Postoperative Mesenchymal Circulating Tumor Cell Detection Monitoring of Minimal Residual Disease in Colorectal Cancer

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Abstract: Objective To explore the clinical diagnostic value of mesenchymal circulating tumor cell (MCTC) detection to assist the minimal residual disease (MRD) monitoring and prognosis assessment of patients with colorectal cancer. Methods The peripheral blood test samples of 147 patients with colorectal cancer who underwent surgical treatment from January 2018 to December 2019 were collected. CytoSorter® microfluidic nanochip method combined with MCTC-specific antibody enrichment detection reagents were used to analyze the auxiliary MCTC detection after surgery. The feasibility of evaluating the prognosis of colorectal cancer. Results 147 cases of colorectal cancer peripheral blood MCTC detection results showed that the detection rate of MCTC was 72.8% (107/147). The postoperative MCTC level has no significant correlation with the patient's gender (P=0.3177), age (P=0.4983), primary tumor location (P=0.0715), and tumor invasion depth (P=0.4174), but it has a significant correlation with the patient's lymph node metastasis (P<0.0001) and clinicopathological stage (P<0.0001). There was a significant difference in the number of MCTCs between colorectal cancer patients with distant metastasis and colorectal cancer without distant metastasis (P<0.0001). Patients with a higher number of MCTCs had a higher risk of distant metastasis after surgery. The postoperative MCTC level can evaluate the progression-free survival and overall survival of patients with colorectal cancer. The progression-free survival (P<0.0001) and overall survival (P <0.0001) of patients with MCTC≥ 3/4mL are significantly shorter than MCTC 3/4mL Of colorectal cancer patients. Conclusion The detection of mesenchymal CTC in patients with colorectal cancer can assist in prognostic evaluation, and the presence of MCTC may indicate the higher risk of tumor metastasis.

1. Introduction

Colorectal cancer is a common malignant tumor of digestive system. China's latest statistics show that colorectal cancer incidence rate ranks fourth in malignant tumors, and mortality rate ranks fifth in [1]. Although the survival rate of colorectal cancer has improved in recent years, about 50% of patients still have recurrence or distant metastasis [2]. Studies have shown that circulating tumor cells (CTC) can assist in the monitoring of minimal residual disease (MRD) [3-4]. And CTC detection can be used as an independent prediction tool for colorectal cancer prognosis [5]. CTC detection is mostly based on epithelial phenotype CTC or total CTC. There are few reports on interstitial phenotype CTC specific enrichment detection to assist colorectal cancer prognosis evaluation and metastasis monitoring. In this study, cell surface vimentin (CSV) interstitial CTC was used for mctc specific enrichment detection of colorectal cancer, and the relationship between postoperative mctc and colorectal cancer prognosis was analyzed to explore the application value of CSV based mctc detection in postoperative MRD monitoring of colorectal cancer.

2. One Materials and Methods

2.1 One Point One Specimen Source

147 newly diagnosed colorectal cancer patients who could receive surgical treatment in the hospital from January 2018 to December 2019 were collected. All patients with colorectal cancer were confirmed by histopathology. The average age of 147 patients with colorectal cancer was (57.9 ± 11.7) years (age range: 20 ~ 87 years); There were 93 males and 54 females. Pathological types: tubular villous adenoma of ascending colon, sigmoid colon cancer, transverse colon cancer, rectal cancer, etc. According to the American Joint Committee on cancer (AJCC), the TNM stages of patients were determined, including 20 patients in stage I, 60 patients in stage II and 67 patients in stage III. Inclusion criteria: ① patients with colorectal cancer diagnosed by imaging, pathology or cytology; ② No contraindications; ③ Have perfect clinical and pathological information; ④ No serious diseases such as heart, liver and kidney, and no history of other malignant tumors; ⑤ Patients who voluntarily participated in the study and signed informed consent. Exclusion criteria: ① pregnant or lactating patients; ② Patients who have or are suffering from other neoplastic diseases; ③ Patients with poor compliance and unable to follow up on schedule; ④ Patients unwilling to sign informed consent.

2.2 One Point Two Method

All enrolled patients collected peripheral blood samples 4-6 weeks after operation, uniformly used 5ml heparin sodium to collect blood vessels, discarded the initial 2ml peripheral blood, and collected the blood of the median elbow vein as CTC test samples. After blood collection, they gently reversed and mixed for 5-8 times to fully mix the anticoagulant with the blood. After obtaining the samples, they shall be stored at room temperature (15 ℃ ~ 30 ℃), and CTC detection shall be carried out within 6h. Using cytosorter ® CTC analyzer and interstitial CTC detection kit were used for enrichment detection. CTC observation and cell counting were performed by Nikon ti-e inverted fluorescence microscope, and typical mctc immunofluorescence photos and bright field photos were taken.

Criteria for mctc test results: ① cells were round, oval or long; The cell diameter was greater than 10mm. The cell morphology and edge were intact under fluorescence. The complete cell morphology and nucleus were observed under light microscope. ② Immunofluorescence staining: the cells in green and blue were judged as interstitial circulating tumor cells, and the immunofluorescence staining characteristics of MCTC were CSV + / Hoechst + / CD45 -; The cells in orange and blue were judged as leukocytes, and the immunofluorescence staining characteristics of leukocytes were CSV - / Hoechst + / CD45 +. ③ Results more than two trained experimenters determined that MCTC ⩾ 1 / 4ml was positive.

2.3 One Point Three Statistical Analysis

SPSS20 The data of this study were statistically analyzed by 0 software. The measurement data were expressed by mean ± standard deviation (` x ± s), and the counting data were expressed by percentage (%). The correlation between the number of mctc and the clinicopathological characteristics of patients with colorectal cancer was analyzed by C2 test, $P < 0.05$, which was statistically significant.

3. Result

3.1 Detection Results of Interstitial CTC in Colorectal Cancer

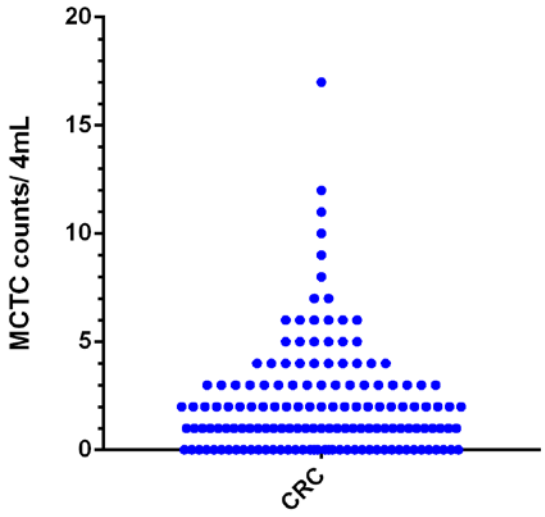

Fig.1 Quantitative Distribution of Postoperative MCTC in Patients with Colorectal Cancer

The results showed that the distribution of CTC detection quantity in 147 peripheral blood samples was 0 ~ 17 / 4ml, and the CTC detection rate was 72.8% (107 / 147), as shown in Figure 1. Different types of colorectal cancer and different pathological stages of colorectal cancer have good detection of MCTC.

3.2 Correlation between MCTC Level and Clinicopathological Features in Patients with Colorectal Cancer after Surgical Treatment

Peripheral blood MCTC was detected in patients with colorectal cancer after operation. The correlation between the number of postoperative MCTC and patients' age, gender, tumor location, depth of tumor invasion, lymph node metastasis and clinicopathological stage was analyzed. The experimental results showed that when the threshold was set to 3, the level of postoperative mctc was related to patients' age ($P = 0.4983$), gender ($P = 0.3177$) There was no significant correlation between tumor location ($P = 0.0715$) and depth of tumor invasion ($P = 0.4174$), but it was significantly correlated with tumor lymph node metastasis ($P < 0.0001$) and clinicopathological stage ($P < 0.0001$).

3.3 Correlation between Postoperative MCTC Number and Metastasis and Prognosis of Colorectal Cancer

All enrolled patients were followed up for an average follow-up time of (31.8 ± 4.1) months. The results showed that patients with a higher number of mctc had a higher risk of postoperative distal metastasis. There was a significant difference in the number of MCTC between colorectal cancer patients with distal metastasis and colorectal cancer patients without distal metastasis ($P < 0.0001$). The number of postoperative MCTC was used to analyze the progression free survival (PFS) of operable colorectal cancer patients. The results showed that there were significant differences in progression free survival ($P < 0.0001$) and overall survival ($P < 0.0001$) between colorectal cancer patients with MCTC $\geqslant$ 3 / 4ml and colorectal cancer patients with MCTC < 3 / 4ml.

4. Conclusion

Traditional colorectal cancer CTC is mostly based on epithelial phenotype, and CTC is used to monitor local recurrence or metastasis. Interstitial phenotypic CTC has more clinical value in tumor prognosis evaluation, efficacy monitoring, recurrence and metastasis monitoring and disease progression monitoring. In this study, CSV was used to enrich and detect colorectal cancer interstitial phenotype CTC by microfluidic chip and immune capture method. Compared with less than 50% based on colorectal cancer epithelial CTC, the detection rate of colorectal cancer

postoperative interstitial CTC can reach 72.8% (107 / 147). This may have important clinical significance for the auxiliary diagnosis of colorectal cancer.

Tumor cells can fall off and enter the circulatory system during the operation of removing the primary focus, increasing the possibility of tumor metastasis. At present, most studies believe that postoperative CTC detection can better predict the survival status of patients with colorectal cancer. The results of this study show that CTC detection can evaluate the prognosis of colorectal cancer patients 4-6 weeks after operation. Patients with a higher number of mctc have a higher risk of postoperative distal metastasis. The detection of peripheral blood mctc has certain clinical significance in the evaluation of postoperative recurrence and metastasis of colorectal cancer. Because this study is a single center prospective study with limited sample size and short follow-up time, the statistical impact of clinical variables on survival may be limited. Therefore, further large-scale prospective trials are needed to confirm the role of CTC in postoperative recurrence and metastasis monitoring and prognosis evaluation of colorectal cancer.

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