Correlation Analysis of Clinical Pathological Characteristics of Circulating Tumor Cells and Lung Cancer

Xu Chuanbin, Bai Ling, Huang Zedi, Hu Anmei, Qiu Jinhuang, Zeng Jun, Lai Rensheng

Bao'An Central Hospital of Shenzhe, Shenzhen City, Guangdong Province, 518100, China

Keywords: Tuberculosis, Low dose ct, Circulating tumor cells, Microporous filtration membrane, He staining, Pathological cytology diagnosis

Abstract: Objective To investigate the correlation of circulating tumor cells and clinical pathological pathology of lung cancer. Methods The institutes were selected from March 2019 (CTC actual test), and 26 patients with suspected malignant signs were selected. 5 mL of venous blood was taken, and the chip was slit with a CTC biopsy-A10 cycle tumor cell (CTC). After some slices, the filter was stained with Sumu-Ird (HE). Psychologists observe CTC and cytology (CTM) under mirror and issued a double blind cytology. Results 1, a 42-year-old male, the right lung outer substrate segment (SE4, IM125-131) showed about 6 mm size grinding glass nodule × 6 mm, no evilness, and the size of the left base before the left lung The border of 3mm $\times 2$ mm is clear. Abnormal CTC cells were visible under the light microscope, but did not see CTM. Three cases of malignant CTC cells report. The results of immunohistochemistry show CK7 (++), TTF1 (++), NAPSIN A (++). Case 2,61 years old women repeated cough more than 3 months × 22 mm, malignant is not excluded. CT value 25 Hu, CT enhancement 80 Hu. He dyeing on the peripheral blood CTC separator filter. Eroscope was found to be abnormal in 17 cases of CTC cells and did not see CTM. Celloscopic diagnosis under mirror is consistent with squamous carcinoma. Pathological diagnosis is squamous cell carcinoma. The results of immunohistochemistry showed P63 (+), CK5 / 6 (+), P40 (+). Conclusion CT imaging examination is combined with LCHC. It is the focus on changing Fish staining for He dyeing, light microscopic patient diagnosis, and a key discipline of CTC diagnosis of CTC, changing the fuzzy report of the biological industry, and cancel fluorescent microscope reagents. In 2 cases of the above discussion, 26 CTCs were detected together. Among them, 19 cases of malignity were suspected, 16 positive were positive (84.2%). This can be obtained, a conventional cytology method CTC dyeing tuberculosis has a good application prospect.

1. Introduction

Lung cancer is one of the most serious diseases that threaten human health. The incidence increased year by year, and the death rate ranked first in all malignant tumors. The distant transfer is the main reason for death. The emergence of circulating tumor cells (CTC) is the prerequisite for the distant transfer of tumors and is the key to forming metastasis. Studies have shown that CTC can not only effectively determine the prognosis of patients with lung cancer, but also to predict and monitor the effects of adjuvant therapy. This study was designed to CT primary sieve tuberculosis, screening with malignant tendencies, or non-eliminating patients, testing CTC, surgical biopsy, verifying the diagnosis value of CTC.

2. Information and Method

2.1 General Information

All patients in the observation group were informed, including standards: Observing group suspected of malignant tuberculosis> 0.6mm less than 2.0mm, (1) has pathological diagnosis before and after detection, initial diagnosis (2) All patient medical records and image information is complete, not accepted before treatment Allowation or biological immunotherapy (3) Emotional

stability, can be combined with doctors to complete related inspections; exclusion criteria: (1) mental abnormalities, abnormal coagulation functions or cognitive function; (2) Diagnosis of malignant tumors, itself Immunological diseases or infectious diseases. The other 2 cases of non-malignant nodule are a negative control group. From March to 2021, 2019, 19 patients with pneumatic and suspected malignant sickness were low-dose CT examination. Among them, 2 cases of typical cases were discussed in detail. Case 1, male, 42 years old, right lung outfit segment (SE4, IM125-131) has a grinding glass nodule, a size of about $6 \text{ mm} \times 6 \text{ mm}$, malignant sickness is not excluded; case 2, female, 61 years old, repeated cough More than 3 months, the lower right lung door junction 27 mm × 22 mm, and the malignant tumor is not excluded by imaging.

2.2 Method

2.2.1 Ctc Cell Separation

According to the size difference of tumor epithelial cells and non-tumor cells, 19 cases were isolated from CTCBiopsy-A10 circulating tumor cell separators (1) specimen collection. The blood of 5 ml of empty abdomen was collected on the second day of the morning, and EDTA was anticoagulated. In order to ensure the quality of the specimen, 1 ml of venous blood was collected with 2 mL of anti-coagulation pipe, and the test product was collected by the formal specimen, and 5 ml was collected with a BD-EDTA anti-coagulation pipe. Pretreatment is carried out 2 hours after storage at room temperature. If it is more than 2 hours, it should be placed in a refrigerator ° C in 4 o'clock and should be used within 8 hours. Samples were stored at 4 ° C for 30 minutes (2) sample pretreatment: 1,375 µ 15 ml of centrifuge tube was added to 3 mL to add saline. The blood sample was 5 ml, and 15 ml of centrifuge tube (add 8 ml). Gamble salt water to 15 ml, inverted 8 times, stand for 10 min, predetermined cell (3) sample separation: Put the pre-solid sample into the membrane filter tube, the separation process is semi-automated (4) rinsing: plus 4ml PBS washed filtration, repeating twice (5) methanol fixation: add 1 min after adding 500 to the filter film µ, emptying the methanol (6) from the filter, lower the lower portion of the slide, will The membrane face is posted on the bottom of the slide, as far as possible, naturally dry 5 min (7) transfer film: add a drop of methanol in the middle of the slide, sandwich the dried filter, and move it with methanol to load The middle of the slide, try to be confined, naturally for 5 minutes.

2.2.2 Cell Hf Dyeing

The filtration film oven was dried at 50 ° C for 20-30 minutes; add 1 min 30s, washing with water, washing with water, 80% ethanol in addition to the water, drip iris 20-30S, 80% ethanol removal, 95% ethanol 30S, 100% ethanol 30S, dikylene chlorobenzene 40S-1 min, xylene 30s, 1 minute of transparent agent, 1 drip neutral inclusion, air dry. Observation under the light microscope, which is diagnosed by experienced pathologists.

2.2.3 Diagnostic Indicator

CTC: a single cycle tumor cell; CTM: is a group of 3 or more circulating tumor cells. It can be identified as circulating tumor cells, or cells that cannot be accurately identified by mutual overlap without accurately identifying non-blood source cells having nuclear differential characteristics, and there is no petal cluster around the filter hole. CTC Screening Positive Standard Refer to the International Conflicted Rarmeter HE staining form and volume of 2 to 3 times the normal blood cells. The morphology and quantity of each vision CTC cell cell in the group met a normal distribution. The above CTC is positive in the filter membrane, but is negative in the screening. The pathological diagnosis of clinical cells requires comprehensive reference clinical examination and imaging diagnosis. Comprehensive evaluation of doctors with tumor tissue cytology

3. Result

3.1 Pulmonary Tuning Cycle Tumor Cell Clinical Pathological Cytology Case 1

Case 1, male, 42 years old, right lung outsolating section (SE4, IM125-131) has a grinding

glass-like nodule, a size of about $6mm \times 6mm$, does not exclude malignant signs; in addition, the size of the left lower base base is $3mm \times 2mm$ border is clear. B ultrasound inspection of liver, gallbladder, spleen, and pancreas have no abnormalities. The CTC separator peripheral blood film production examination is seen in CTC, and the nuclear depth enters a large exception. No CTM was found, 3 cases of malignant cells were reported from CTC. The pathology is diagnosed as "micro-invasive gland cancer". The immunohistochemical results are CK7 (++), TTF1 (++), NAPSIN A (++).

3.2 Pulmonary Tuning Cycle Cycle Cell Clinical Pathological Cytology Case 2

Case 2,61 years old, repeated cough for more than 3 months, CT cleaning the right under the right under the upper right lung door, size $27\text{mm} \times 22\text{mm}$, does not exclude malignancy. The CT value is 25 Hu and the enhanced CT value is 80 Hu. B ultrasound: liver, gallbladder, spleen, pancreas, normal genital system. The peripheral blood CTC separator filter film found 17 cases of CTC, nuclear staining, a small amount of red dyeing and fouling, and did not see CTM. The diagnosis of CTC is consistent with squamous cell carcinoma. The pathological diagnosis of squamous cell carcinoma under fiber bronchoscopy is p63 (+), CK5 / 6 (+), P40 (+).

4. Discussion

According to NCCN Guide, 2016 Asian Consensus Guide and 2018 Chinese Tongjun Section Treatment Expert Consensus, Observation and Followup is the main method of low risk nullings <10mm, but at the same time increase the psychological pressure of patients, improved anxiety scores, affecting The quality of life of patients. Studies have shown that: tumor cell CTC leaves solid tumor into peripheral blood is the growth stage of early lung cancer circulation systems, which will lead to risk of occult micro-transfer and distant metastasis. This study analyzed two typical cases. Case 1 CT scan found that the right lung outer base segment (SE4, IM125-131) has a grinding glass-like nodule, a size of about 6 mm × size of 6 mm and 3 mm × CT reported 2 mm boundary clear, and does not exclude hyperplasia nodules; The pathological diagnosis of minimally invasive surgery is "minimally invasive agriculture cancer". Case 1 showed 0.6 cm nodules very small, but also reported to CTC cells in the blood. Combined with postoperative tissue morphology, immunohistochemistry and HE staining were diagnosed as adenocarcinoma. Therefore, the pathological CTC screening positive report can enhance CT imaging performance, do not exclude malignant tendency diagnosis, improve diagnosis and positive rate, improve patient early surgical compliance, help recommend "8mm small nodules CTC test, improve guide Update, proved that the pathological cytology screening report is of great significance for the diagnosis of the elimination of false positives and supporting negative CT.

Acknowledgment

Project Level: district level Source: Baoan District Medical and Health basic Research Project Subject number: 2009JD403

Topic: Application of Multidisciplinary Clinical Genome Technology in Liquid Biopsies and Pathology of Pulmonary Nodules Diagnosed by Image

References

[1] Sun Jian. Correlation between peripheral blood circulating tumor cell expression and lyric protein 19 fragment and fibrinogen in patients with lung cancer [J] .Chinese Health Engineering, 2020,19 (01): 88-89.

[2] Gong Jia, Xu Feng, Zhou Yimei, Wu Yufang, Xie Pingfang. Correlation between clinical pathological characteristics of circulating tumor cells and early breast cancer [J] .Journal of Central South University (Medical Sciences), 2019,44 (09): 1016-1022.