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RESEARCH ARTICLE

Monitoring Treatment Response in Rectal Cancer through Circulating Tumor Cell Dynamics: A Pilot Clinical Study

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ABSTRACT

Background: Circulating tumor cells (CTCs) are increasingly recognized as a minimally invasive biomarker with significant potential in oncologic monitoring and prognostication. This study aimed to assess the dynamics of CTC levels in patients with rectal cancer undergoing multimodal treatment and evaluate their potential role in therapeutic decision-making.

Methods: We conducted a prospective, observational study of 56 patients with histologically confirmed rectal adenocarcinoma. Patients underwent either primary surgical resection or neoadjuvant chemoradiotherapy (CRT) followed by surgery. Peripheral blood samples were collected at defined intervals and analyzed using the CytoTrack CT11™ system to detect and quantify CTCs.

Results: Pretreatment CTCs were detected in 16.1% of patients. In the primary surgery group, all preoperatively positive CTC cases became negative postoperatively, although transient positivity was observed in two cases at 1 week post-surgery. In the CRT group, 35.7% of patients exhibited detectable CTCs during treatment, with complete clearance after surgery. The dynamic change in CTC levels correlated with therapeutic response and potential recurrence risk.

Conclusions: This pilot study highlights the clinical relevance of CTC monitoring in rectal cancer. CTC dynamics appear to reflect treatment efficacy and may serve as an early indicator of response. These findings support the development of a randomized clinical trial comparing rectal cancer treatments with and without neoadjuvant therapy, using CTC trends as a primary outcome measure.

1 | Introduction

Multimodal treatment is the standard of care for patients with rectal cancer. Surgical resection is a crucial component of treatment, although outcomes are influenced by the stage of the disease at diagnosis. For patients with locally advanced rectal cancer (stage cT3-T4) and/or regional lymph node involvement (cN+), neoadjuvant chemoradiotherapy (CRT) is typically recommended before surgical intervention [1–3]. However, the indication for neoadjuvant treatment in patients with stage III rectal cancer, particularly those with cT1–cT3 carcinoma that does not infiltrate

the fascia recti propria (as assessed by preoperative magnetic resonance imaging), remains a topic of ongoing debate [4–7].

A multidisciplinary approach, including surgery, radiotherapy, and systemic therapy, is essential for optimal treatment of rectal cancer. However, determining the effectiveness of individual treatment modalities and predicting patient prognosis is challenging, as existing standard clinical parameters, such as overall survival or disease-free interval, do not provide sufficiently rapid feedback [8–10]. Therefore, new biomarkers are being sought that could improve monitoring of treatment response and allow for more accurate prognosis assessment [11–13].

Circulating tumor cells (CTCs) represent a promising biomarker with great potential for evaluating the effectiveness of anticancer therapy [13, 14]. CTCs are tumor cells that are shed from the primary tumor into the peripheral blood, and their detection can be performed through so-called "liquid biopsy" exploiting immunomagnetic-, immunohistochemical-, and PCR-based methods [15]. The advantage of this approach is that it is a minimally invasive method that can be easily repeated at different times of treatment [9]. Existing studies show that changes in CTC levels correlate with treatment response and can serve as a prognostic indicator of the risk of recurrence or disease progression [10–12, 16, 17].

In the present study, we focus on evaluating the impact of multimodal treatment (neoadjuvant chemoradiotherapy and surgical resection) on the dynamics of CTC levels in patients with rectal cancer. We believe that monitoring changes in CTC levels can provide valuable information on the effectiveness of individual treatment modalities and assist clinicians in making optimal therapeutic decisions for each patient.

2 | Methods

2.1 | Study Design and Patient Selection

This prospective, observational clinical trial was conducted at the University Hospital, targeting patients with histologically confirmed rectal adenocarcinoma. During the study period (May 2, 2023 - August 30, 2024), all patients aged 18 years or older with rectal cancer located within 15 cm from the anal verge were considered for inclusion. Patients with metastatic disease (stage IV), recurrent rectal cancer, or other concurrent malignancies were excluded from the study.

The primary goal of the study was to monitor changes in CTC levels in the peripheral blood of patients undergoing multimodal treatment of rectal cancer. Peripheral blood samples were collected from each patient at specific time intervals to determine the presence and quantity of CTCs. All participants provided written informed consent, and the study was approved by the Ethics Committee of the University Hospital Ostrava (ref. number 8/2023).

2.2 | Treatment Protocol

The management of study patients was guided by multidisciplinary team discussions and followed the NCCN guidelines. Patients with rectal cancer classified as cT1-3, N0, M0, and negative circumferential resection margin (CRM-) were indicated for primary surgery, which involved radical rectal resection with total mesorectal excision or tumor-specific mesorectal excision, depending on tumor location.

Neoadjuvant treatment, consisting of concomitant long-course radiotherapy and chemotherapy, was indicated for patients with rectal cancer classified as cT3-4, any N, M0, CRM+ as well as for those with any T, N+, M0 disease. Surgery, which included radical rectal resection with total mesorectal excision or tumor-specific mesorectal excision, was scheduled 6–10 weeks after the completion of neoadjuvant treatment. Adjuvant systemic therapy was administered postoperatively based on pathological findings and clinical judgment.

2.3 | Sample Collection and CTC Detection

Peripheral blood samples were collected from each patient undergoing CRT at multiple time points: (1) before the initiation of CRT, (2) 1 week and (3) 1 month after the initiation of CRT, (4) preoperatively (1–2 weeks before surgery), (5) 1 week postoperatively, and (6) 1 month postoperatively. For patients undergoing primary surgery, blood samples were collected at the following time points: (1) preoperatively (1–2 weeks before surgery), (2) 1 week postoperatively, and (3) 1 month postoperatively.

Peripheral blood samples were collected into Cell-Free DNA BCT tubes (Streck). Samples were transported daily to the laboratory for processing. Following centrifugation at 2500 g for 15 min, the buffy coat was isolated, and residual red blood cells were lysed using FACS Lysing Solution (BD Biosciences).

Isolated nuclear cells were permeabilized (Intrastain, DAKO) and immunostained at 4°C for 60 min in the dark using a mixture of anti-CD45 antibodies (clone HI30, APC conjugate), anti-pancytokeratin antibodies (clones AE1/AE3 and C-11, Alexa Fluor® 488 conjugate), anti-EpCAM antibodies (clone VU1D9, Alexa Fluor® 555 conjugate), and DAPI. After two washing steps in 1%BSA in PBS and 0.5% BSA in PBS, cells were resuspended in water, applied onto CytoDisc™ glass disks (2 C A/S), air-dried, and mounted using mounting medium.

CytoDiscs were scanned using the CytoTrack CT11 fluorescence microscope. Scanning was performed in an outward spiral pattern with a 488 nm laser, and fluorescence signals were captured. Potential CTC candidates (hotspots) were identified based on green fluorescence and subjected to operator review. CTCs were defined by morphology (round shape, $>4\,\mu m$), DAPI-positive nucleus, pancytokeratin and/or EpCAM positivity, and CD45 negativity.

2.4 | Data Collection and Statistical Analysis

Clinical data, including patient demographics, tumor characteristics, and treatment details, were collected from medical

records and recorded in a secure database. CTC levels at each time point were documented, and their changes throughout the treatment course were analyzed. Descriptive statistics were used to summarize patient characteristics and CTC counts. The *t*-test for two samples with equal variances and Fisher's exact test at the 5% significance level were used to compare the characteristics and clinical outcomes of the two groups of patients. The evaluation was performed using Stata version 17 (StataCorp LLC, College Station TX USA).

3 | Results

A total of 56 patients (16 women, 40 men) with rectal cancer were prospectively enrolled in this study. The patient demographics and clinical characteristics are presented in Table 1. The mean height of the distal tumor margin was 10.0 ± 5.8 cm. Clinical (preoperative) stage I rectal carcinoma was diagnosed in 25.0% of patients, stage II in 21.4%, and stage III in 53.6%. Primary surgery was performed in 42 (75.0%) patients, while 14 (25.0%) underwent neoadjuvant chemoradiotherapy (CRT). Surgery was performed 6–8 weeks following the final radiotherapy session. The most common surgical procedure was anterior rectal resection.

As presented in Table 2, CTCs were detected in the peripheral blood of 9 patients (16.1%) before the initiation of multimodal treatment—8 patients from the primary surgery group and 1 patient from the neoadjuvant chemoradiotherapy (CRT) group.

In the primary surgery subgroup, all 8 patients with preoperative CTC positivity converted to negative status following resection. Notably, two additional patients who were initially CTC-negative developed transient CTC positivity one week postoperatively, which resolved by the 1-month follow-up. In the CRT group, 5 out of 14 patients (35.7%) exhibited detectable CTCs at one or more time points during neoadjuvant treatment (weeks 1 or 4, or preoperatively), although only one of these patients had CTCs before treatment initiation. All patients in this group showed complete CTC clearance after surgery. Individual CTC dynamics in patients with at least one positive CTC sample are illustrated in swimmer plots (Figures 1 and 2).

4 | Discussion

Circulating tumor cells (CTCs) have emerged as a promising biomarker in oncology, offering a minimally invasive approach for disease monitoring through liquid biopsy. Unlike traditional tissue biopsies, which are limited by spatial and temporal constraints, liquid biopsy enables real-time assessment of tumor dynamics, treatment response, and disease progression [13, 14, 18]. The enumeration and molecular characterization of CTCs provide valuable insights into tumor biology, metastatic potential, and resistance mechanisms, making them an essential tool in precision oncology. Several studies have demonstrated that CTC detection correlates with prognosis and therapeutic efficacy in various malignancies, including colorectal cancer, underscoring their potential role in guiding clinical decision-making [10–13, 15–18].

Our study demonstrates that the dynamics of circulating tumor cells (CTCs) in the peripheral blood of patients with rectal cancer may serve as a valuable indicator of treatment response. CTCs were detected in 16.1% of patients before the initiation of multimodal therapy, with a significant reduction in the

TABLE 1 | Demographic and clinical characteristics of the study cohort.

Parameter	Primary surgery ($n = 42$)	Surgery after CRT ($n = 14$)	Total ($n = 56$)	*p*-value
Age (years)	63.3 ± 7.5	68.2 ± 9.3	64.9 ± 8.7	0.051
Gender				
Female	13 (31.0)	3 (21.4)	16 (28.6)	
Male	29 (69.0)	11 (78.6)	40 (71.4)	0.734
BMI (kg/m^2)	27.4 ± 4.8	25.1 ± 4.1	26.2 ± 4.6	0.114
ASA classification				
I	4 (9.5)	3 (21.4)	7 (12.5)	
II	14 (33.3)	3 (21.4)	17 (30.4)	
III	24 (57.2)	8 (57.2)	32 (57.1)	0.455
Height of tumor (cm)	9.9 ± 6.3	7.1 ± 4.1	10.0 ± 5.8	0.121
Cancer stage (clinical)				
I	14 (33.3)	0 (0.0)	14 (25.0)	
II	9 (21.4)	3 (21.4)	12 (21.4)	
III	19 (45.3)	11 (78.6)	30 (53.6)	0.023
Type of surgery				
Anterior resection	36 (85.7)	10 (71.4)	46 (82.1)	
Abdominoperineal excision	6 (14.3)	4 (28.6)	10 (17.9)	0.247

Note: Values are *n* (%) or mean ± SD.

TABLE 2 | Detection of CTCs in peripheral blood at different treatment time points.

Time point	Primary surgery ($n = 42$)	Surgery after CRT ($n = 14$)	Total ($n = 56$)	p-value
Pretreatment CTCs				
Positive	8 (19.1)	1 (7.1)	9 (16.1)	
Negative	34 (80.9)	13 (92.9)	47 (83.9)	0.424
During neoadjuvant treatment				
Positive		5 (35.7)	5 (8.9)	
Negative		9 (64.3)	9 (16.1)	
Postoperative CTCs				
Positive	2 (4.8)	0 (0.0)	2 (3.6)	
Negative	40 (95.2)	14 (100.0)	54 (96.4)	1.000

Note: Values are *n* (%).

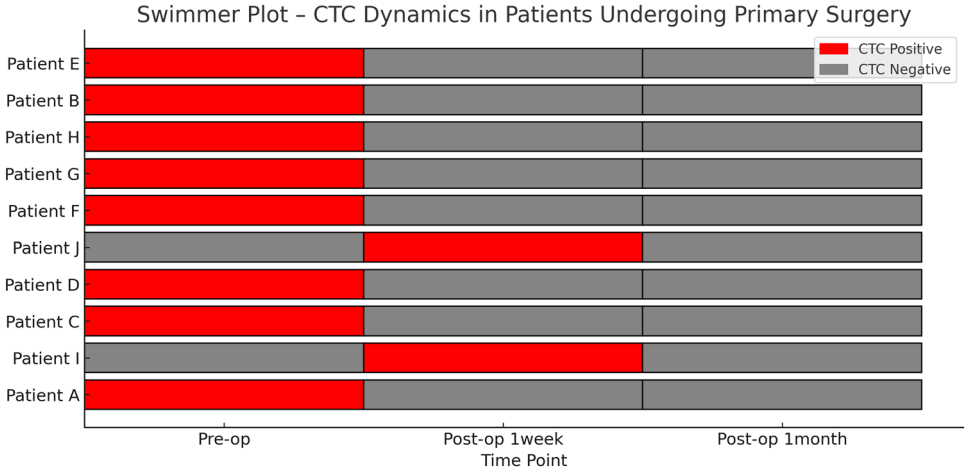

FIGURE 1 | Swimmer plot of CTC dynamics in patients undergoing primary surgical resection.

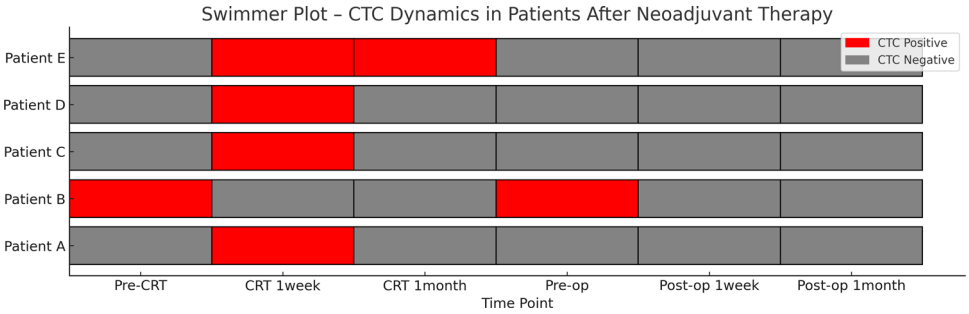

FIGURE 2 | Swimmer plot of CTC dynamics in patients undergoing neoadjuvant chemoradiotherapy followed by surgery.

proportion of positive samples throughout treatment. In patients undergoing primary surgical treatment, none of the initially CTC-positive cases exhibited detectable CTCs postoperatively. However, transient CTC detection was observed in two cases during the early postoperative period. Among patients receiving neoadjuvant chemoradiotherapy (CRT) followed by surgery, CTCs were detected in 35.7% of cases during neoadjuvant treatment, yet none remained detectable after surgery.

These findings are consistent with previous studies indicating that surgical resection significantly reduces the number of detectable CTCs in peripheral blood. A study by Smith et al. reported a similar effect, demonstrating a marked decrease in CTC detection following radical surgery [19]. Additionally, studies by Johnson et al. and Martinez et al. have corroborated that persistent CTCs post-neoadjuvant therapy are associated with an increased risk of disease recurrence and poorer prognosis [20, 21].

Our data support the hypothesis that neoadjuvant therapy may transiently increase CTC detection due to the release of tumor cells into circulation. This phenomenon has been described in studies by Lee et al. and Brown et al. who observed a temporary

rise in CTC counts during early treatment phases, followed by a subsequent decline after therapy completion. This pattern may reflect tumor cell destruction and remodeling of the tumor microenvironment [22, 23].

Several studies have established a strong correlation between the presence of CTCs and patient prognosis in colorectal cancer. Persistent detection of CTCs after treatment has been linked to a higher risk of disease recurrence and reduced overall survival. For instance, Smith et al. reported that patients with detectable CTCs following curative-intent surgery had a significantly lower disease-free survival rate compared to those without detectable CTCs [19]. Similarly, a study by Johnson et al. demonstrated that elevated CTC levels before and after treatment were associated with a poorer prognosis, reinforcing their potential role as a prognostic biomarker [20]. Furthermore, Martinez et al. highlighted that dynamic changes in CTC counts during neoadjuvant therapy could predict treatment response and long-term outcomes [21]. Given these findings, the clinical utility of CTC monitoring should be further explored in larger, prospective trials to validate its prognostic significance.

An important consideration is the variability in CTC detection methodologies, which complicates interinstitutional comparisons. Our study employed the CytoTrack CT11™ system; however, alternative approaches such as microfluidic platforms and molecular detection methods are utilized in other studies, thereby limiting direct comparability [9, 13, 14].

The present study represents a pilot project aimed at obtaining initial data on the role of CTCs in rectal cancer management. The primary limitation of our study is the relatively small patient cohort and short follow-up duration, precluding definitive conclusions regarding the prognostic significance of CTC persistence or clearance. However, based on these preliminary findings, we plan to expand our research through the design and implementation of a randomized clinical trial, in which the primary objective will be to compare the treatment outcomes of rectal cancer patients with and without neoadjuvant therapy, using CTC level dynamics as a key evaluation parameter. Future research should incorporate larger patient populations and extended follow-up to better elucidate the relationship between CTC dynamics and clinical outcomes.

In conclusion, our findings underscore the potential role of CTC detection in evaluating the efficacy of multimodal treatment for rectal cancer. While surgical resection effectively eliminates CTCs, the transient increase observed during neoadjuvant therapy warrants further investigation to determine its clinical implications. Future studies should aim to standardize detection methodologies and a more comprehensive understanding of CTCs as prognostic biomarkers in oncologic surgery.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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