carefully adjusting the microhelix
Mucous columnar cells	Irregular	Mucous vacuoles can be seen in the middle of the cells, phagocytic granules can be found in the vacuoles, and cilia can often be found at the top of the cells
Ciliated cuboid cells	Mulberry	Cell size is small, cytoplasm is small, and crowded arrangement. The cilia and terminal bars are not obvious, they can only be found on one side of the cell mass by carefully adjusting the microhelix
Reactive ciliated cells	Adenoid	The nucleus is located at the basal part and enlarges obviously. The cytoplasm is abundant. The cilia and terminal bars can be seen at the top of the cell

recorded into histological and cytological groups, respectively. Statistical analysis was performed using SPSS 16.0 software package (SPSS, Inc. Chicago, IL, USA).

Results

First of all, we need to interpret the normal cell groups that are easy to be confused with adenocarcinoma cells. The key to interpret these normal cell groups is to find ciliated cells. Common ciliated cells include the following four types, and their morphological characteristics are shown in Table 1 (Fig. 1A–D).

In 136 cases of lung adenocarcinoma confirmed by histology, cytology was diagnosed as adenocarcinoma cells, cancer cells, suspicious cancer cells, and atypical proliferative cells, respectively. Among them, 101 cases were diagnosed as adenocarcinoma cells by cytology, accounting for 74.26% of the total cases. The cytological morphological basis of various interpretation results is shown in Table 2.

The common morphological characteristics of 136 cases of lung adenocarcinoma cells and their relationship with the background were reviewed. The results showed that the cytological diagnosis results were closely related to nuclear enlargement and cell arrangements. The main basis for the interpretation of adenocarcinoma cells is the enlargement of individual nucleus, the arrangements of multistage papillary, and the general enlargement of nuclei, while the main clue for the interpretation of suspicious cancer cells and dysplasia

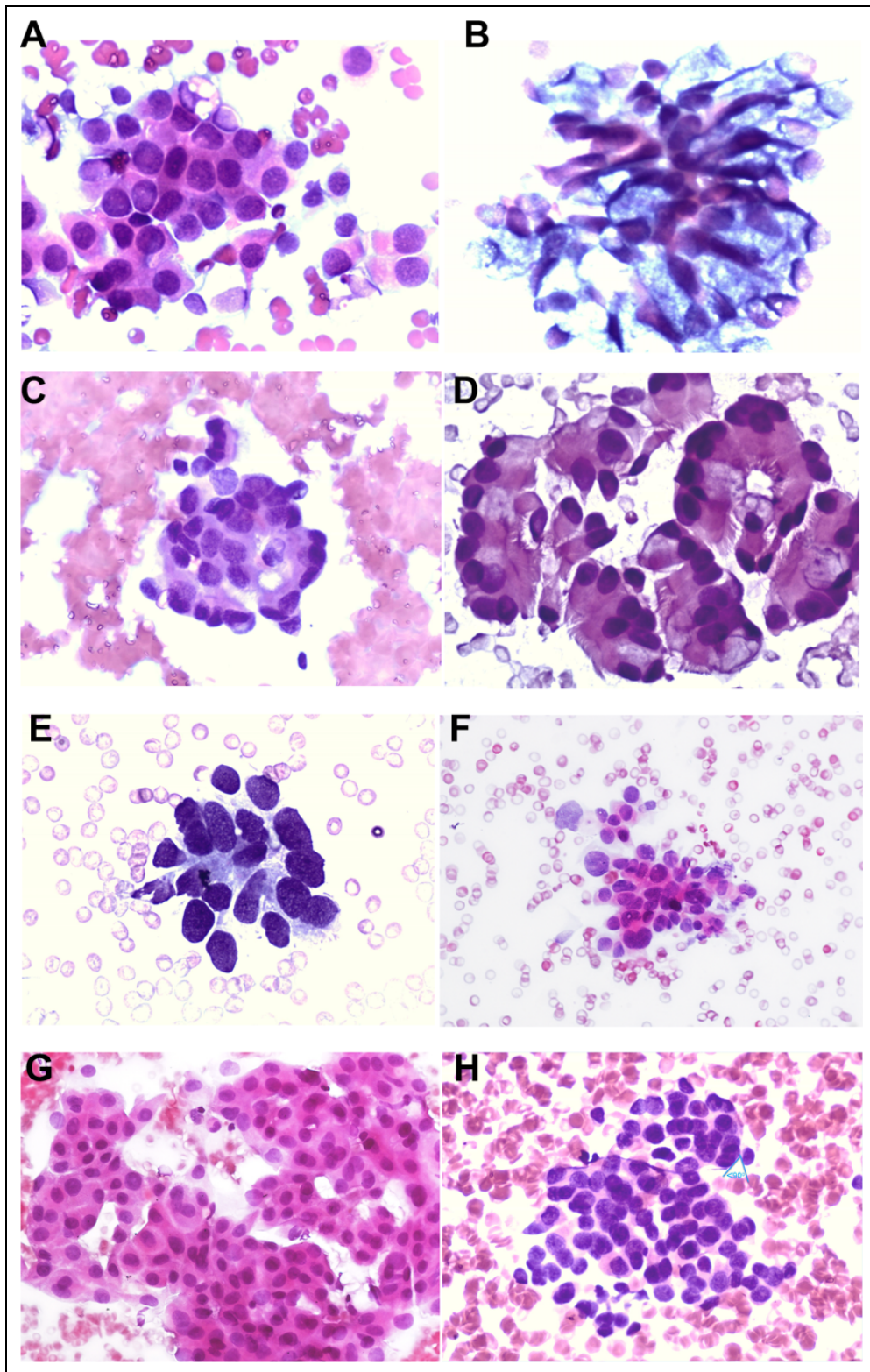


Figure 1. Morphological characteristics of ciliated cells and adenocarcinoma cells in both bronchial brushing and TBNA (Papanicolaou stain, $\times 400$). (A) On the left side of the picture, a cluster of ciliated columnar cells are seen from the top, but the cilia and endplate are not clear; on the right lower corner, three ciliated columnar cells are seen from the side, and the cilia and endplate are clear. (B) A cluster of mucinous columnar cells are arranged in an adenoid way, the nuclei are located in the basement, and a lot of mucinous vacuoles are in the cytoplasm. (C) A cluster of ciliated cuboid cells is arranged in a plum pattern. Cilia and terminal bars can be found in the cracks at the bottom through careful observation. (D) Several rows of reactive ciliated cells are arranged in palisade, the enlarged nuclei are located in the basal part, and (to be Continued.)

Table 2. Diagnosis Results of 136 Patients with Lung Adenocarcinoma by Bronchial Brushing and Needle Aspiration Cytology.

Cytological diagnosis	n	%	Morphological characteristics
Adenocarcinoma cells	101	74.26	The cancer cells showed atypical adenoid arrangement or multiple papillary pattern and three-dimensional sense was obvious. The cancer cells were significantly larger than the surrounding benign cells, or obviously different in size
Carcinoma cells	11	8.09	The cancer cells were only nest-like but three-dimensional sense of them was not obvious. It is difficult to distinguish adenocarcinoma cell from squamous cell carcinoma by morphological characteristics
Suspicious cancer cells	20	14.71	The cells were atypical adenoid or mulberry, papillary protrusion was not obvious, cell size was similar, but there was no cilia and terminal bars
Atypical proliferative cells	4	2.94	The cells showed obvious atypical, but the morphological characteristics tended to be benign. These cells might be benign cells around the cancer

cells comes from escape cells. The detailed results are shown in Table 3 (Fig. 1E–H).

Discussion

We had made a detailed discussion on the cytological characteristics and arrangements of small cell lung cancer¹⁰. However, accurate interpretation of the morphological characteristics and arrangements of lung adenocarcinoma cytology has not been reported. Ravaioli et al. believed that the best way to interpret lung adenocarcinoma cells was to combine nucleolus with medium/small cell mass¹¹, and it was also reported that accurate cell typing of nonsmall cell lung cancer (NSCLC) could be achieved by TBNA, but the specific morphological characteristics of NSCLC were not discussed in detail^{12–14}. In this study, we found that the premise of accurate interpretation of lung adenocarcinoma cytology is to first understand the morphological characteristics of

normal bronchial epithelial cells. The normal epithelial cells that are often confused with lung adenocarcinoma cells were ciliated columnar cells, mucinous columnar cells, ciliated cubic cells, and reactive ciliated cells, and both the cilia and terminal bars in these cells were often covered up due to the angle, which led to these cells being mistaken for adenocarcinoma cells. In order to avoid false positive results, it is necessary to carefully observe the surrounding or central fissures of these cell clusters. Once the cilia and terminal bars are found, it can be determined that these cells are normal cells. Even reactive ciliated cells, no matter how obvious the nuclear enlargement, are benign cells as long as cilia and terminal bars are found.

In 136 cases of lung adenocarcinoma in this study, 101 cases (74.26%) were diagnosed by cytology. In addition to the obvious malignant features of the cells in these lesions, the characteristics of the origin of the glandular epithelium are also obvious, such as the atypical arrangements of glandular cavities. The results suggest that the cytological diagnosis obtained by fibrobronchoscopy has an important clinical application value in the diagnosis of lung adenocarcinoma. The cytology of 11 cases showed only malignant features, lacking the characteristics of glandular epithelial origin, so they could only be diagnosed as cancer cells. Further classification needs to be confirmed by immunohistochemistry. In another 20 cases, the malignant features of the cytology were not very clear, just suspicious, so we only reported the suspicious cancer cells. These lesions need to further observe the arrangement and morphological characteristics of the cells, so as to find a reliable clue for the diagnosis of adenocarcinoma cells. In addition, there are 4 cases diagnosed as reactive cells, and they had some heteromorphism, but lack of malignant characteristics, so they might be benign cells around the cancer.

In order to provide an accurate cytological diagnosis for the clinic as much as possible, reviewing the cytological diagnosis results of 136 cases of lung adenocarcinoma in this study, we found that the nuclear characteristics and arrangements are very important for the interpretation of the diagnosis results. In the nuclear characteristics, we must observe the extent of nuclear enlargement, and choose the appropriate contrast cells. One comparison method was that if the majority of nuclei in the cell mass were more than three times larger than the lymphocytes or red blood cells in the background, this was probably the most reliable basis for the interpretation of adenocarcinoma cells. However, only 24 cases met this standard, accounting for 23.76% of the

Figure 1. (Continued). the cilia and terminal bars can be seen at the top of the cells. (E) A cluster of adenocarcinoma cells are arranged in an atypical adenoid cavity, the majority of nuclei in the cell cluster were more than three times larger than the red blood cells in the background. (F) A cluster of adenocarcinoma cells are arranged in an atypical adenoid cavity, two cells in the upper left corner of the cell cluster are more than three times larger than other cells. (G) Several clusters of adenocarcinoma cells are arranged in multistage papillary pattern, and the nucleus size is similar. (H) A cluster of adenocarcinoma cells is arranged in the shape of plum blossom petals, with a similar size of nucleus. One escape cell can be found in the upper left corner of the cell cluster, and one escape trend cell can be found in the upper right corner. The angle between the escape trend cell and the cell cluster is less than 90°. TBNA: transbronchial needle aspiration.

Table 3. The Morphological Basis of Cytological Diagnosis for Bronchial Brushing and TBNA of Patients with Lung Adenocarcinoma.

Group	n	Diagnostic		Basic (%)	
		All cells+	Individual cell+	Multistage papillary	Escape cells
Adenocarcinoma cells	101	24 (23.76)	43 (42.57)	26 (25.74)	8 (7.92)
Carcinoma cells	11	4 (36.36)	2 (18.18)	2 (18.18)	3 (27.27)
Suspicious cancer cells	20	0	0	2 (10.00)	18 (90.00)
Atypical proliferative cells	4	0	0	0	4 (100)

TBNA: transbronchial needle aspiration; All cells+: all cell nuclei are enlarged; Individual cell+: individual cell nucleus is enlarged.

adenocarcinoma cell group. The other method was the cells in the cell mass were compared with each other, if a few nuclei in the cell mass were more than 3 times larger than other nuclei, this was probably another reliable standard for judging adenocarcinoma cells. In this study, the number of cases meeting this standard was the most, 43 cases, accounting for 42.57% of the adenocarcinoma cell group. This result suggests that it may be the most helpful for us to focus on the intercomparison within cell clusters in the interpretation of adenocarcinoma cells.

The arrangements of cells can be divided into multistage papillary arrangements of most cells and escape state of a few cells from the original cell cluster. The arrangements of cells were the problem that were easy to be ignored by cytologists and rarely reported in the literature. Most cytologists thought that cytological diagnosis was only based on the morphological features of cells. However, the results of this study showed that 25.74% of adenocarcinoma cells were interpreted according to multistage papillary arrangements. The results suggest that the multiple papillary arrangements have an irreplaceable role in the interpretation of adenocarcinoma cells. In this study, 11 cases were diagnosed as cancer cells, and the cytology only showed malignant manifestations, but the characteristics of glandular epithelial origin were lacking. The number of cases in this group was small, which needs to be further explored in the future.

In the group of suspected cancer cells, the size of cancer cells was similar, arranged in plum or papillary pattern, and the only clue to the adenocarcinoma cell was the discovery of escape cells. The arrangements of these cells were characterized by complete separation from the original cell cluster. In the 4 cases of atypical proliferative cell group, no escape cells were found, only marginal cells protruded outwards, and the angle between them and cell cluster was sharp, showing escape trend, so they were called escape trend cells. Reviewing the benign lesions in the control group, we found that the marginal cells in the benign lesions also showed escape trend, but these cells did not break away from the original cell clusters and the angle between them and cell cluster was obtuse, therefore, such cells can neither be called escape cells nor escape trend cells. Through the comparative analysis of benign and malignant cell clusters, we have had a deeper understanding of the concept of both escape cells and escape trend cells, which is of great clinical

significance for the future diagnosis of adenocarcinoma cells and the reduction of the proportion of false negative caused by suspicious cancer cells or atypical proliferative cells.

Conclusion

The degree of nuclear enlargement and the arrangements of cells are very important for the cytological interpretation of both bronchial brushing and TBNA of lung adenocarcinoma. Nuclear enlargement more than three times is often a malignant feature, multiple papillary arrangements is another manifestation of lung adenocarcinoma cells, and the interpretation of escape cells and escape trend cells is an important clue for the diagnosis of adenocarcinoma cells. Therefore, it is of great significance for clinical cytological diagnosis to accurately identify the cilia and endplates of benign cells as well as the nuclear enlargement, arrangement model, and escape status of adenocarcinoma cells.

Ethical Approval

Ethical approval was obtained for the experimental procedures by the Ethics Committee of the First Hospital of China Medical University, Shenyang, China.

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the First Hospital of China Medical University's (APPROVAL NUMBER/2016-125) approved protocols. This article does not contain any studies with animals.

Statement of Informed Consent

Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.


Declaration of Conflicting Interests

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References

1. YF ZU, Wang XC, Chen Y, Wang JY, Liu X, Li X, li CW, Xie YC, Luo Y, Shang XQ, Guo V. Thyroid transcription factor 1 represses the expression of ki-67 and induces apoptosis in non-small cell lung cancer. *Oncol Rep.* 2012;28(5):1544–1550.
2. Oktay E, Oflazoglu U, Varol Y, Tanriverdi O, Mermur N, Arda HU, Demir L, Keskin O, Ahmadli T, Somali I, Oztop I, et al. The prognostic role of thyroid transcription factor-1 in lung adenocarcinoma. *J Cancer Res Ther.* 2018;14(Supplement): S1201–S1208.
3. Sackett MK, Salomão DR, Donovan JL, Yi ES, Aubry MC. Diagnostic concordance of histologic lung cancer type between bronchial biopsy and cytology specimens taken during the same bronchoscopic procedure. *Arch Pathol Lab Med.* 2010; 134(10):1504–1512.
4. Li WN, Wang DF, Zhao YB, Qiu XS, Wang EH, Wu GP. Comparative analysis for diagnostic yield of small cell lung cancer by cytology and histology during the same Bronchoscopic procedure. *Clin Lung Cancer.* 2017;18(5):e357–e361.
5. Mak VH, Johnston ID, Hetzel MR, Grubb C. Value of washings and brushings at fiberoptic bronchoscopy in the diagnosis of lung cancer. *Thorax.* 1990;45(5):373–376.
6. Matsuda M, Horai T, Nakamura S, Nishio H, Sakuma T, Ikegami H, Tateishi R. Bronchial brushing and bronchial biopsy: comparison of diagnostic accuracy and cell typing reliability in lung cancer. *Thorax.* 1986;41(6):475–478.
7. Zhang Y, Xie F, Mao X, Zheng X, Li Y, Zhu L, Sun J. Determining factors of endobronchial ultrasound-guided transbronchial needle aspiration specimens for lung cancer subtyping and molecular testing. *Endosc Ultrasound.* 2019; 8(6):404–411.
8. Liu A, Qian L, Zhong Y, Lu X, Zhao Y. Endobronchial ultrasound guided transbronchial needle aspiration combining with immunohistochemistry and genotype in lung cancer: a single-center, 55 cases retrospective study. *Ann Med Surg (Lond).* 2017;23:1–7.
9. Hou G, Miao Y, Hu XJ, Wang W, Wang QY, Wu GP, Wang EH, Kang J. The optimal sequence for bronchial brushing and forceps biopsy in lung cancer diagnosis: a random control study. *J Thorac Dis.* 2016;8(3):520–526.
10. Li WN, Wang DF, Zhao YB, Qiu XS, Wang EH, Wu GP. Comparative analysis for diagnostic yield of small cell lung cancer by cytology and histology during the same bronchoscopic procedure. *Clin Lung Cancer.* 2017;18(5):e357–e361.
11. Ravaioli S, Bravaccini S, Tumedei MM, Pironi F, Candoli P, Puccetti M. Easily detectable cytomorphological features to evaluate during ROSE for rapid lung cancer diagnosis: from cytology to histology. *Oncotarget.* 2017; 8(7): 11199–11205.
12. Nizzoli R, Tiseo M, Gelsomino F, Bartolotti M, Majori M, Ferrari L, De Filippo M, Rindi G, Silini EM, Guazzi A, Ardizoni A. Accuracy of fine needle aspiration cytology in the pathological typing of non-small cell lung cancer. *J Thorac Oncol.* 2011;6(3):489–493.
13. Wallace WA, Rassi DM. Accuracy of cell typing in nonsmall cell lung cancer by EBUS/EUS-FNA cytological samples. *Eur Respir J.* 2011;38(4):911–917.
14. Dong Z, Li H, Jiang H, Wu C. Evaluation of cytology in lung cancer diagnosis based on EBUS-TBNA. *J Cytol.* 2017;34(2): 73–77.