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Circulating Tumor Cell Detection

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Clinical Reference Manual for Circulating Tumor Cell Detection in Colorectal Cancer

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Preface

Colorectal cancer (CRC) incidence and mortality are rising globally, ranking third in cancer threats. According to the "Analysis of the Prevalence of Malignant Tumors in China in 2015" published by Academician He Jie and others in 2019, the number of CRC cases in China in 2015 was 388,000, with 187,000 deaths, making it a disease that poses a significant threat to people's health and life.

At present, common treatments for CRC include surgery, radiotherapy, targeted therapy, and immunotherapy. Advanced diagnostics and treatments are improving CRC patient outcomes. The current overall 5-year survival rate and 10-year survival rate for CRC patients have reached 65% and 58% respectively. This suggests that about half of CRC patients experience tumor recurrence or metastasis during the progression of the disease. Therefore, clinicians need more and more accurate new tumor markers to monitor disease progression and accurately judge the prognosis of CRC patients, alongside imaging and traditional serum tumor markers.

Circulating tumor cells (CTCs) are tumor cells in the peripheral blood of patients. First observed by Dr. Ashworth in 1869, their significance as valuable tumor markers was recognized around 2000, revolutionizing clinical diagnosis and treatment. In 2008, Dr. Cohen and others in the USA published a research result on CTCs in patients with metastatic colorectal cancer, showing that for patients with metastatic colorectal cancer, when the number of CTCs in their peripheral blood is more than or equal to 3 per 7.5 mL, their prognosis is significantly worse; and the predictive accuracy of patient treatment prognosis is greatly improved when CTC detection is combined with imaging evaluation. Based on this research, the U.S. FDA approved CTC detection for the prognosis assessment of patients with metastatic colorectal cancer in the same year. Subsequently, various countries have conducted multiple clinical trials on CTC detection at different stages of colorectal cancer, exploring the clinical significance and detection value of CTCs in different diagnostic and treatment stages of colorectal cancer from various angles.Next, the manual discuss the clinical significance of CTC detection in different diagnostic and treatment stages of colorectal cancer.

Application of Circulating Tumor Cells (CTC) Detection in the Diagnosis and Treatment of Colorectal Cancer

Prediction and evaluation on the therapeutic effect of unresectable advanced colorectal cancer.

Evidence-based clinical outcomes suggest that the CTC detection frequency among patients with unresectable advanced colorectal cancer undergoing systemic treatment (including chemotherapy or combined targeted therapy):

1. Perform a baseline CTC test one week before systemic treatment (including chemotherapy and targeted therapy): if the CTC count is above the threshold, the patient is considered as poor prognosis, recommending to evaluate the necessity of further treatment, or seek a new treatment.
2. For chemotherapy patients (including combined targeted therapy): perform the second CTC test before the start of the third course of treatment after the end of two courses of treatment; if both CTC tests are above the threshold, it indicates that the treatment effect is poor, recommending to change treatment plan; if the second CTC test is below the threshold, or it is still above the threshold but there is a significant drop (＜50%) compared to the baseline value, it indicates that the current treatment is effective, recommending to maintain the treatment plan.
3. If the treatment plan is changed, CTC should be tested again after two courses of treatment (or two months) and side-by-side comparing with the previous two results. If the CTC count is still above the threshold, the necessity of further treatment is recommended to re-evaluated, or seek new treatments.
4. After all systemic treatments, if the CTC count exceeds the threshold, it indicates that the patient's prognosis is poor, the necessity of further treatment is recommended to re-evaluated, or seek new treatments. CTC outcomes are a strong supplement to the traditional imaging evaluation. Patients with different CTC results for the same image evaluation, their prognosis will be different.

- Patients with image evaluation of SD/PD, if CTC is below the threshold, it is expected that the patient still has a better treatment effect;
- Patients with imaging evaluation of SD/PR, if CTC is above the threshold, it is predicted the presence of a poorer treatment effect, therefore, the combination of image evaluation and CTC detection will be more accurate in evaluating the treatment effect.

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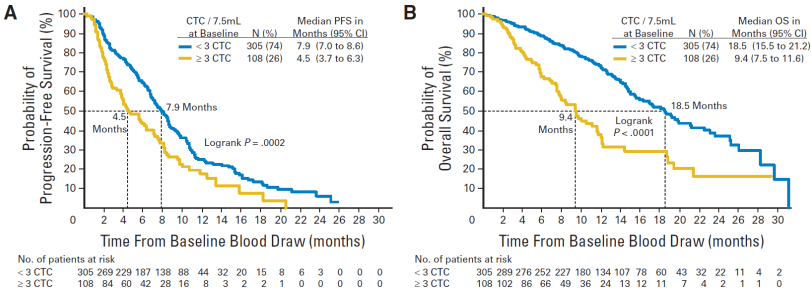
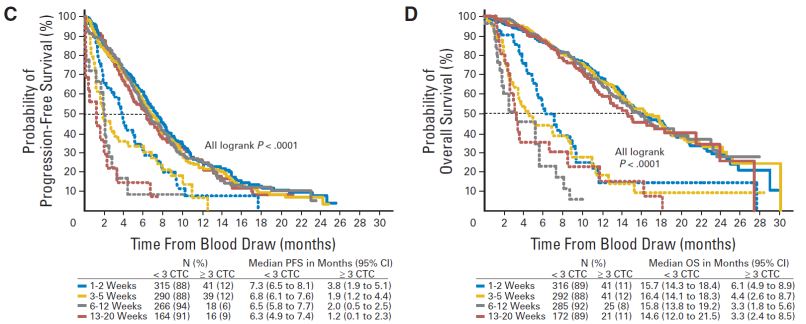
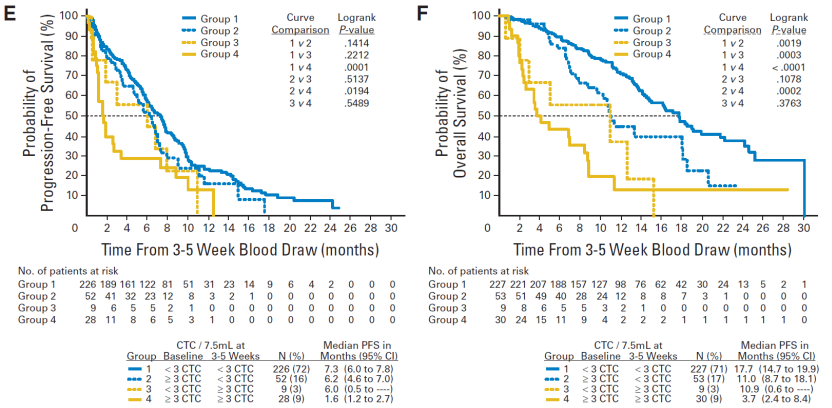
For patients with unresectable advanced colorectal cancer, the main treatment plan is systemic treatment, including combinations of chemotherapy and targeted therapy. These patients need specific biomarkers to assess the prognosis and timely monitor the treatment effect, so that clinicians can understand the disease progression and the effect of treatment, deciding the next treatment measures. Clinical trials on 340 metastatic colorectal cancer patients participated CTC tests before and after first to third line of systemic treatments showed that the CTC detection before treatment has good prognostic evaluation value. Patients with a CTC counts at ≥3/75mL CTC are significantly worse than other at both overall survival (OS) and disease-free survival (PFS) (Figure below).



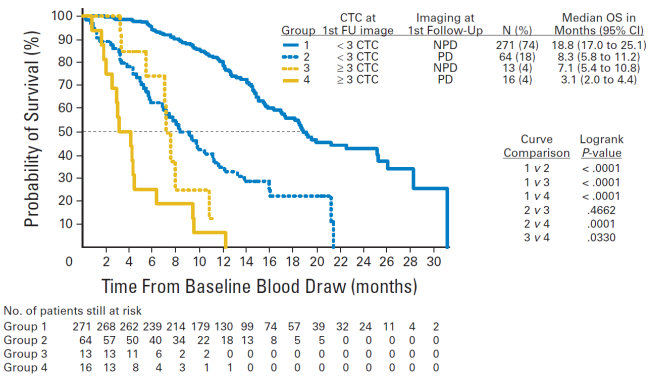
In term of the detection results after patient treatment, whether it is 1-2 weeks after treatment, or 3-5 weeks, 6-12 weeks, 13-20 weeks later, patients with a CTC counts at ≥3/7.5mL have significant worse treatment effect than other patients (Figure below).



Comparing the CTC counts before treatment and at 3-5 weeks after treatment, the research indicated that the treatment effect of patients at <3/7.5mL CTC counts before and after treatment is the best, while at ≥3/7.5mL the treatment effect is the worst.



The research compared CTC counts of patients 3-5 weeks post-treatment and correlation with image evaluations. Findings: (1) PD patients with CTC counts <3/7.5mL still had good treatment effects; (2) NPD patients with CTC counts ≥ 3/7.5mL had similar treatment effects to the previous group (Figure below). Thus, CTC detection, approved by the US FDA in 2008, is a valuable supplement to post-treatment image evaluations, aiding in distinguishing treatment beneficiaries, facilitating timely treatment effect evaluations, and planning subsequent treatment strategies.



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Other clinical team reported similar results in 2010. 467 patients with advanced colorectal cancer undergoing systemic treatment, at a relatively high CTC counts, have a worse prognosis. For patients with DC (CR, PR, and SD) image evaluation 9 weeks after treatment, patients with high CTC counts have significantly worse prognosis (Figure below).

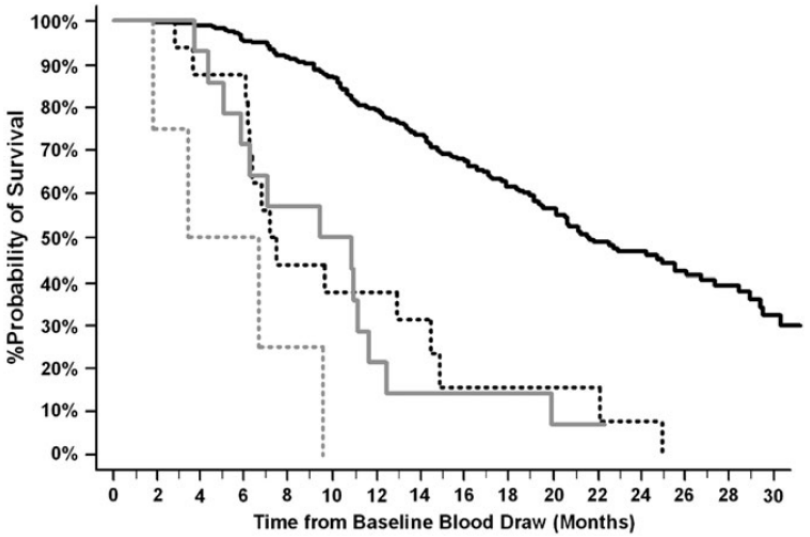

Figure 4. The combination of circulating tumour cells (CTC) and computed tomography imaging results: the overall survival in 273 patients with low CTC after 1–2 weeks and disease control at first tumour evaluation (solid black line), in 16 patients with low CTC and progressive disease (PD) at first tumour evaluation (dashed black line), in 14 patients with high CTC and disease control (solid grey line), and in 4 patients with high CTC and PD (dashed grey line).

Numerous clinical researchers, including Sastre J (2012), Aggarwal C (2013), ZhangD (2017), WangL (2019), etc., in different countries, have conducted different systemic treatment plans for advanced colorectal cancer. All found that the baseline CTC count before treatment can indicate the prognosis of patients undergoing chemotherapy, and if CTC counts increases after treatment, the prognosis is worse to great extent.

Risk assessment: surgical treatment and local treatment of metastatic lesions in metastatic colorectal cancer

Evidence-based clinical outcomes suggest that the CTC detection frequency among patients with unresectable advanced colorectal cancer undergoing systemic treatment (including chemotherapy or combined targeted therapy):

1. Perform a baseline CTC test one week before systemic treatment (including chemotherapy and targeted therapy): if the CTC count is above the threshold, the therapy is considered as poor prognosis, recommending to evaluate the necessity of further treatment, or seek a new treatment.
2. Patients undergoing a new therapy is recommended to have the second CTC test near before the third treatment course.
   - If both CTC tests exceed the threshold, consider changing a therapy plan.
   - If the second CTC test is below the threshold or significantly reduced (over 50%) from the baseline, consider maintaining the current plan.
3. Before surgery, the third CTC test is advisable.
   - If the CTC count exceeds the threshold, re-evaluate surgical risk and necessity.
4. After an adjuvant therapy, the fourth CTC test is recommendable.
   - If the CTC count still exceeds the threshold, consider strengthening patient follow-up and possibly extending the treatment cycle.

According to the “Chinese Society of Clinical Oncology (CSCO) 《Colorectal Cancer Diagnosis and Treatment Guidelines (2019)》 ” and “NCCN Colorectal Cancer Guidelines 2019 Edition”:

Surgical resection and local treatment of metastatic lesions can offer hope for some metastatic colorectal cancer patients. European Society for Medical Oncology (ESMO) guidelines suggest converting patients with only lung or liver metastasis into a curable patient through systemic and local treatment. When considering treatment plans, prognosis assessment is crucial. Classic prognostic indicators include CRS scores, KRAS, NRAS, and BRAF mutations, but these cannot accurately stratify prognosis, necessitating new tumor markers for prognosis evaluation.

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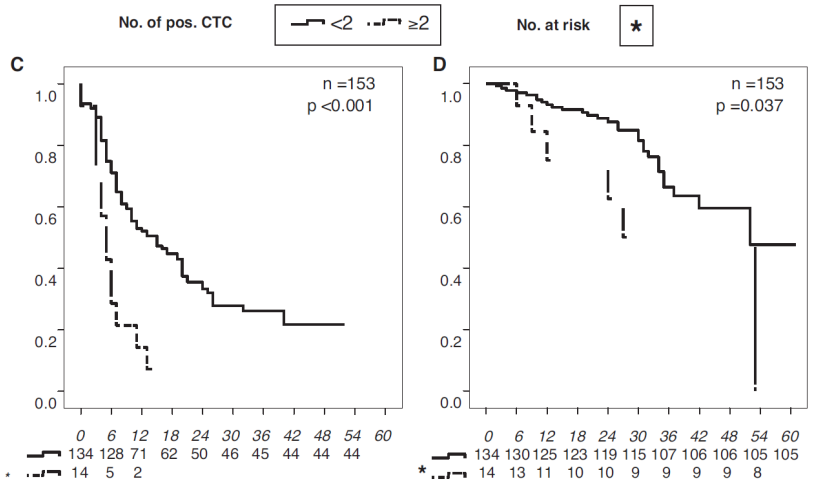
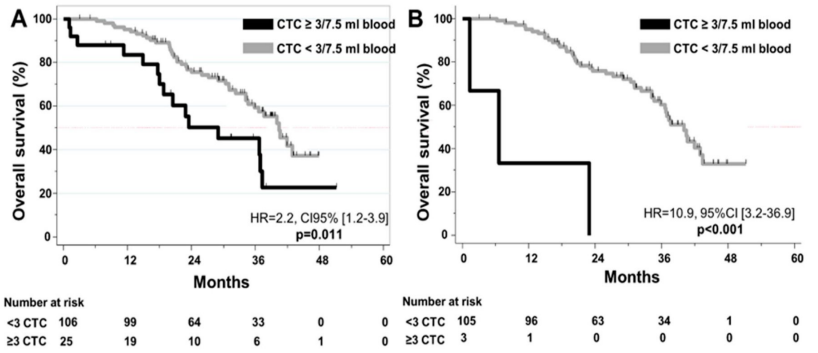
CTC has been shown by numerous clinical studies to have good prognostic evaluation performance in systemic treatment of metastatic colorectal cancer. The question arises whether it can assess surgical risk after curative treatment. In 2015, a team from Norway was tracking 194 patients who had developed colorectal cancer metastasis, of which 153 patients eventually underwent surgical resection, while the remaining 41 patients could be unresected. There was a significant difference in CTCs counts in the peripheral blood of these two subgroups of patients (P<0.001). Among the colorectal cancer patients who underwent surgical resection, patients with a CTC count equal to or higher than the threshold also had a significantly different prognosis from the other group (P<0.001) (Figure below). These research results suggest that CTC detection can predict the surgical risk of colorectal cancer patients with liver metastasis.



Recently, an international clinical team reported on 132 potentially resectable colorectal cancer liver metastasis patients in 《Cells》. The results showed that the baseline CTC counts before treatment was related to the percentage of liver metastasis infiltration in patients (p=0.001) and was related to the synchronicity of LM (p=0.04). A high CTC counts at baseline, or a high CTC counts 4 weeks after treatment was related to the patients' shorter OS (baseline ≥3 CTCs, HR=2.2, 95%CI [1.2;3.9], p=0.011; 4 weeks =3 CTCs, HR=10.9, 95%CI[3.2;36.9], p<0.001) (Figure below).



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Risk assessment and monitoring: Recurrence/Metastasis After Surgery for Resectable Non-metastatic Colorectal Cancer Patients

Based on the results of evidence-based clinical research, it is recommended that for non-metastatic colorectal cancer patients planning to undergo radical surgery, the following CTC detection timing and frequencies should be considered:

1.Perform the first CTC test as a baseline value in one week before surgery.

2.Perform the second CTC test, one month after the surgery. If the postoperative CTC is above the threshold, consider adjuvant therapy, or strengthen follow-up frequency.

3.Consider performing the third CTC test after adjuvant therapy. If CTC counts is still above the threshold, consider extending treatment course or strengthening follow-up.

4. Perform CTC test very 6-12 months after treatment.

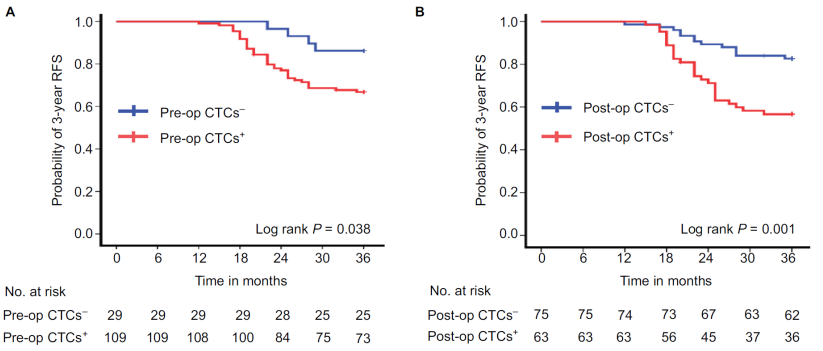
5. Consider performing a CTC test every 2-3 years.

6. Five years after radical surgery,

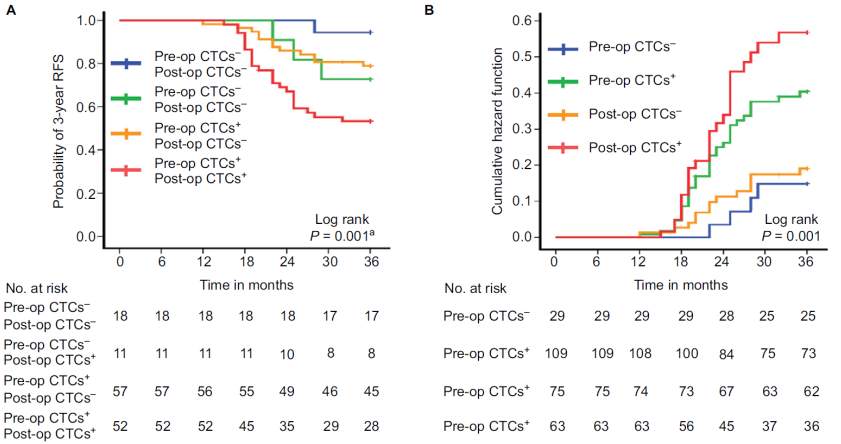
- if CTC counts detected during the postoperative follow-up period exceeds the threshold, consider strengthening the follow-up frequency and retesting CTC.
- if CTC count is above the threshold for two consecutive tests, it suggests that the patient has a higher risk of recurrence. Consider strengthening follow-up frequency, performing detailed examinations, or preventive interventions to mitigate the risk of recurrence/metastasis.

For non-metastatic colorectal cancer patients, radical surgery and combined treatment are the frequently used therapeutic strategy. With post-surgery relapse or metastasis in about 50% of colorectal cancer patients being a leading cause of death in early to mid-stage cases, post-surgery risk assessment is vital. At present, clinical postoperative follow-up mainly relies on CEA and CA199 as serum biological markers for predicting the therapeutic effect of colorectal tumors, but the sensitivity and specificity of these markers are relatively low. A dynamic detection of CTCs is a feasible technique in clinical practice for identifying recurrence and metastasis risks in early to mid-stage colorectal cancer post-treatment.

A top academic university reported a research on the correlation between CTCs counts before and after surgery and the recurrence/metastasis of patients after surgery in gastrointestinal tumor patients. They used the CTCBIQPSY® platform to detect the CTC count in the peripheral blood of 138 patients who underwent radical resection surgery before and one month after surgery. They found that the positive CTC before and after surgery can effectively prompt: the disease-free survival periods of patients is shorter without a recurrence (Figure below).



Multivariate analysis shows post-surgery CTC is a key predictor of 3-year Recurrence Free Survival (RFS). Patients with positive CTC both pre- and post-surgery have worse prognosis. Hence, CTC tests are crucial before and after surgery.



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In 2013, Italy Team monitored 76 patients who underwent surgical removal of colorectal cancer. Among the 69 patients who underwent radical surgery, 16 were found to have high levels of CTC after surgery, and 12 of these (75%) experienced cancer recurrence. Post-surgery high CTC was the only independent factor for cancer recurrence. For patients with no CTC detected, the Progression-Free Survival (PFS) increased from 16% to 86%, reducing the risk of tumor recurrence by over 90%. They concluded that high post-surgery CTC can accurately predict tumor recurrence, suggesting that CTC assessment can optimize the management of colorectal cancer patients.

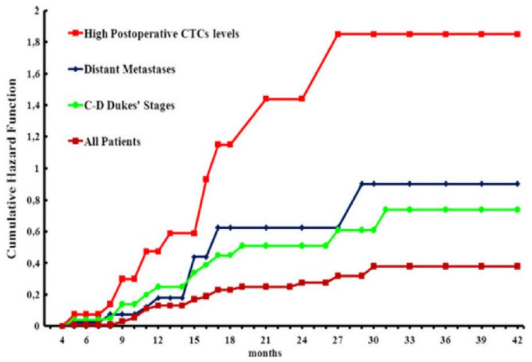

Fig. 4 Three-year cumulative hazard function of cancer relapse according to high postoperative CTC levels, distant metastases, advanced Dukes' stages, and complete series

Colorectal Cancer (CRC) High-Risk Group Screening

According to Chinese guidelines, adults over 45, especially those with high-risk factors, should regularly undergo CRC and Circulating Tumor Cell (CTC) screening.

- **CRC High-Risk Groups**

1. History of colorectal polyps.
2.Undergone therapeutic colorectal cancer resection.
3.Direct relatives diagnosed with rectal cancer or colorectal adenoma.
4.Long-standing inflammatory bowel disease.
5.suspected to have hereditary syndromes, e.g. Lynch syndrome or Familial Adenomatous Polyposis.
6. History of abdominal or pelvic radiation for tumors.

- **CRC Risk Factors**

1.High intake of fat and cholesterol;
2.Insufficient intake of fresh vegetables and fruits; obesity;
3.Lack of physical activity, chronic intestinal inflammation;
4.Long-term constipation;
5.Related genetic mutations;

The National Cancer Center of China in 2019 reported: nearly 4 million new cancer cases were diagnosed in China, with 388,000 new cases and about 187,000 deaths from CRC. CRC screening methods include fecal occult blood test and colonoscopy, but acceptance is low due to the invasiveness of colonoscopy.

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A prospective clinical study published in 2019 by clinical experts from Chang Gung Hospital in Taiwan, which included 667 subjects, showed that the detection rate of CRC precancerous lesions by CTC was 79.2%, close to the effect of colonoscopy (76%-94%).

The sensitivity for stages I-IV CRC was as high as 95.2%, surpassing the screening effect of the fecal occult blood test (FOBT) (62%-79%), and was comparable to the effect of colonoscopy (75%-93%). Therefore, using CTC detection as a method for early CRC screening is reasonable and feasible.

Table 2. Sensitivity of CMx test and CTC[a] counts grouped by disease stage

Stage	Sensitivity[a,b] (%)	Lower 95% CI (%)	Upper 95% CI (%)
Adenoma	79.2	70.8	86.0
CRC stage I	89.2	79.1	95.6
CRC stage II	97.9	92.5	99.7
CRC stage III	95.7	90.2	98.6
CRC stage IV	97.4	86.5	99.9
CRC stage I–IV	95.2	92.2	97.3
Adenomas/ CRC	90.1	87.6	93.3

CI, confidence interval; CMx, CellMax; CTC, circulating tumor cell, CRC, colorectal cancer.
[a]Based on a cutoff of 0.53 in logistic regression analyses.
[b]Odds ratio for (log_{10}) CTC count is 66.8 (95% CI: 9.6, 687.8).

Afterword

The use of CTC, whether in the early or late stages of colorectal cancer, significantly indicates a poorer prognosis for patients, including shorter Progression-Free Survival (PFS) and Overall Survival (OS). The change in CTCs counts before and after treatment can effectively reflect or predict the effect of treatment. Dynamic CTC monitoring can provide more accurate information for doctors and patients at different stages of CRC diagnosis and treatment, thereby helping doctors formulate more accurate treatment plans. In January 2019, the Minimally Invasive Surgery Professional Committee of the Chinese Research Hospital Association published the “Expert Consensus on the Application of Circulating Tumor Cell Detection in Colorectal Cancer (2018)” in the Journal of Laparoscopic Surgery, summarizing that CTC detection in CRC plays a preliminary role in dispelling doubts and guidance, indicating that CTC detection can be an effective and feasible detection method for the clinical diagnosis and treatment of CRC. Of course, at this stage, CTC detection still faces some unresolved issues, such as further analysis of CTC subtypes, further refinement of the guiding role of CRC staging, and quantification of the significance of follow-up and guidance of perioperative treatment options.

Therefore, clinicians are expected to conduct more in-depth clinical research on these issues while using CTC detection to serve patients, priding more sufficient and accurate clinical evidence for CTC detection.

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