Prognostic Value of Preoperative Circulating Tumor Cell Counts in Patients with UICC-Stage I-IV Colorectal Cancer

Thaer Abdalla (thaer.abdalla@gmail.com)
Department of General, Thoracic and Visceral Surgery, University Medical Center Hamburg-Eppendorf, Germany

Jan Meiners
Department of General, Thoracic and Visceral Surgery, University Medical Center Hamburg-Eppendorf, Germany

Alexandra König
Department of General Surgery, Hospital Wilhelmshaven

Nathaniel Melling
Department of General, Thoracic and Visceral Surgery, University Medical Center Hamburg-Eppendorf, Germany

Karl Karstens
Department of General, Thoracic and Visceral Surgery, University Medical Center Hamburg-Eppendorf, Germany

Tobias Gorges
Department of Tumor Biology, University Medical Center Hamburg-Eppendorf

Sabine Riethdorf
Department of Tumor Biology, University Medical Center Hamburg-Eppendorf

Jakob Izbicki
Department of General, Thoracic and Visceral Surgery, University Medical Center Hamburg-Eppendorf, Germany

Klaus Pantel
Department of Tumor Biology, University Medical Center Hamburg-Eppendorf

Matthias Reeh
Department of General, Thoracic and Visceral Surgery, University Medical Center Hamburg-Eppendorf, Germany

---

Research Article

Keywords: colorectal cancer, circulating tumor cells, CellSearch, prognostic markers

Posted Date: December 21st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-129872/v1

Abstract

The detection of CTCs in peripheral blood is one of the most promising approaches to identify disseminated disease in colorectal cancer (CRC). This study aims to evaluate the prognostic relevance of preoperative CTCs using the Cellsearch® system (CS)in patients, who underwent resection with curative intent of different stages of colorectal cancer (UICC I-IV). CTC analysis was performed in 68 CRC patients at UICC stages I-IV immediately before surgery. Data were correlated with clinicopathological parameters and patient outcomes. One or more CTCs/7.5 mL were detected in 45.6% (31/68) of patients. CTCs were detected in all stages of the Union of International Cancer Control (UICC), in stage I (1/4, 25%), in stage II (4/12, 33.3%), in stage III (5/19, 26.3%) in stage IV (21/33, 63.6%).The detection of CTCs was associated to the UICC stage (p = 0.035) and to the presence of distant overt metastases (p = 0.014). The presence of ≥ 1 CTCs/ 7.5 ml correlated significantly with shorter progression-free (p = 0.013) and overall survival (p = 0.014). Multivariate analyses showed that preoperative CTCs are an independent prognostic indicator for overall survival (HR, 2.68; 95% CI, 1.05–6.92 7; p = 0.039, ≥ 1 CTC). In conclusion, detection of CTCs is an independent and strong prognostic factor in CRC, which might improve the identification of high-risk patients in future clinical trials.

Introduction

Colorectal cancer (CRC) is the 3rd most commonly diagnosed cancer and the 4th leading cause of death in the world with 1.3 million new cases annually (1). Two-thirds of the patients with CRC present with localized and potentially curable disease at diagnosis (TNM Stage I-III) (2). In these patients, surgery remains the most important treatment modality.

Currently, the overall 5-year survival is 65% (1). This increased in the last four decades after the introduction of screening programs and the concept of adjuvant chemotherapy (3, 4). Surgical technique also evolved with the introduction of total mesorectal excision (TME) for rectal cancer and central venous ligature (CVL) with complete mesocolic excision (CME) for colon cancer, where sharp dissection in the embryological planes increases lymph node yields, subsequently improving staging and survival(5, 6). Nevertheless, tumor recurrence or spread to distant sites and formation of metastases still occur in 20% of the patients and is the leading cause of death in these patients (7). Even in patients with apparently early stages TNM (I-II), local recurrence or distant metastases occur despite proper treatment (8).

Tumor persistence and progression occur mainly due to circulating tumor cells (CTCs) which are seeded from the primary tumor and target distant organs, where they eventually mature and cause a secondary metastasis (9). Liquid biopsy for identification of CTC preoperatively is of proven utility in predicting prognosis in breast, colon, and prostate cancer. (10–12).

The 8th AJCC Cancer Staging Manual expanded the definitions of Tis, T4a, and M1 and nodal micrometastasis in CRC (13). However, unlike in breast cancer, new staging categories like M0 (i+), in which CTC or disseminated tumor cells in bone marrow are detected, are still lacking (14).

Identifying CTCs preoperatively in liquid biopsies could provide information to solve common dilemmas in CRC (15), like patient selection for adjuvant chemotherapy in stage II CRC as well as selecting patients for D2 or D3 lymphadenectomy in right hemicolectomy.

The isolation and molecular analysis of CTCs in peripheral blood is one of the most promising approaches to identify disseminated disease, in order to upgrade or downgrade the multimodal therapy. The CellSearch® (CS) (Menarini, Silicon Biosystems, Bologna, Italy), a known method for quantification of CTCs based on the expression of the epithelial cell adhesion molecule (EpCAM) and of keratin, is the first standardized system approved by the U.S. Food and Drug Administration for capturing and detection of CTCs derived from metastatic breast and prostate cancer as well as metastatic CRC (10, 16).

The aim of this study is to assess the preoperative value of CTCs in different stages of CRC on the overall survival and progression-free survival using CS.

Method And Material

Study Design

This prospective study was conducted at the University Hospital Hamburg-Eppendorf in Germany and enrolled 68 patients with different stages of colorectal cancer, who underwent surgical resection for CRC. Informed consent was obtained from all patients. The study was approved by the medical ethics committee of the Chamber of Physicians of Hamburg.

This study included only patients who underwent primary resection for colorectal cancer as well as patients after neoadjuvant radiotherapy for rectal cancer. Peripheral blood samples for CTC analysis were collected immediately before surgery and postoperatively during the first 96 hours. Follow up was conducted according to S3 German Guidelines (17). Events considered were death, local recurrence, and distant metastasis. Overall survival was the time from operation to death or last follow-up; and progression-free survival was defined as the time from operation to the diagnosis of tumor recurrence.

CTC Analysis

CTC analysis was performed using CS as previously described(18). Blood samples (7.5 mL) were collected in CellSave preservative tubes, stored at room temperature and processed within 96 hours after blood collection, according to the manufacturer’s instructions. The accuracy and reproducibility of CS have been described previously (18, 19). The presence of a nucleus, cytokeratin expression, round or oval cell morphology, and absent CD45 expression were the criteria for CTCs(18).

Statistical Analysis

SPSS statistic software version 25 was used. Histological characteristics were expressed as descriptive statistics. The x^2 was used to investigate the association between CTCs and histopathological parameters. Survival rates were determined using the Kaplan–Meier method and were compared using the log-rank test. A multivariate analysis of factors that might influence OS was performed using the Cox proportional hazards regression model. The results were presented as hazard ratios with 95% CI. All comparisons were two-tailed. A *p*-value of less than 0.05 was considered statistically significant.

All authors had access to the study data and had reviewed and approved the final manuscript.

Results

Patient Characteristics and CTC Detection

CTC analysis was performed in blood samples from 68 patients preoperatively. 38 patients had CTC analysis within the first 96 hours after the procedure. Forty-three patients were nodal positive. Distant metastasis was present in 34 patients at the time of operation (Table 1). Of the 68 patients, 44 were males. The median age was 64.7 years (range, 18–88 years).

Table 1
Site of Metastasis

Site of Metastasis	Number of Patients
Lungs	4
Peritoneum	9
Liver	21

Applying a cut-off of ≥ 1 CTC, 31 out of 68 (45%) patients were CTC-positive preoperatively and 13 out of 38 (19.1%) patients were postoperatively CTC-positive. In 5 out of 13 patients, CTCs were not present preoperatively.

We assessed the correlation between CTC detection preoperatively with sex, age and the following histopathologic parameters: Grade of differentiation (G), tumor Invasion (T), nodal status (N), metastases (M), Union of International cancer control (UICC-Stage), HER2, KRAS, MSI mutations and tumor location (Table 2). The detection of CTCs was related to the presence of distant metastases *(p = 0.014)* and to the Union of International cancer control stage *(p = 0.035)*. Other parameters did not significantly correlate with CTC positivity preoperatively. Postoperatively, CTC detection did not correlate with any of the above-mentioned parameters.

Table 2
Patient Characteristics and Correlation of CTCs at Baseline with Clinicopathological Parameters

Variables	Preoperative ≥ 1 CTC		
	All	CTC-Positive	p-value
All	68	31 (45.6%)	
Age			0.812
< 65	20	8 (40%)	
65–74	28	13 (46.4%)	
≥ 75	20	10 (50%)	
Sex			0.320
Male	44	18 (40.9%)	
Female	24	13 (54.2%)	
Grade			0.636
G1	1	0 (0%)	
G2	48	23 (49.9%)	
G3	13	6 (46.2%)	
No Grading*	6		
Tumor size			0.270
T1	3	0 (0%)	
T2	8	4 (50%)	
T3	38	16 (42.1%)	
T4	19	11 (57.9%)	
Nodal status			0.707
N0	25	12 (48%)	
N1	14	5 (35.7%)	
N2	29	14 (48.3%)	
Metastatic stage			0.014

Legend: *P*-value Indicates significance according to the χ2 test when CTC-negative patients are compared with CTC-positive patients. Round parentheses indicate percentages. Other* Transverse colon, synchronous colorectal cancer. No Grading* after radiotherapy for rectal cancer, instead of Dworak's system of tumor regression.

Variables	Preoperative ≥ 1 CTC		
	All	CTC-Positive	p-value
M0	34	10 (29.4%)	
M1	34	21 (61.8%)	
UICC Stage			0.035
Stage I	4	1 (25%)	
Stage II	12	4 (33.3%)	
Stage III	19	5 (26.3%)	
Stage IV	33	21 (63.6%)	
Tumor site			0.291
Right side	17	5 (29.4%)	
Left side	10	6 (60%)	
Rectum	27	14 (51.9%)	
Other*	4	1 (25%)	
HER2-Mutation			0.527
negative	44	22(50%)	
positive	4	2 (50%)	
missing	20	7 (35%)	
KRAS-Mutation			0.345
negative	24	10 (41.7%)	
positive	29	16(55.2%)	
missing	15	5 (33.3%)	
MSI			0.718
present	5	2 (40%)	
not present	25	13 (52%)	
missing	38	16 (42.1%)	

Legend: *P*-value Indicates significance according to the $\chi 2$ test when CTC-negative patients are compared with CTC-positive patients. Round parentheses indicate percentages. Other* Transverse colon, synchronous colorectal cancer. No Grading* after radiotherapy for rectal cancer, instead of Dworak's system of tumor regression.

Univariate and Multivariate Analysis of Survival

The median survival time was 32 months. Kaplan-Meier's univariate analysis showed that patients with ≥ 1 CTC had significantly shorter progression-free ($p = 0.008$) as well as overall survival ($p = 0.008$) compared to CTC-negative patients (Fig. 1).

Also, presence of distant metastases at baseline was associated with shorter OS (p-value 0.002) and shorter PFS (p-value < 0.001) (Fig. 3). However, postoperative CTCs did not correlate with OS (p-value = 0.829) and PFS (p-value 0.876) (Fig. 2).

Nine clinicopathological factors were analyzed using Cox-Regression analysis. Only 4 factors correlated with survival in univariate analysis (Table 3). These included age, metastatic disease, UICC stage, preoperative CTC detection ($p < 0.05$). Gender, postoperative CTC detection, and other factors were not related to survival in the univariate analysis.

Table 3
Univariate and Multivariate analysis of overall survival in patients with CRC

Univariate Analysis				**Multivariate Analysis**		
	HR	**95% CI**	p	**HR**	**95% CI**	p
Age, < 65,65–74, ≥ 75	2.95	1.50–5.80	0.002	2.81	1.52–5.18	0.001
Sex, male vs female	0.833	0.37–1.87	0.650	1.2	0.52–2.73	0.660
Grade of Differentiation, G1-3	1.30	0.51–3.33	0.585			
T, T1-T4	1.26	0.73–2.19	0.392			
N, N0- N2	1.09	0.67–1.78	0.710			
Metastatic stage, M0 vs M1	3.01	1.11–8.20	0.019	1.55	0.49–2.89	0.668
UICC Stage	2.07	1.09–3.92	0.009	1.55	0.49–4.89	0.285
Preoperative CTCs, neg vs pos	2.85	1.25–6.46	0.012	2.68	1.05–6.92	0.039
Postoperative CTCs, neg vs pos	1.53	0.43–5.51	0.515			
Legend: *p* Indicates significance according to cox regression analysis comparing the specified variables. HR indicates hazard ratio.						

The four factors significantly related to survival in univariate analysis were evaluated in the multivariate analysis, which showed that only advanced age and preoperative CTC detection were independent prognosticators of an unfavourable OS (Table 3).

Discussion

Despite advances in multimodal treatment, CRC is the second most common cause of cancer death worldwide (1). CTCs play a pivotal role in disease progression, metastasis, and recurrence (20). Aside from TNM classification and residual tumor status, novel tools and staging systems are needed for adequate prognostic staging and for guiding multimodal therapy (21). CTC identification has proven to be an important prognostic factor in breast (22). For this reason, a decade ago, 7th AJCC Cancer Staging Manual introduced the new category M0(i) for breast cancer, which is defined by the presence of circulating or disseminated tumor cells not exceeding 0.2 mm detectable in bone marrow, circulating blood or other non-regional tissues of non-metastatic patients. In contrast, such a category is still lacking in the current CRC Staging system (23) due to the fact that the assessment of CTCs in CRC is still controversial (16) and different technical platforms have provided conflicting results (24–26). Here, we applied the FDA-cleared Cellsearch® system, which uses immunomagnetic enrichment (EpCAM) and immunocytochemical (cytokeratin, CD45, DAPI) analysis. Compared to immunocytochemistry, CS is standardized, more time efficient and provides better CTC enrichment(16, 18, 27). In our cohort, we found CTCs in 45.6% % of the patients before surgical resection. CTCs were present in all stages of CRC. Even in early stage CRC, UICC I (T1-2, N0, M0), one of four patients had detectable CTCs.

Up to now, there is no consensus regarding the threshold used to define CTC positivity in CRC(16). Cohen et al have used the cut-off of ≥ 3 CTC/7.5 ml for defining CTC positivity in metastatic CRC(12), while others have shown that cut-offs ≥ 1 CTC/7.5 ml and ≥ 2 CTC/7.5 ml were also associated with poor prognosis in CRC(28, 29).

We used a strict cut-off of ≥ 1 CTC/7.5 ml. CTC detection before surgery was associated significantly with a shorter progression-free (P = 0.008) and overall survival (P = 0.008). Multivariate analyses identified CTCs as a strong, independent, prognostic indicator for overall survival.

Many studies have shown inferior survival for right-sided tumors (32, 33), so we assessed the effect of tumor location on CTC enumeration. Right-sided colon cancer (RCC), left-sided colon cancer (LCC) and rectal cancer (RC) were considered independently, in light of the “three entities” hypothesis in colorectal cancer(34–36). Although more CTCs were present in LCC compared to RCC and RC, this was not statistically significant. A previous publication from Nicolazzo et al. showed similar results (34). However, larger cohorts are needed to deliver a robust conclusion about tumor sidedness and CTC detection.

From a clinical perspective, using CTCs in peripheral blood allows assessing cancer prognosis in a non-invasive and easily applicable method (16) that allows real-time monitoring of tumor dynamics (37). Our present study supports that preoperative CTC detection in CRC identifies patients with shorter OS and PFS, which might contribute to an improved stratification of high-risk CRC patients. Nevertheless, larger validation studies are required before implementation of CTC detection into tumor staging classification of CRC.

Conclusion

Preoperative CTC detection is an important prognostic marker for survival in CRC, which can be further developed as enrichment tool to study a high-risk population of CRC patients in clinical trials.

Declarations

Author contributions

M.R and K.P. conceived the idea and designed the study. J.M. and K.K. contributed to the collection of the samples and clinical data. S.R., J.T., K.P analyzed the blood samples for CTC-detection. J.I., A.K. and N.M. supervised the study. T.A. analyzed the data and wrote the main manuscript text. M.R., T.A., K.P. S.R. were major contributors in writing the manuscript. All the authors reviewed the paper.

Additional information

The authors declare no competing interest. No grants or fellowships were used to support the paper. All procedures were under good clinical practice and within the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all patients.

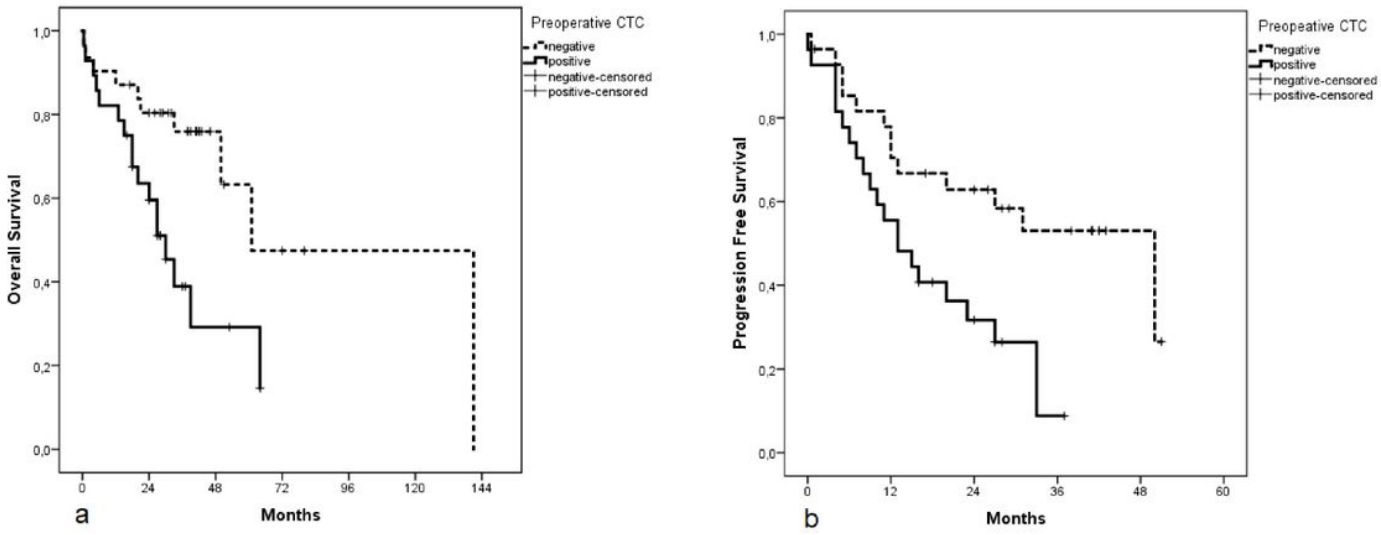
References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-86.
2. Allemani C, Rachet B, Weir HK, Richardson LC, Lepage C, Faivre J, et al. Colorectal cancer survival in the USA and Europe: a CONCORD high-resolution study. BMJ Open. 2013;3(9):e003055.
3. Strey CW, Wullstein C, Adamina M, Agha A, Aselmann H, Becker T, et al. Laparoscopic right hemicolectomy with CME: standardization using the "critical view" concept. Surg Endosc. 2018;32(12):5021–30.
4. Lirici MM, Huscher CG. Techniques and technology evolution of rectal cancer surgery: a history of more than a hundred years. Minim Invasive Ther Allied Technol. 2016;25(5):226–33.
5. Hohenberger W, Weber K, Matzel K, Papadopoulos T, Merkel S. Standardized surgery for colonic cancer: complete mesocolic excision and central ligation–technical notes and outcome. Colorectal Dis. 2009;11(4):354–64; discussion 64 – 5.
6. Heald RJ, Santiago I, Pares O, Carvalho C, Figueiredo N. The Perfect Total Mesorectal Excision Obviates the Need for Anything Else in the Management of Most Rectal Cancers. Clin Colon Rectal Surg. 2017;30(5):324–32.
7. Labianca R, Nordlinger B, Beretta GD, Mosconi S, Mandala M, Cervantes A, et al. Early colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013;24 Suppl 6:vi64-72.

8. Young PE, Womeldorph CM, Johnson EK, Maykel JA, Brucher B, Stojadinovic A, et al. Early detection of colorectal cancer recurrence in patients undergoing surgery with curative intent: current status and challenges. J Cancer. 2014;5(4):262–71.
9. Liberko M, Kolostova K, Bobek V. Essentials of circulating tumor cells for clinical research and practice. Crit Rev Oncol Hematol. 2013;88(2):338–56.
10. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2004;351(8):781–91.
11. de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res. 2008;14(19):6302–9.
12. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, et al. 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.
13. Weiser MR. AJCC 8th Edition: Colorectal Cancer. Ann Surg Oncol. 2018;25(6):1454–5.
14. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th Edition of the AJCC Cancer Staging Manual and the Future of TNM. Annals of Surgical Oncology. 2010;17(6):1471–4.
15. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med. 2016;8(346):346ra92.
16. Huang X, Gao P, Song Y, Sun J, Chen X, Zhao J, et al. Meta-analysis of the prognostic value of circulating tumor cells detected with the CellSearch System in colorectal cancer. BMC Cancer. 2015;15:202.
17. Pox C, Aretz S, Bischoff SC, Graeven U, Hass M, Heussner P, et al. [S3-guideline colorectal cancer version 1.0]. Z Gastroenterol. 2013;51(8):753–854.
18. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, et al. 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.
19. Riethdorf S, Fritsche H, Muller V, Rau T, Schindlbeck C, Rack B, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. Clin Cancer Res. 2007;13(3):920–8.
20. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nature Reviews Cancer. 2003;3(6):453–8.
21. Hoeppner J, Kulemann B. Circulating Tumor Cells in Esophageal Cancer. Oncol Res Treat. 2017;40(7–8):417–22.
22. Giuliano M, Giordano A, Jackson S, De Giorgi U, Mego M, Cohen EN, et al. Circulating tumor cells as early predictors of metastatic spread in breast cancer patients with limited metastatic dissemination. Breast Cancer Res. 2014;16(5):440.

23. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol. 2010;17(6):1471–4.
24. Kuvendjiska J, Bronsert P, Martini V, Lang S, Pitman MB, Hoeppner J, et al. Non-Metastatic Esophageal Adenocarcinoma: Circulating Tumor Cells in the Course of Multimodal Tumor Treatment. Cancers (Basel). 2019;11(3).
25. 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.
26. Vojtechova G, Benesova L, Belsanova B, Minarikova P, Levy M, Lipska L, et al. Monitoring of Circulating Tumor Cells by a Combination of Immunomagnetic Enrichment and RT-PCR in Colorectal Cancer Patients Undergoing Surgery. Adv Clin Exp Med. 2016;25(6):1273–9.
27. Riethdorf S, Fritsche H, Müller V, Rau T, Schindlbeck C, Rack B, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. Clin Cancer Res. 2007;13(3):920–8.
28. Seeberg LT, Waage A, Brunborg C, Hugenschmidt H, Renolen A, Stav I, et al. Circulating tumor cells in patients with colorectal liver metastasis predict impaired survival. Ann Surg. 2015;261(1):164–71.
29. Gazzaniga P, Raimondi C, Gradilone A, Biondi Zoccai G, Nicolazzo C, Gandini O, et al. Circulating tumor cells in metastatic colorectal cancer: do we need an alternative cutoff? J Cancer Res Clin Oncol. 2013;139(8):1411–6.
30. Shankaran V, Obel J, Benson AB, 3rd. Predicting response to EGFR inhibitors in metastatic colorectal cancer: current practice and future directions. Oncologist. 2010;15(2):157–67.
31. Frederick BA, Helfrich BA, Coldren CD, Zheng D, Chan D, Bunn PA, Jr., et al. Epithelial to mesenchymal transition predicts gefitinib resistance in cell lines of head and neck squamous cell carcinoma and non-small cell lung carcinoma. Mol Cancer Ther. 2007;6(6):1683–91.
32. Lee MM, MacKinlay A, Semira C, Schieber C, Jimeno Yepes AJ, Lee B, et al. Stage-based Variation in the Effect of Primary Tumor Side on All Stages of Colorectal Cancer Recurrence and Survival. Clin Colorectal Cancer. 2018;17(3):e569-e77.
33. Mendis S, Beck S, Lee B, Lee M, Wong R, Kosmider S, et al. Right versus left sided metastatic colorectal cancer: Teasing out clinicopathologic drivers of disparity in survival. Asia Pac J Clin Oncol. 2019;15(3):136–43.
34. Nicolazzo C, Raimondi C, Gradilone A, Emiliani A, Zeuner A, Francescangeli F, et al. Circulating Tumor Cells in Right- and Left-Sided Colorectal Cancer. Cancers (Basel). 2019;11(8).
35. Salem ME, Yin J, Renfro LA, Weinberg BA, Maughan T, Adams R, et al. Rectal versus left-sided colon cancers: Clinicopathological differences observed in a pooled analysis of 4,182 patients enrolled to 8 clinical trials from the ARCAD database. Journal of Clinical Oncology. 2017;35(4_suppl):675-.
36. Li FY, Lai MD. Colorectal cancer, one entity or three. J Zhejiang Univ Sci B. 2009;10(3):219–29.
37. Junqueira-Neto S, Batista IA, Costa JL, Melo SA. Liquid Biopsy beyond Circulating Tumor Cells and Cell-Free DNA. Acta Cytol. 2019;63(6):479–88.

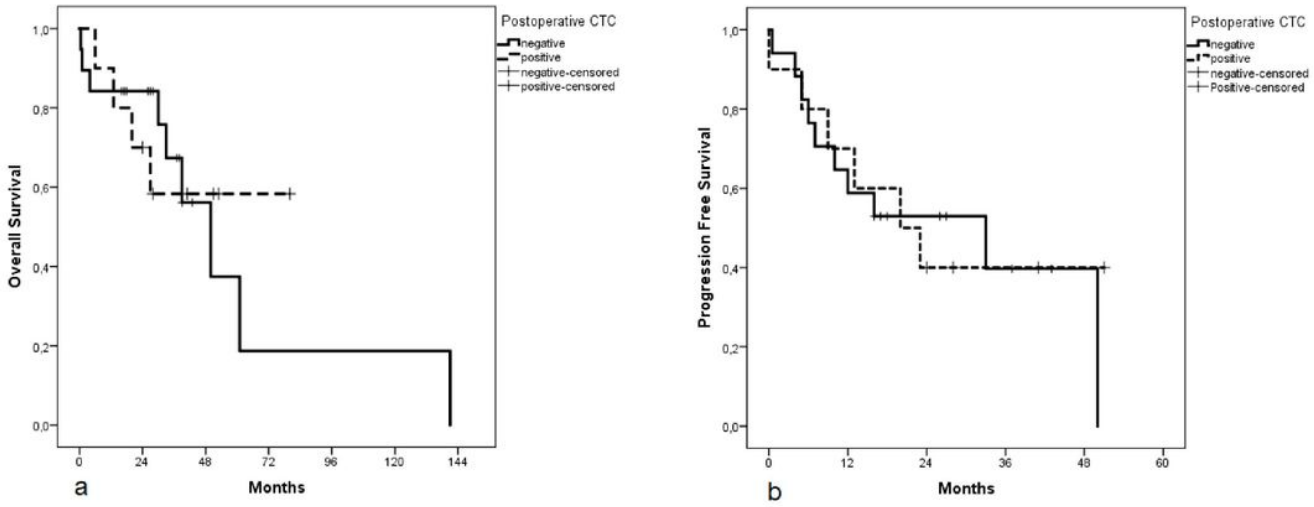
Figures



	Log-Rank (Mantel-Cox) Chi-Square	*p*-value
Figure A (OS)	6.962	0.008
Figure B (PFS)	7.072	0.008

Figure 1

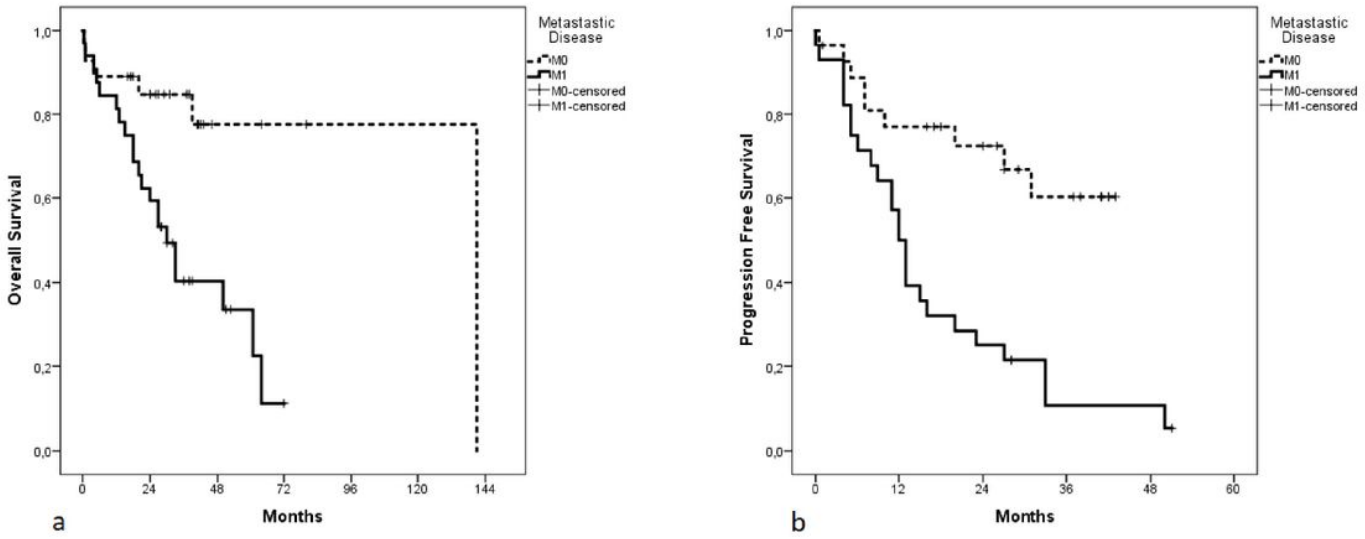
Kaplan-Meier Survival Analysis for overall survival and progression free survival according topreoperative CTCs. Figure a: Overall survival in patients with CRC, positive vs. negative preoperative CTCs. Figure b: Progression-free survival in patients with CRC, positive vs. negative preoperative CTCs. P-value Indicates significance according to Log-Rank (Mantel-Cox) test when CTC-negative patients are compared with CTC-positive patients preoperatively.



	Log-Rank (Mantel-Cox)	
	Chi-square	*p*-value
Figure a (OS)	0.047	0.829
Figure b (PFS)	0.024	0.876

Figure 2

Kaplan-Meier Analysis for overall survival and progression free survival according to postoperative CTCs. Figure a: Overall survival in patients with CRC, positive vs. negative postoperative CTCs. Figure b: Progression-free survival in patients with CRC, positive vs. negative postoperative CTCs. P-value Indicates significance according to Log-Rank (Mantel-Cox) test when CTC-negative patients are compared with CTC-positive patients postoperatively.



	Log-Rank (Mantel-Cox)	
	Chi-Square	*p*-value
Figure a (OS)	9.262	0.002
Figure b (PFS)	12.447	< 0.001

Figure 3

Kaplan-Meier analysis for overall survival and progression-free survival in mCRC. Figure a: Overall survival in patients with CRC, metastatic vs. non-metastatic disease. Figure b: Progression-free survival in patients with CRC, metastatic vs. non-metastatic disease. P-value Indicates significance according to Log-Rank (Mantel-Cox) test when mCRC is compared to non-mCRC.