

The Clinical Value of Detecting the Level of Exfoliated Cells in Pleural Effusion by Flow Cytometry in the Differential Diagnosis of Non-Small Cell Lung Cancer and Benign Lung Diseases

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Abstract: *Objective:* To explore the value of flow cytometry (FCM) in detecting the level of exfoliated cells in pleural effusion in the differential diagnosis of non-small cell lung cancer and benign lung diseases. *Methods:* Clinical data of patients with non-small cell lung cancer who were hospitalized in Hebei hospital from June 2019 to March 2022 were collected. A total of 98 patients were included, and 63 patients with alveolar lung disease were screened during the same period, and the two groups of patients were analyzed. *Results:* Compared with alveolar lung disease group, FCM detection and analysis showed that the level of exfoliated cells in the pleural effusion of non-small cell lung cancer (NSCLC) patients was 99 (3-969)/100,000, and patients with alveolar lung disease was 4 (0 ~ 19)/100,000. Additionally, compared with the alveolar lung disease group, the level of exfoliated cells in the pleural effusion of patients with non-small cell lung cancer (NSCLC) was significantly increased ($P < 0.001$). The diagnostic efficacy of FCM for detecting pleural fluid exfoliated cells in non-small cell lung cancer was assessed using ROC curves and using 95% CI (-11.1, -13.2) with a sensitivity of 0.75 and specificity of 0.94, and the diagnostic efficacy of FCM for detecting pleural fluid exfoliated cells in alveolar lung disease was assessed using 95% CI (-11.1, -13.2) with a sensitivity of 0.71 and specificity of 0.87. *Conclusion:* Flow cytometry has a wider range of clinical applications, simple operation, low cost, and high sensitivity, which makes it of great significance in disease diagnosis.

Keywords: Flow cytometry detection; Non-small cell lung cancer; Benign lung disease

Online publication: May 31, 2023

1. Introduction

Lung cancer is a disease with highest morbidity and mortality rates among malignant tumors in the world. Because lung cancer has no obvious symptoms in the early stage, it is easy to be misdiagnosed as pneumonia or other lung diseases. Therefore, early and accurate diagnosis has a great impact on the prognosis of patients. As a routine inspection item for lung cancer patients, the examination of pleural effusion exfoliated cells is great significance. The detection of pleural effusion exfoliated cells is an important diagnostic index in the diagnosis and treatment of lung cancer, especially in patients with non-small cell lung cancer (NSCLC). When the cells are negative for tumor markers, their diagnostic value is greater. Although the level of pleural fluid exfoliated cells is low in lung cancer, it may become continuously elevated as the tumor progresses. As one of the main differentiating markers between malignant and non-malignant tumors, it is important for postoperative efficacy assessment, recurrence monitoring, and prognosis judgment of NSCLC patients. However, currently the most commonly used clinical detection methods are pleural

effusion cytology examination, chest CT examination, etc., but they have certain limitations. In recent years, flow cytometry (FCM) has become a research hotspot in disease diagnosis. It has the advantages of high cell count, rapidity and high sensitivity, and has good application value. In lung cancer, FCM can show the cellular differences between NSCLC and alveolar lung disease (Pulmonary bullous disease), but the diagnostic value is still unclear for the identification of NSCLC and alveolar lung. This study retrospectively analyzed the level of exfoliated cells in pleural effusion of patients with NSCLC, explored its value in the differential diagnosis of NSCLC and benign lung diseases, and provide new information for clinicians in the diagnosis and treatment of lung cancer and benign lung diseases. The ideas and methods have a certain guiding significance.

2. Materials and methods

2.1. Study subjects

Clinical data of patients with NSCLC who were hospitalized in Hebei hospital from June 2019 to March 2022 were collected. The age range of the patients was 35-80 years old, and the median age was 59 years old. A total of 98 patients were included, including 63 males and 35 females, and all cases were diagnosed as NSCLC by histopathology. A total of 63 patients with concurrent alveolar lung disease were screened, including 39 males and 24 females.

2.2. Methods

Immunohistochemical staining was used to detect exfoliated cells in pleural effusion in lung cancer tissues. FCM reagent was provided by Sigma Company in the United States. The concentration of the antibody was fixed at 1:100, washed with 10% neutral buffer, and the target cells was detected by FCM. The optical density of tumor cells was detected using DAPI chromogenic kit.

2.3. Statistical method

All the data in this study were processed by SPSS 22.0 statistical software. The measurement data were expressed as mean \pm standard deviation ($\bar{x} \pm s$), expressed by t test, and the adoption rate (%) of enumeration data was expressed by chi-square (χ^2) test.

3. Results

3.1. Analysis of FCM test results

FCM detection and analysis showed that compared with the alveolar lung disease group, the level of exfoliated cells in the pleural fluid of patients with NSCLC was 99(3-969)/100,000, while the level of exfoliated cells in the pleural fluid of the alveolar lung disease group is 4(0-19)/100,000. Compared with the alveolar lung disease group, the level of exfoliated cells in the pleural effusion of patients with NSCLC was significantly increased. The independent sample t-test was used to compare the level of exfoliated cells in the pleural fluid of NSCLC and alveolar lung disease, $P < 0.001$, as shown in **Table 1**.

3.2. Efficiency of FCM in detecting exfoliated cells in pleural effusion

The diagnostic efficacy of FCM for detecting pleural fluid exfoliated cells in NSCLC was assessed using ROC curves and using 95% CI (-11.1, -13.2) with a sensitivity of 0.75 and specificity of 0.94, and the diagnostic efficacy of FCM for detecting pleural fluid exfoliated cells in alveolar lung disease was assessed using 95% CI (-11.1, -13.2) with a sensitivity of 0.71 and specificity of 0.87 (**Table 1**).

Table 1. Efficiency of FCM in detecting exfoliated cells in pleural effusion

Group	Sensitivity	Specificity	95% CI
NSCLC	0.75	0.94	(-11.1, -13.2)
Alveolar lung disease	0.71	0.87	(-11.1, -13.2)

4. Discussion

Lung cancer is a disease with highest incidence rate among malignant tumors. About 400,000 people die due to lung cancer every year world-wide, and more than 500,000 people are diagnosed with lung cancer every year. In China, there are about 300,000 new cases of lung cancer reported every year, and it has the highest incident rate of lung cancer world-wide. At present, NSCLC accounts for about 50% of all malignant tumors, and has high degree of malignancy with poor prognosis. Due to the lack of specificity in clinical manifestations and the high invasiveness of NSCLC, it is easy to misdiagnose this disease, therefore early diagnosis is the key to improve the efficacy and prognosis. Exfoliated cytology of pleural effusion is currently a commonly used diagnostic method in clinical practice, but this method is easily affected by other diseases such as pleura, pleural effusion, and other factors. Therefore, it is necessary to improve the diagnostic rate clinically by combining clinical and other examinations methods [1-6]. Single or smaller alveoli will show no signs, chest tightness, shortness of breath, and dyspnea mainly in larger volumes or multiple large pulmonary alveoli. If there is shortness of breath, cough, dyspnea, cyanosis, movement of the trachea and mediastinum, and sensation of tinnitus, it should be a spontaneous pneumothorax. Lung holes of different sizes can be seen under X-ray, and transparent holes of different degrees can be seen, but it is different with lung sac and spontaneous pneumothorax. CT scan is a significance method for the identification of these two. In addition, in an epidemiological study of lung cancer, 0.19% of men with emphysema did not develop overly large emphysema, while 6.1% of patients had large air bubbles.

Pleural effusion exfoliated cells are one of the common cell components in lung cancer, and their content in lung cancer is very low, about $2\sim 5\times 10^9$ /mL. Although a large number of studies have shown that exfoliated cells in pleural effusion can reflect changes in lung tissue cells, their differential diagnosis value for lung cancer and benign lung diseases is still unclear. The sensitivity of pleural effusion exfoliated cells in the diagnosis of lung cancer is 75% to 90%, and the specificity is 98% to 100%, but the sensitivity and specificity are both low. Therefore, it is essential to improve the detection value of exfoliated cells in pleural effusion to improve the detection rate of lung cancer. FCM is currently the most widely used cytological detection technique, which can simultaneously display the characteristics and status of various malignant tumor cells [7-9]. Pulmonary cyst is a common benign lesion, which mostly occurs in infants and adolescents, and with the highest incidence in children. Cystic lesions are composed of different fluids and commonly found in parabronchial cysts, followed by endobronchial cysts. Cystic lesions can be divided into various types according to the liquid composition in the cyst, such as hydration cystic cyst, fat cystic cyst, etc. However, these types of diagnosis depend on the fluid composition in the cyst, such as cells, lipid particles, and various tissue fragments in the cyst fluid, therefore it is difficult for diagnosis. The results of detecting exfoliated cells in pleural effusion by FCM showed that FCM has high sensitivity and specificity in detecting cystic lesions, and the detected patients were consistent with the pathological results. Therefore, we applied FCM to detect the level of exfoliated cells in the patient's pleural fluid, and by combining the clinical and pathological findings, it can provide an accurate diagnosis of pulmonary cysts. It plays an important role in the diagnosis of lung cysts.

Pleural effusion exfoliated cells are the most important cell components in lung cancer, which can reflect the postoperative curative effect of patients with NSCLC, especially play an important role in the judgment of pulmonary metastases. The results of this study showed that the level of exfoliated cells in

pleural effusion in the NSCLC group was significantly higher than in the alveolar lung disease group ($P < 0.05$), and the level of exfoliated cells in the pleural effusion in the NSCLC group was higher than in the alveolar lung disease group ($P < 0.05$). With the progression of the tumor, the exfoliated cells in the pleural effusion may continue to increase, suggesting that it has a certain value in the diagnosis and monitoring of NSCLC. The level of exfoliated cells in pleural effusion after lung cancer treatment can be used as one of the variables for clinical evaluation to determine the treatment effect, and it is essential for the selection of postoperative adjuvant therapy, prognosis judgment, and recurrence monitoring of lung cancer. This study showed that in the NSCLC group, the level of exfoliated cells in pleural effusion had no significant correlation with tumor stage, lymph node metastasis, TNM stage, and degree of differentiation; while in the alveolar lung disease group, the level of exfoliated cells in pleural effusion had no significant correlation with tumor stage and TNM stage ($P > 0.05$).

In patients with NSCLC, the detection rate of pleural effusion exfoliated cells is low, and the positive rate is differed in different pathological types of lung cancer. At present, the detection of pleural effusion exfoliated cells is mainly based on the pleural effusion exfoliated cell detection card to determine whether the patient has pleural effusion. Although this examination can provide a certain reference value for clinical practice, it cannot be used as the only parameter for the diagnosis of NSCLC because it cannot fully reflect the cause, type, and source of pleural effusion. In addition, the detection of exfoliated cells in pleural effusion has certain limitations on the source of pleural effusion. If only relying on FCM to determine the source of pleural effusion exfoliated cells in patients with NSCLC, there will be a certain degree of missed diagnosis, so further research is needed to determine its accuracy in diagnosis [10-15].

FCM plays an important role in the clinical diagnosis of lung cancer, postoperative curative effect evaluation and prognosis judgment, but because it needs to use the advantages of optical microscopy, it also has certain limitations, therefore FCM has its limitations in clinical application, such as not directly reflecting the actual level and distribution of cells, having certain false positives or false negatives, having large differences in different testing sites for the same patient, only showing the distribution of cells, but not directly reflecting their proliferation and apoptosis status, etc. In addition, there are some limitations in the study: i. Different tumor pathological types and stages have different effects on the level of exfoliated cells in patients' pleural effusion; ii. Currently, there are false positive or false negative phenomena in the detection of pleural effusion exfoliated cells, which may be related to the staining protocol during the detection; iii. Different analytical methods used by different laboratories may lead to discrepancies in the results; and iv. Could not obtain enough sample from some patients, so the study should be conducted after expanding the sample size.

In conclusion, the advantages of wide range of clinical applications, ease of operation, low cost, and high sensitivity of FMC make it as an important tool in disease diagnosis.

Disclosure statement

The authors declare no conflict of interest.

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