Minimal residual disease and prognostic significance of circulating tumour cells in early-stage colorectal cancer

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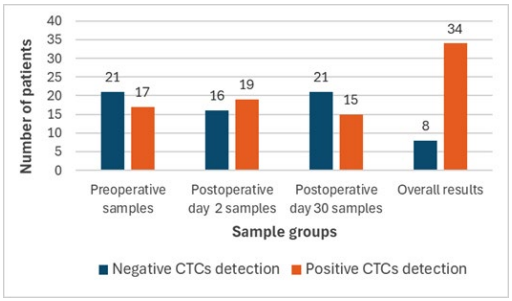
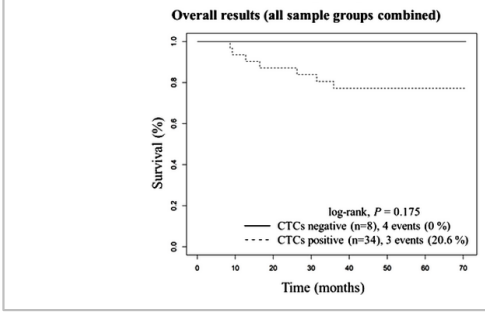
Aim. The recurrence rate of colorectal cancer remains high even after radical surgery. Existing criteria for administering adjuvant treatment lack sufficient precision, often leading to undertreatment or overtreatment. This study investigates circulating tumour cells (CTCs) as a potential prognostic biomarker to improve the accuracy of patient selection.
Methods. Forty-six colorectal cancer patients without distant metastases who underwent radical surgery were enrolled in this prospective study conducted at Tomas Bata Hospital in Zlín. The study protocol was approved by the hospital's Ethics Committee. Circulating tumour cells (CTCs) were measured in peripheral blood samples collected preoperatively, on the second postoperative day, and one month after surgery. CTCs were detected and characterized using semiautomated microscopy. Comprehensive clinicopathological data were recorded as part of standardized perioperative care. The prognostic significance of CTCs was evaluated based on the time to recurrence (TTR) parameter.
Results and Conclusion. Growing evidence from circulating tumour cell (CTC) research supports their potential role as a valuable biomarker in cancer management. In this study involving stage I–III colorectal cancer patients, a recurrence rate of 20% was observed among individuals with detectable CTCs, whereas no recurrence occurred in patients with a sustained absence of CTCs. Despite this apparent difference, the time to recurrence (TTR) did not differ significantly between the groups (log-rank test, *P*=0.175). Although the result was not statistically significant, the observed trend suggests a possible prognostic value of CTCs that merits investigation in a larger cohort. Furthermore, neither individual time-point measurements nor dynamic changes in CTC levels demonstrated a significant correlation with TTR.

Prognostic Significance Of Circulating Tumour Cells In Early-stage Colorectal Cancer

The search for novel prognostic and predictive biomarkers in solid tumors is ongoing. Circulating tumor cells (CTCs) represent a promising candidate in this context. In this study, we quantified CTCs in the peripheral blood of patients with early-stage colon cancer at three distinct time points using the CytoTrack® system.



The CytoTrack® method successfully detected circulating tumor cells (CTCs) in patients with early-stage colorectal cancer. However, no significant association was found between the presence of CTCs and clinicopathological characteristics. Time to recurrence (TTR) was used as the endpoint to evaluate the prognostic value of CTCs. Although TTR did not differ significantly between CTC-positive and CTC-negative groups (log-rank test, *P*=0.175), the observed trend suggests a potential prognostic relevance of CTCs that warrants further investigation in a larger patient cohort.



CTCs may have prognostic potential in early-stage colorectal cancer, warranting further investigation in larger cohorts.

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INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnosed malignancy globally, affecting nearly two million individuals each year and accounting for approximately 10% of all new cancer cases[1]. More than half of these diagnoses occur in developed countries, implicating lifestyle-related risk factors in its etiology[2]. After lung cancer, CRC is also the second leading cause of cancer-related mortality worldwide[1]. Prognosis is highly dependent on the stage at diagnosis; in Europe, the five-year overall survival rates are 89.1% for stage I, 81.2% for stage II, 69.4% for stage III, and only 15.4% for stage IV disease[3]. Consequently, early detection is essential for effective disease management. Another crucial prognostic factor is the risk of recurrence, which correlates strongly with disease stage. Reported recurrence rates are 7.4%, 16%, 33.5%, and 54.5% for stages I, II, IIIa + b, and IIIc, respectively[4]. These figures underscore the need for improved biomarkers capable of enabling earlier detection and more accurate recurrence prediction to enhance patient outcomes.

Adjuvant chemotherapy, particularly with fluorouracil (5-FU), has long been a standard component of postoperative management in CRC. Its benefit was first substantiated in 1988 by a meta-analysis conducted by Buyse et al., which demonstrated a survival advantage for patients receiving 5-FU compared to surgery alone, with a mortality odds ratio of 0.83 (95% CI: 0.70–0.98) (ref.[5]). Current treatment guidelines rely predominantly on clinicopathological parameters, recommending 3 to 6 months of adjuvant chemotherapy for all stage III patients[6]. For patients with stage II disease, adjuvant therapy is typically advised only in the presence of high-risk features, such as large tumour size, vascular or lymphatic invasion, and poor differentiation[7]. Given the recurrence rates observed, these criteria are insufficiently accurate, potentially leading to both undertreatment and overtreatment. The toxicity associated with chemotherapy further complicates the decision-making process, emphasizing the need for more individualized treatment strategies[8,9]. To this end, novel biomarkers that can provide information on recurrence risk and guide therapy decisions are of critical interest.

Circulating tumour cells (CTCs) are defined as malignant cells that have detached from either primary or metastatic tumour sites and entered the peripheral bloodstream[10]. The first observation of CTCs dates back to 1869, when Ashworth reported their presence in a patient with advanced breast cancer[11]. In that historical context, no modern detection tools were available, and the identification of CTCs reflected a high tumour burden. Typically, CTCs are extremely rare, occurring at a frequency of approximately 1 cell per 10^6–10^7 white blood cells[12]. They are also characterized by a short half-life of 1 to 2.4 hours, which, while technically challenging, makes them a dynamic biomarker for real-time monitoring of tumour biology[13].

Various methodologies have been developed to detect and analyze CTCs, generally comprising three key steps: enrichment, detection, and downstream analysis[14]. Based on the enrichment strategy, detection techniques are typically divided into two major categories: label-independent and label-dependent methods. Label-independent approaches exploit the physical properties of tumour cells, such as size, density, or electrical characteristics. These include size-based filtration, density gradient centrifugation, dielectrophoresis, and microfluidic chip technologies[15]. Label-dependent methods, by contrast, rely on immune affinity and typically target epithelial surface markers such as EpCAM (epithelial cell adhesion molecule). The CellSearch system, which utilizes immunomagnetic beads targeting EpCAM, is the only CTC detection platform approved by the U.S. Food and Drug Administration (FDA) for prognostic assessment in metastatic colorectal, breast, and prostate cancers[16,17]. However, during epithelial–mesenchymal transition (EMT), tumour cells may lose epithelial markers like EpCAM, leading to potential false-negative results in EpCAM-based detection systems[18].

In response to these limitations, newer enrichment-free technologies have been developed. Among them, the CytoTrack system employs automated fluorescent microscopy for CTC identification. This technique involves staining cells with a mixture of immunofluorescent dyes, placing them on glass discs, and scanning for CTCs using a semiautomated system. A key advantage of CytoTrack is its capacity for single-cell isolation and characterization, including non-epithelial phenotypes, as staining can be customized based on tumour-specific gene expression profiles[19].

A growing body of evidence supports the utility of CTCs in multiple clinical contexts, including early detection, prognosis, treatment monitoring, and post-treatment surveillance[10]. Notably, their role in detecting minimal residual disease (MRD) is emerging as particularly promising. MRD refers to residual malignant cells that persist after potentially curative treatments, such as surgery or adjuvant therapy, but are undetectable by conventional imaging or biomarkers. Technological advances have enabled the application of CTCs for MRD detection in colorectal cancer, offering the possibility of refining recurrence risk stratification and guiding adjuvant therapy decisions[20]. If validated as a reliable MRD biomarker, CTCs could fundamentally change the current paradigm—potentially sparing low-risk patients from unnecessary chemotherapy, while ensuring that high-risk individuals receive appropriate systemic treatment, even when traditional factors suggest otherwise.

Accordingly, we conducted a study utilizing the CytoTrack system to monitor CTCs in patients with resectable, non-metastatic colorectal cancer treated at our surgical department. We aimed to evaluate the impact of CTC presence on time to recurrence, thereby assessing their potential as a prognostic tool in clinical decision-making.

MATERIALS AND METHODS

Patients

This prospective study was conducted between February 2019 and May 2022, enrolling a total of 46

patients. The trial was registered at ClinicalTrials.gov (NCT02314871) and adhered to the principles of the Declaration of Helsinki. Approval was granted by the Institutional Review Boards, and written informed consent was obtained from all participants prior to enrollment.

Inclusion criteria were: age ≥18 years, the ability to provide informed consent, and planned open radical surgery for histologically confirmed colorectal cancer. Exclusion criteria included prior surgery for colorectal cancer, presence of another malignancy not in permanent remission, ongoing immunosuppressive or corticosteroid therapy, surgery performed within 30 days prior to enrollment, and active acute or chronic infections. Eligible patients were identified and recruited in the preoperative setting.

Comprehensive baseline data were collected, including demographic information, medical history, physical status, American Society of Anesthesiologists (ASA) classification, chronic medication use, and preoperative laboratory results. A standardized perioperative protocol was followed for all patients. This protocol included premedication administration on the day before and the day of surgery, and a 400 mL carbohydrate-rich beverage containing 50 g of carbohydrates given two hours prior to the procedure.

Anaesthetic management was also standardized and included defined protocols for medication administration, fluid therapy, haemodynamic targets, diuresis, core temperature maintenance, transfusion thresholds, and prophylaxis for postoperative nausea and vomiting. Analgesic protocols were applied consistently in both preoperative and postoperative phases. The duration of anaesthesia, type and length of surgical procedure, and intraoperative data were recorded.

All surgeries were performed with curative intent (radical resection). Postoperative care adhered to standardized protocols encompassing analgesia management, fluid resuscitation, haemodynamic optimization, glycaemic control, transfusion practices, and antiemetic strategies. Postoperative complications were documented and graded according to the Clavien–Dindo classification system. Additional recorded data included intraoperative blood loss, transfusion requirements, adverse events, and final histopathological findings with corresponding tumour classification.

All collected clinical and pathological data were securely stored in patient-specific electronic case report forms using the academic cloud-based platform ClinData (https://clindata.imtm.cz).

Sample collection and detection methods

Peripheral blood samples (9 mL each) were collected from all enrolled patients at three time points: preoperatively (prior to induction of anaesthesia), on postoperative day 2, and one month following surgery. Blood was drawn from peripheral veins, typically the right cubital vein or dorsal vein of the right hand, and collected into Cell-Free DNA BCT® stabilization tubes (Streck, Omaha, NE, USA) to preserve cellular integrity during transport.

Samples were transported via express courier service to the Institute of Molecular and Translational Medicine, Palacky University, and processed immediately upon arrival. Each sample underwent centrifugation at 2,500 × g for 15 min to separate the buffy coat. The buffy coat was subsequently isolated, stabilized using FACS Lysing Solution (BD Biosciences, San Jose, CA, USA), and stained with the CytoTrack® reagent kit (2C A/S, Copenhagen, Denmark). This kit contains a combination of fluorescently labelled antibodies including anti-EpCAM, anti-pan cytokeratin (panCK), anti-CD45, and the nuclear dye DAPI.

Following staining, samples were applied to glass CytoDiscs™ and scanned using the CytoTrack CT11™ automated fluorescence microscope (2C A/S, Copenhagen, Denmark). Circulating tumour cells (CTCs) were identified and enumerated according to standardized morphological and immunophenotypic criteria, including nuclear positivity (DAPI+), epithelial marker expression (CK20+, EpCAM+), and absence of leukocyte marker expression (CD45−), consistent with established guidelines.

Follow-up

Both short-term and long-term follow-up assessments were conducted. Short-term follow-up, performed approximately one month postoperatively, included documentation of hospital length of stay, postoperative complications, and histopathological evaluation of the resected tumour. Additional laboratory analyses were carried out, including measurements of tumour markers, C-reactive protein (CRP), and standard biochemical parameters.

Long-term follow-up extended from the date of surgery until January 1, 2025. During this period, patients were monitored for oncological outcomes, specifically focusing on recurrence and overall survival.

Data analysis

Statistical analyses were performed using R software, version 4.4.2 (R Core Team, 2018). Demographic and clinicopathological variables were analysed in relation to the presence of circulating tumour cells (CTCs) and time to recurrence (TTR). The prognostic impact of selected variables on TTR was assessed using Cox proportional hazards regression models. Kaplan–Meier survival curves were constructed to visualize TTR distributions, and the log-rank test was used to assess differences between groups. All statistical tests were two-sided, with a significance threshold set at $P<0.05$.

RESULTS

Clinical-pathological characteristics

A total of 46 patients were initially enrolled in the study. Of these, four patients were excluded from the final analysis: two due to intraoperative detection of liver

metastases and two due to a pathological complete response in the resected specimen. The final study cohort thus comprised 42 patients, including 29 men (69%) and 13 women (31%) (Table 1).

The median age of the cohort was 69.7 years (range 48–86 years). No patients were classified as underweight. The median body mass index (BMI) was 27.7 kg/m², with values ranging from 18.8 to 42.6 kg/m².

Tumour staging according to the TNM classification revealed that 12 patients (28.6%) had stage I disease, 21 patients (50%) had stage II, and 9 patients (21.4%) had stage III colorectal cancer. In terms of tumour grade, 21 tumours (50%) were classified as moderately differentiated, 9 (21.4%) as well-differentiated, and 12 (28.6%) as poorly differentiated.

Regarding tumour location, 25 patients (59.5%) presented with right-sided tumours (involving the caecum, ascending colon, hepatic flexure, or transverse colon), while 17 patients (40.5%) had left-sided tumours (in the descending colon, sigmoid colon, or rectum). The precise anatomical distribution of tumour locations is illustrated in Figure 1.

Surgical and preoperative health status

Assessment of preoperative health status using the American Society of Anaesthesiologists (ASA) physical status classification revealed that the majority of patients were classified as ASA II (22 out of 42; 52.4%). Fifteen patients (35.7%) were classified as ASA III, and five patients (11.9%) as ASA I. As previously noted, all surgical procedures were performed using an open approach; no patients underwent laparoscopic or robotic-assisted surgery. The median duration of surgery was 147.5 minutes, with operative times ranging from 70 to 275 minutes.

Circulating tumour cells detection rate

Circulating tumour cells (CTCs) were detected in at least one of the three peripheral blood samples in 34 out

Table 1. Clinicopathological characteristics of colorectal cancer patients.

Clinicopathological characteristics Number of patients = 42	
Sex	
Male	29 (69%)
Female	13 (31%)
ASA	
I	5 (11.9%)
II	22 (52.4%)
II	15 (35.7%)
UICC stage	
I	12 (28.6%)
II	21 (50%)
II	9 (21.4%)
T stage	
T1	2 (4.8%)
T2	10 (23.8%)
T3	29 (69%)
T4	1 (2.4%)
N stage	
N0	33 (78.6%)
N1	9 (21.4%)
Grade	
1	9 (21.4%)
2	21 (50%)
3	12 (28.6%)
Tumour location	
Right	25 (59.5%)
Left	17 (40.5%)
	Median (q1 –q3)
Age	69.7 (63.24–77.65)
BMI	27.7 (25.42–32.46)

ASA, American Society of Anesthesiologists; UICC, Union of International Cancer Control; T, tumour; N, nodus; BMI, Body mass index.

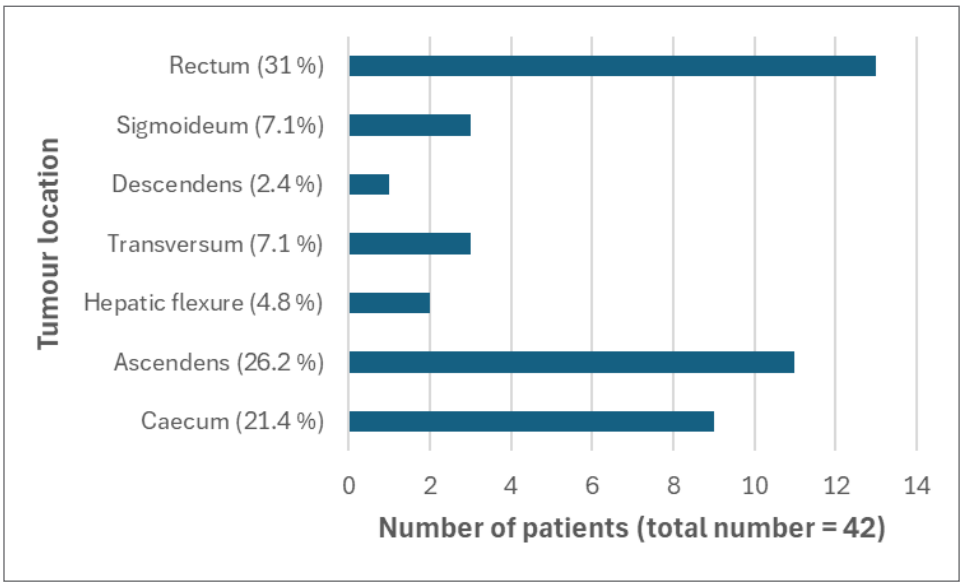

Fig. 1. The precise anatomical distribution of tumour locations of colorectal cancer patients.

of 42 patients (81%), while no CTCs were identified in any of the samples in 8 patients (19%), representing the overall CTC detection rate in this cohort.

It is important to note that a limited number of samples were either damaged or lost during transport and were therefore excluded from the final analysis. Ultimately, 38 preoperative blood samples were successfully analysed, making this the most complete dataset among the three time points. The number of analysable samples collected on postoperative day 2 and one month postoperatively was 35 and 36, respectively (Fig. 2).

Stage-dependent detection rate of circulating tumour cells

Circulating tumour cells (CTCs) were detected in at least one of the three peripheral blood samples in 66.7% (8/12) of patients with stage I disease, 90.5% (19/21) with stage II, and 77.8% (7/9) with stage III. However, the differences in overall detection rates across stages were not statistically significant (Fisher's exact test, P=0.229).

A similar pattern was observed when analysing individual time points. In preoperative samples, CTCs were detected in 27.3% (3/11) of stage I patients, 55.6% (10/18) of stage II, and 44.4% (4/9) of stage III patients (P=0.410). On postoperative day 2, detection rates were 44.4% (4/9) in stage I, 66.7% (12/18) in stage II, and 37.5% (3/8) in stage III (P=0.331). At postoperative day 30, CTCs were identified in 50% (4/8) of stage I patients, 40% (8/20) of stage II, and 37.5% (3/8) of stage III patients (P=0.905).

Although none of the differences reached statistical significance, there appears to be a trend toward higher

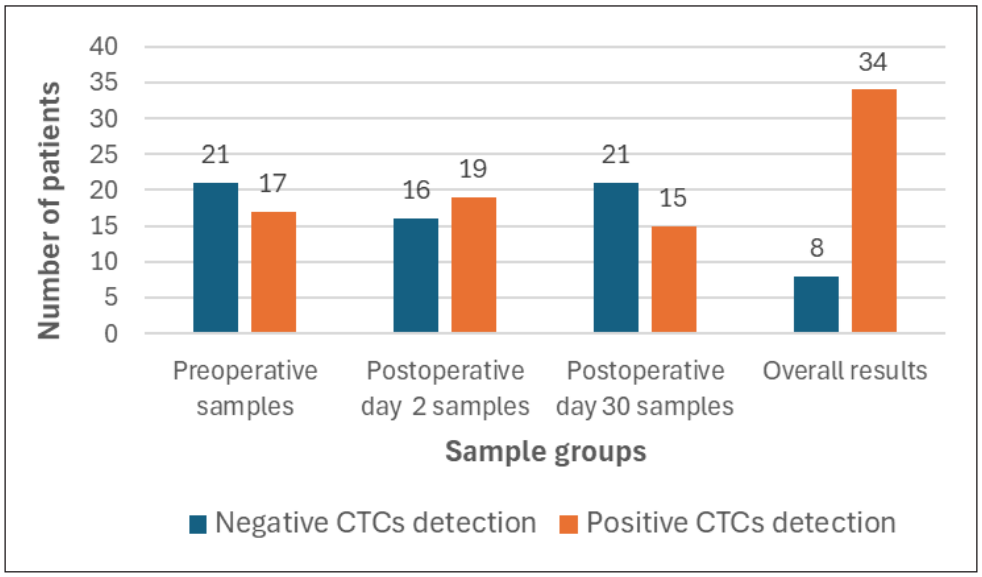

Fig. 2. The number of patients with and without detectable circulating tumour cells (CTCs) in peripheral blood samples was recorded at three time points: preoperatively, on postoperative day 2, and 30 days after surgery. Additionally, the overall CTC detection rate across all time points was calculated.

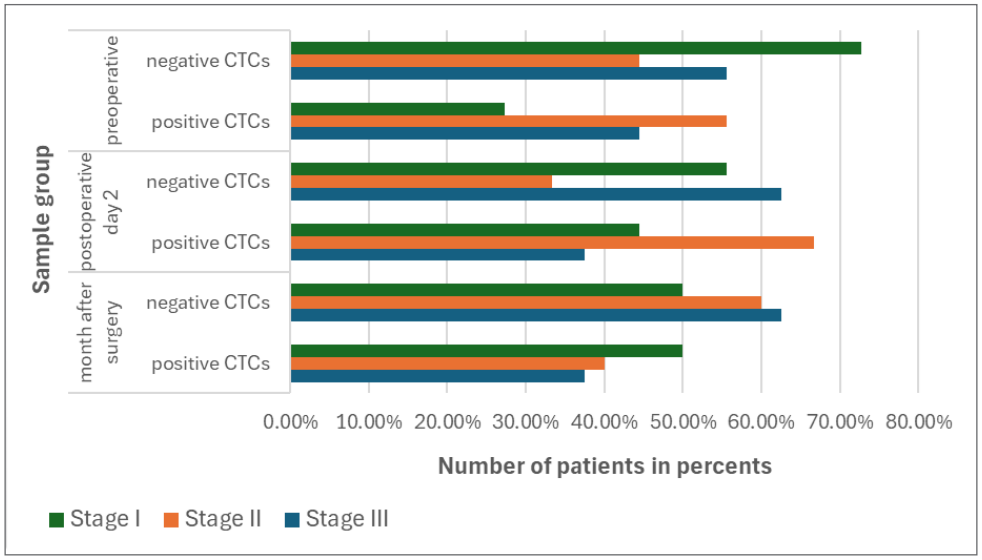

Fig. 3. Detection rates of circulating tumour cells (CTCs) in three consecutive peripheral blood samples collected from patients with stage I–III colorectal cancer.

CTC detection rates in stage II and III patients, particularly in preoperative and early postoperative (day 2) samples (Fig. 3). This trend warrants further investigation in a larger patient cohort to confirm its potential clinical relevance.

Prognostic value of circulating tumour cells

The total study duration was 71 months, with a median follow-up time of 53 months. During this period, disease recurrence occurred in 7 patients (16%), with a median time to recurrence (TTR) of 16.5 months. An additional 7 patients died; however, none of the deaths were directly attributable to cancer progression (e.g., causes included sepsis or pneumonia). Therefore, overall survival (OS) was not analysed in this study.

The prognostic significance of circulating tumour cells (CTCs) was evaluated using a univariate Cox proportional hazards model, with TTR as the primary endpoint. Patients were stratified into two groups: those with detectable CTCs in at least one of the three peripheral blood samples (n=34) and those with no detectable CTCs across all samples (n=8). No statistically significant difference in TTR was observed between the two groups (log-rank test, P=0.175). However, a notable trend was evident: none of the patients without detectable CTCs experienced recurrence during the follow-up period, whereas in the CTC-positive group, the recurrence rate was 20.6%, with a median TTR of 16.5 months.

A similar pattern was observed for blood samples collected on postoperative day 2, where the difference in TTR between CTC-positive and CTC-negative patients was also not statistically significant (log-rank test, P=0.719). Detailed survival data and Kaplan–Meier curves are presented in Table 2 and Fig. 4.

Clearance of circulating tumour cells (CTCs) was observed in 38.5% (15/39) of patients with stage I–III colorectal cancer. These patients had detectable CTCs either preoperatively or on postoperative day 2, but no CTCs were detected in blood samples collected one month after surgery. For the purpose of analysis, this group was compared to the remaining patients who did not exhibit CTC clearance, excluding three individuals who were CTC-negative at all three time points. No statistically significant difference in time to recurrence (TTR) was observed between the CTC clearance and non-clearance groups (log-rank test, P=0.291) (Table 2).

DISCUSSION

This study aimed to evaluate multiple aspects of circulating tumour cells (CTCs) detection in peripheral blood at three distinct time points, preoperatively (on the day of surgery), postoperative day 2, and one month after surgery in patients with stage I–III colorectal cancer (CRC). Our first objective was to assess factors influencing CTCs positivity, particularly the potential association between tumour stage and CTC detection.

CTCs concentrations are generally low and exhibit substantial variability in localized disease[21]. In previously published studies, CTCs are most frequently detected in patients with stage IV CRC and a high burden of liver or lung metastases[22,23]. Despite the absence of distant metastases in our study cohort, the overall CTCs detection rate was relatively high, with 81% of patients showing CTC positivity in at least one sample. However, no statistically significant correlation was observed between advancing stage and increased CTCs detection in our cohort, which may be attributable to the limited sample size. Similar findings were reported by Baek et al., who analysed blood samples from 88 patients and found no significant association between CTCs detection and tumour stage (P=0.811) (ref.[24]). Conversely, a meta-analysis that included 12 studies with 2,363 non-metastatic CRCs patients reported that 8 of those studies demonstrated a significant association between CTCs positivity and advanced stage[21]. In our dataset, although a slightly higher proportion of stage II patients were CTC-positive compared to stage I, particularly in the preoperative and early postoperative samples, this difference did not reach statistical significance. Eliasová et al. similarly reported that CTCs were detected across all disease stages, with the highest detection rate in stage II patients (92.86%) in a cohort of 98 individuals[25]. These findings suggest that tumour stage alone may not be the

Table 2. Time to recurrence (TTR) in stage I–III colorectal cancer patients based on circulating tumour cell (CTC) positivity in individual peripheral blood samples. Results are expressed as hazard ratios (HR) with corresponding statistical comparisons.

		TTR					
		number of patients	recurrences	3yrs nonrecurrence	log-rank test p-value	HR	Cox model p-value
Preoperative samples	negative	21	4 (19 %)	78.3 % ± 9.7 %	0.976		
	positive	17	3 (17.6 %)	80 % ± 10.3 %		0.98	0.976
Postoperative day 2 samples	negative	16	3 (18.8 %)	79.4 %± 10.6 %	0.719		
	positive	19	3 (15.8 %)	82.6 %± 9.1 %		0.75	0.720
Postoperative day 30 samples	negative	21	4 (19 %)	79.4 %± 9.2 %	0.675		
	negative	15	2 (13.3 %)	85.7 %± 9.4 %		0.70	0.676
Overall results	negative	8	0 (0 %)	100 %± 0%	0.175		
	positive	34	7 (20.6 %)	77.2 %± 7.6%		—	—
Clearance	no	24	3 (12.5%)	86.4 %± 7.3 %	0.290		
	yes	15	4 (26.7 %)	71.4 %± 12.1 %		2.20	0.303

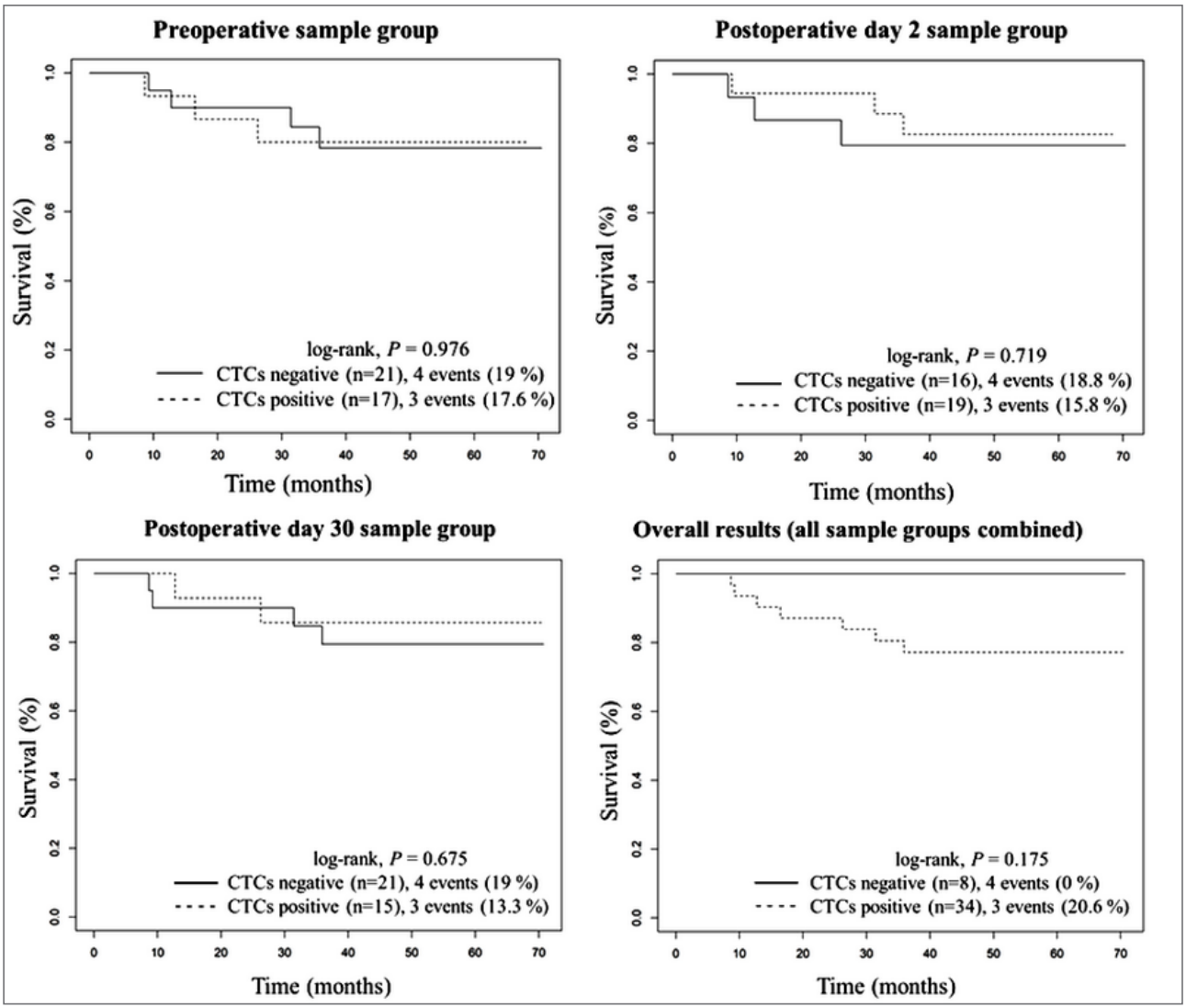

Fig. 4. Kaplan–Meier curves illustrating time to recurrence (TTR) in stage I–III colorectal cancer patients, comparing those with detectable circulating tumour cells (CTCs) in peripheral blood samples collected before and after surgery to those without detectable CTCs.

primary determinant of CTC presence. Other clinicopathological variables, such as tumour dedifferentiation and insanitation, may play a role in the release of CTCs into the bloodstream. Furthermore, technical factors such as blood sample volume and timing may affect detection rates. While 7.5 mL of peripheral blood is typically analysed, increasing the sample volume could enhance sensitivity.

Our second objective was to evaluate the prognostic utility of CTCs in non-metastatic CRC. Although CTCs have been validated as prognostic biomarkers in metastatic disease, their role in earlier stages remains under investigation. Several recent meta-analyses have explored the prognostic relevance of CTCs in localized or locoregional CRC. However, in our cohort, the presence of CTCs in any of the three blood samples was not significantly associated with time to recurrence (TTR), and CTC status failed to reach statistical significance in prognostic modeling.

In contrast, a 2018 meta-analysis by Tan et al., which included 15 studies and 3,129 patients, found that CTC positivity was associated with shorter overall survival (OS) (hazard ratio [HR]=2.36, 95% CI: 1.87–2.97, P=0.006) and progression-free survival (PFS) (HR=1.83, 95% CI: 1.42–2.36, P<0.00001) (ref.[26]). However, this analysis did not differentiate between metastatic and non-metastatic cases. A 2017 meta-analysis specifically focusing on non-metastatic CRC included 20 studies with 3,687 patients and demonstrated a strong association between CTC positivity and poor prognosis. Patients with detectable CTCs had significantly higher risks of disease progression (HR=2.57, 95% CI: 1.64–4.02, P<0.001) and reduced OS (HR=2.41, 95% CI: 1.66–3.51, P=0.002) (ref.[27]).

Nevertheless, not all studies support these associations. For example, Sotelo et al. investigated 519 patients with stage III CRC and reported no significant impact of CTCs on disease-free survival (DFS) (HR=0.97, P=0.85) or OS (HR=1.03, P=0.89) when ≥1 CTC was present in 7.5 mL of preoperative peripheral blood[28]. Similarly, another study assessing pulmonary venous blood collected during pulmonary metastasectomy in 24 patients with

CRC lung metastases found no correlation between CTC counts and either PFS or OS (ref.[29]).

One potential explanation for the absence of significant associations in our study may be the limited cohort size. This hypothesis is supported by a meta-analysis by Lu et al., which stratified studies based on sample size. Studies with fewer than 100 patients failed to show statistically significant associations between CTC positivity and DFS or OS, whereas larger studies did[27].

While our findings did not demonstrate statistical significance, they did reveal a consistent trend. Patients who were CTC-negative across all time points experienced no recurrence during follow-up, in contrast to a 20.6% recurrence rate among patients with at least one CTC-positive sample. This suggests that CTC negativity rather than positivity could serve as a valuable marker for identifying patients at low risk of recurrence. Such information may have practical implications for guiding decisions regarding adjuvant therapy. Importantly, none of the deaths observed in our study cohort were directly attributable to cancer progression therefore overall survival was not included in the analysis of CTC prognostic value.

Lastly, we aimed to evaluate the prognostic significance of CTCs clearance in non-metastatic colorectal cancer (CRC). While the dynamics of CTCs levels and their clearance have been extensively studied in metastatic malignancies, data in early-stage disease are limited. In metastatic prostate and breast cancer, failure to clear CTCs or an increase in CTCs counts during treatment has been associated with significantly worse outcomes[30,31]. In metastatic CRC, Souza e Silva et al. investigated CTCs kinetics in a cohort of 54 patients by comparing baseline CTCs levels to those measured at the first follow-up. Patients who achieved CTCs clearance demonstrated improved progression-free survival (PFS) of 14.7 months compared to 10.3 months in those with persistent CTCs, and 6.9 months in patients whose status shifted from CTC-negative to CTC-positive (P=0.6) (ref.[32]). Although this finding did not reach statistical significance, it suggested a potential prognostic role of CTCs kinetics. A larger prospective multicentre study involving 430 patients with metastatic CRC further supported this concept. Patients who presented with ≥3 CTCs at baseline and showed a reduction to <3 CTCs within 3–5 weeks had significantly longer survival compared to those with persistently elevated CTCs (PFS: 6.2 vs. 1.6 months, P=0.02; OS: 11.0 vs. 3.7 months, P=0.0002) (ref.[33]). However, not all studies report consistent findings. In a study of 81 patients with stage IV non-small cell lung cancer, CTCs levels measured at various time points during therapy revealed an unexpected trend: patients who showed an increase in CTCs during the first three months of treatment had improved OS and PFS (P=0.0478), suggesting that in some tumour types or treatment contexts, the prognostic role of CTCs dynamics may differ[34].

In our study, no statistically significant difference was observed between patients with and without CTCs clearance, defined as initial CTCs positivity followed by the absence of CTCs in the one-month postoperative blood sample. To the best of our knowledge, no prior study has specifically investigated the prognostic significance of perioperative CTCs clearance in patients with stage I–III colorectal cancer (CRC). Although our findings did not demonstrate statistical significance and were limited by the relatively small sample size, they provide preliminary evidence that supports the need for further investigation. Larger, well-designed prospective studies are warranted to validate CTC clearance as a potential prognostic biomarker in early-stage CRC.

Most of the previously cited studies evaluating circulating tumour cells have utilized either the CellSearch® system or reverse transcription polymerase chain reaction (RT-PCR) for detection. In contrast, our study employed the CytoTrack® detection system, a less commonly used but promising method for CTCs identification. As of January 2025, only one other study indexed in PubMed® has assessed CTCs using CytoTrack in the context of non-metastatic colorectal cancer. That study included a cohort of just 20 patients and reported a low LTC detection rate–positive findings in only 3 out of 40 blood samples. Moreover, the prognostic significance of CTCs was not investigated in that study[35]. These factors highlight the relevance of our findings, particularly in demonstrating the feasibility, detection rate, and potential clinical utility of this relatively novel technology.

The primary limitation of our study was the small sample size, which likely influenced the statistical power and limited the ability to detect significant associations between CTCs status and clinical outcomes. Current evidence suggests that larger patient cohorts are critical to establishing the prognostic value of CTCs with adequate confidence. Another limitation is the inclusion of patients receiving both neoadjuvant and adjuvant therapies, which introduces treatment heterogeneity and may influence CTCs dynamics independently of tumour biology. Furthermore, the median follow-up duration, although sufficient for preliminary conclusions, may not have been long enough to capture all recurrence events in patients with non-metastatic disease.

Future research should aim to address these limitations by enrolling larger, homogeneous patient populations and extending follow-up durations. Longitudinal blood sampling at multiple time points beyond the first postoperative month could offer additional insights into the kinetics of CTCs clearance or re-emergence. These findings could then be correlated with radiologic recurrence to enhance early detection strategies and personalize postoperative surveillance in CRC patients.

CONCLUSION

Although not statistically significant, our study yielded several noteworthy findings. The sustained absence of circulating tumour cells in the peripheral blood of patients with non-metastatic colorectal cancer was associated with an extremely low recurrence rate, suggesting the potential utility as a negative prognostic indicator. Importantly, no association was observed between standard clinicopathological characteristics and CTCs presence in our cohort.

A distinguishing feature of our study is the use of a relatively novel detection technique, semi-automated fluorescence microscopy via the CytoTrack® system without prior CTCs enrichment. This contrasts with many prior studies that relied on enrichment-based platforms such as CellSearch® or RT-PCR. Despite our relatively small and heterogeneous patient population, the results contribute meaningful data to the existing body of literature and underscore the need for larger, prospective studies to validate the role of CTCs dynamics in the management of non-metastatic CRC.

ABBREVIATIONS

ASA,American Society of Anesthesiologists; CTCs, circulating tumour cells; CRC, colorectal cancer; 5-FU, 5-fluorouracil; EpCAM, epithelial cell adhesion molecule; FDA, U.S. Food and Drug Administration; MRD, minimal residual disease; UICC, Union of International Cancer Control; OS, overall survival; TTR, time to recurrence; RT-PCR, reverse transcription polymerase chain reaction.

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REFERENCES

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024;74(3):229-63. doi: 10.3322/caac.21834
2. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, van de Velde CJ, Watanabe T. Colorectal cancer. Nat Rev Dis Primers 2015;1:15065. doi: 10.1038/nrdp.2015.65
3. Cardoso R, Guo F, Heisser T, De Schutter H, Van Damme N, Nilbert MC, Christensen J, Bouvier AM, Bouvier V, Launoy G, Woronoff AS, Cariou M, Robaszkiewicz M, Delafosse P, Poncet F, Walsh PM, Senore C, Rosso S, Lemmens VEPP, Elferink MAG, Tomšič S, Žagar T, Marques ALM, Marcos-Gragera R, Puigdemont M, Galceran J, Carulla M, Sánchez-Gil A, Chirlaque MD, Hoffmeister M, Brenner H. Overall and stage-specific survival of patients with screen-detected colorectal cancer in European countries: A population-based study in 9 countries. Lancet Reg Health Eur 2022;21:100458. doi: 10.1016/j.lanepe.2022.100458
4. Storli PE, Dille-Amdam RG, Skjærseth GH, Gran MV, Myklebust TÅ, Grønbech JE, Bringeland EA. Cumulative incidence of first recurrence after curative treatment of stage I-III colorectal cancer. Competing risk analyses of temporal and anatomic patterns. Acta Oncol 2023;62(12):1822-30. doi: 10.1080/0284186X.2023.2269644
5. Buyse M, Zeleniuch-Jacquotte A, Chalmers TC. Adjuvant therapy of colorectal cancer. Why we still don't know. JAMA 1988;259(24):3571-8.
6. Argilés G, Tabernero J, Labianca R, Hochhauser D, Salazar R, Iveson T, Laurent-Puig P, Quirke P, Yoshino T, Taieb J, Martinelli E, Arnold D; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2020;31(10):1291-305. doi: 10.1016/j.annonc.2020.06.022
7. Morris EJ, Maughan NJ, Forman D, Quirke P. Who to treat with adjuvant therapy in Dukes B/stage II colorectal cancer? The need for high quality pathology. Gut 2007;56(10):1419-25. doi: 10.1136/gut.2006.116830
8. Kannarkatt J, Joseph J, Kurniali PC, Al-Janadi A, Hrinczenko B. Adjuvant Chemotherapy for Stage II Colon Cancer: A Clinical Dilemma. J Oncol Pract 2017;13(4):233-41. doi: 10.1200/JOP.2016.017210
9. Chakrabarti S, Peterson CY, Sriram D, Mahipal A. Early stage colon cancer: Current treatment standards, evolving paradigms, and future directions. World J Gastrointest Oncol 2020;12(8):808-32. doi: 10.4251/wjgo.v12.i8.808
10. Smit DJ, Schneegans S, Pantel K. Clinical applications of circulating tumor cells in patients with solid tumors. Clin Exp Metastasis 2024;41(4):403-11. doi: 10.1007/s10585-024-10267-5
11. Ashworth TR. A case of cancer in which cells similar to those in the tumours were seen in the blood after death. Aust Med J 1869;14:146-7.
12. Hu M, Wang Z, Wu Z, Ding P, Pei R, Wang Q, Xing C. Circulating tumor cells in colorectal cancer in the era of precision medicine. J Mol Med (Berl) 2022;100(2):197-213. doi: 10.1007/s00109-021-02162-3
13. Meng S, Tripathy D, Frenkel EP, Shete S, Naftalis EZ, Huth JF, Beitsch PD, Leitch M, Hoover S, Euhus D, Haley B, Morrison L, Fleming TP, Herlyn D, Terstappen LW, Fehm T, Tucker TF, Lane N, Wang J, Uhr JW. Circulating tumor cells in patients with breast cancer dormancy. Clin Cancer Res 2004;10(24):8152-62. doi: 10.1158/1078-0432.CCR-04-1110
14. Heidrich I, Abdalla TSA, Reeh M, Pantel K. Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as a Liquid Biopsy Marker in Colorectal Cancer. Cancers (Basel) 2021;13(18):4500. doi: 10.3390/cancers13184500
15. Vidlarova M, Rehulkova A, Stejskal P, Prokopova A, Slavik H, Hajduch M, Srovnal J. Recent Advances in Methods for Circulating Tumor Cell Detection. Int J Mol Sci 2023;24(4):3902. doi: 10.3390/ijms24043902
16. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, Tibbe AG, Uhr JW, Terstappen LW. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res 2004;10(20):6897-904. doi: 10.1158/1078-0432.CCR-04-0378
17. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse M, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LW, Meropol NJ. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 2008;26(19):3213-21. doi: 10.1200/JCO.2007.15.8923 Erratum in: J Clin Oncol 2009;27(11):1923.
18. Alix-Panabières C, Mader S, Pantel K. Epithelial-mesenchymal plasticity in circulating tumor cells. J Mol Med (Berl) 2017;95(2):133-42. doi: 10.1007/s00109-016-1500-6
19. Hillig T, Horn P, Nygaard AB, Haugaard AS, Nejlund S, Brandslund I, Sölétormos G. In vitro detection of circulating tumor cells compared by the CytoTrack and CellSearch methods. Tumour Biol 2015;36(6):4597-601. doi: 10.1007/s13277-015-3105-z
20. Salu P, Reindl KM. Advancements in Circulating Tumor Cell Research: Bridging Biology and Clinical Applications. Cancers (Basel) 2024;16(6):1213. doi: 10.3390/cancers16061213
21. Yang C, Zou K, Zheng L, Xiong B. Prognostic and clinicopathological significance of circulating tumor cells detected by RT-PCR in non-metastatic colorectal cancer: a meta-analysis and systematic review. BMC Cancer 2017;17(1):725. doi: 10.1186/s12885-017-3704-8
22. Kaifi JT, Kunkel M, Zhu J, Dicker DT, Gusani N, Yang Z, Sarwani NE, Li G, Kimchi ET, Staveley-O'Carroll KF, El-Deiry WS. Circulating tumor cells are associated with diffuse spread in stage IV colorectal can-

cer patients. Cancer Biol Ther 2013;14(12):1174-81. doi: 10.4161/cbt.26884
23. Kaifi JT, Kunkel M, Dicker DT, Joude J, Allen JE, Das A, Zhu J, Yang Z, Sarwani NE, Li G, Staveley-O'Carroll KF, El-Deiry WS. Circulating tumor cell levels are elevated in colorectal cancer patients with high tumor burden in the liver. Cancer Biol Ther 2015;16(5):690-8. doi: 10.1080/15384047.2015.1026508
24. Baek DH, Kim GH, Song GA, Han IS, Park EY, Kim HS, Jo HJ, Ko SH, Park DY, Cho YK. Clinical Potential of Circulating Tumor Cells in Colorectal Cancer: A Prospective Study. Clin Transl Gastroenterol 2019;10(7):e00055. doi: 10.14309/ctg.0000000000000055
25. Eliasova P, Pinkas M, Kolostova K, Gurlich R, Bobek V. Circulating tumor cells in different stages of colorectal cancer. Folia Histochem Cytobiol 2017;55(1):1-5. doi: 10.5603/FHC.a2017.0005
26. Tan Y, Wu H. The significant prognostic value of circulating tumor cells in colorectal cancer: A systematic review and meta-analysis. Curr Probl Cancer 2018;42(1):95-106. doi: 10.1016/j.currprobl-cancer.2017.11.002
27. Lu YJ, Wang P, Peng J, Wang X, Zhu YW, Shen N. Meta-analysis Reveals the Prognostic Value of Circulating Tumour Cells Detected in the Peripheral Blood in Patients with Non-Metastatic Colorectal Cancer. Sci Rep 2017;7(1):905. doi: 10.1038/s41598-017-01066-y
28. Sotelo MJ, Sastre J, Maestro ML, Veganzones S, Viéitez JM, Alonso V, Grávalos C, Escudero P, Vera R, Aranda E, García-Alfonso P, Gallego-Plazas J, Lopez C, Pericay C, Arrivi A, Vicente P, Ballesteros P, Elez E, López-Ladrón A, Díaz-Rubio E. Role of circulating tumor cells as prognostic marker in resected stage III colorectal cancer. Ann Oncol 2015;26(3):535-41. doi: 10.1093/annonc/mdu568
29. Le UT, Bronsert P, Picardo F, Riethdorf S, Haager B, Rylski B, Czerny M, Beyersdorf F, Wiesemann S, Pantel K, Passlick B, Kaifi JT, Schmid S. Intraoperative detection of circulating tumor cells in pulmonary venous blood during metastasectomy for colorectal lung metastases. Sci Rep 2018;8(1):8751. doi: 10.1038/s41598-018-26410-8 Erratum in: Sci Rep 2020;10(1):7633. doi: 10.1038/s41598-020-64151-9
30. Wang C, Zhang Z, Chong W, Luo R, Myers RE, Gu J, Lin J, Wei Q, Li B, Rebbeck TR, Lu-Yao G, Kelly WK, Yang H. Improved Prognostic Stratification Using Circulating Tumor Cell Clusters in Patients with Metastatic Castration-Resistant Prostate Cancer. Cancers (Basel) 2021;13(2):268. doi: 10.3390/cancers13020268
31. Magbanua MJM, Savenkov O, Asmus EJ, Ballman KV, Scott JH, Park JW, Dickler M, Partridge A, Carey LA, Winer EP, Rugo HS. Clinical Significance of Circulating Tumor Cells in Hormone Receptor-positive Metastatic Breast Cancer Patients who Received Letrozole with or Without Bevacizumab. Clin Cancer Res 2020;26(18):4911-20. doi: 10.1158/1078-0432.CCR-20-1329
32. Souza E Silva V, Chinen LT, Abdallah EA, Damascena A, Paludo J, Chojniak R, Dettino AL, de Mello CA, Alves VS, Fanelli MF. Early detection of poor outcome in patients with metastatic colorectal cancer: tumor kinetics evaluated by circulating tumor cells. Onco Targets Ther 2016;9:7503-13. doi: 10.2147/OTT.S115268
33. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse M, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LW, Meropol NJ. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 2008;26(19):3213-21. doi: 10.1200/JCO.2007.15.8923 Erratum in: J Clin Oncol 2009;27(11):1923.
34. Shishido SN, Carlsson A, Nieva J, Bethel K, Hicks JB, Bazhenova L, Kuhn P. Circulating tumor cells as a response monitor in stage IV non-small cell lung cancer. J Transl Med 2019;17(1):294. doi: 10.1186/s12967-019-2035-8
35. Gogenur M, Hillig T, Gogenur I. CytoTrack Analysis Reveals Low Presence of Circulating Tumor Cells in the Perioperative Period in Patients with Non-metastatic Colorectal Cancer. Anticancer Res 2017;37(6):3099-103. doi: 10.21873/anticanres.11666