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Variations in the Number of Circulating Tumor Cells During the Surgical Sequence for Locally Advanced Rectal Cancer

Dragos-Viorel Scripcariu[1,2], Gabriel Dimofte[1] and Dan Ferariu[3]

[1]*Department of Surgery, Grigore T Popa University of Medicine and Pharmacy, Iași, Romania*
[2]*1st Surgical Unit, Regional Institute of Oncology, Iași, Romania*
[3]*Department of Pathology, Regional Institute of Oncology, Iasi, Romania*

Abstract

Aim: This study aims to assess the variation of the levels of circulating tumor cells during surgical manipulation, by *in vivo* evaluation at three key points during treatment.

Materials and methods: This was a pilot study with 20 included patients with mid and low locally advanced rectal cancer, with neoadjuvant treatment. The device used to evaluate the number of circulating tumor cells in the bloodstream of the patients was the "Detektor Cancer" GILUPI Cell Collector®. The tests were performed at 8 weeks after the end of the neoadjuvant treatment, at three key moments: in the preoperative period, during surgery - after the surgical dissection and at 7 days postoperatively.

Results: There was an increase in the number of circulating tumor cells after the surgical sequence, but no statistical significance could be achieved due to the small number of patients included in the study.

Conclusion: The circulating tumor cell number is a useful biomarker for the prognosis of the patients with colorectal cancer, demonstrated through several studies. However, there is need for standardization in this field of research. Our study, although with visible differences between the preoperative, intraoperative and postoperative values, showed no statistical significance.

Keywords: Locally advanced rectal cancer; Neoadjuvant treatment; Circulating tumor cells; Total mesorectal excision

Abbreviations

CRC: Colorectal Cancer; CTC: Circulating Tumor Cells; EpCAM: Epithelial Cell Surface Adhesion Molecule; LAR: Low Anterior Resection; ELAPER: Extralevator Abdominoperineal Excision of the Rectum

Introduction

Colorectal cancer (CRC) is an important public health issue worldwide, with an ascending incidence, especially in men at a younger age. In the European Union, CRC is the second most frequent type of cancer after breast cancer in both sexes. An incidence of 13.1%, a 5-year prevalence of 13.3% and a mortality of 11.9% make it an important public health issue. Yet, the mortality of this cancer type is on a downward curve, due to the increase in efficiency of diagnosis and treatment. The rectal situation of the tumor is present in over one third of the cases. According to Globocan 2012, incidence (13%) and mortality (11.8%) by CRC in Romania is close to the European average [1-3].

Adenocarcinoma is the most frequent histological type of rectal cancer, which has a reported overall 5-year survival rate of 66.5% [4]. Metastatic CRC has seen major improvements in the last decades, with an increase of the median survival from 14.2 to 29.3 months [5]. The past years have seen the introduction of various aggressive treatment options, which meant the improvement of overall survival of patients with metastatic CRC to 5-year survival rates of 35-60% [6].

Circulating tumor cells (CTCs) are tumor cells derived from either primary tumors or metastases that are circulating in the peripheral blood. The field of CTCs is an attractive instrument for assessing prognosis, monitoring response to therapy, pharmacodynamics studies and selection of therapies in cancer patients [7].

The aim of this study was to assess the variation of the CTCs during surgical manipulation, by *in vivo* evaluation at three key points during treatment.

Material and Methods

Between January 2015 and September 2015, a total of 20 patients were selected, from the cases with locally advanced rectal cancer treated in the Iași Regional Institute of Oncology. They were included in a pilot study to evaluate the status of CTCs after surgical manipulation of the specimen during surgery.

All patients had neoadjuvant chemo radiotherapy and the surgical sequence was applied at least 8 weeks after completion of the neoadjuvant treatment. In all patients total mesorectal excision was performed through open surgery, with either very low anterior resection (LAR) or extralevator abdominoperineal excision of the rectum (ELAPER).

CTCs were collected using two models of the "Detektor Cancer" GILUPI Cell Collector®. This medical device is represented by a functionalized structured medical Seldinger guidewire, with a chimeric monoclonal antibody directed to a cell surface expressed molecule – epithelial cell surface adhesion molecule (EpCAM). The two models that were used were the DC 01 and DC 02 variants, which differ thorough the length of the functionalized harvesting gold tip of the catheter.

The total number of 20 patients was evenly distributed in two batches that were evaluated using the two models of the medical device (Figure 1).

***Corresponding author:** Scripcariu Dragoș-Viorel, Assistant Lecturer, PhD student, Department of Surgery, Faculty of Medicine, Grigore T Popa University of Medicine and Pharmacy, Iași, Romania, 700483, Tel: +40(0)723913425; E-mail: dscripcariu@gmail.com

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The harvesting procedure was performed after a standardized guideline, by trained medical staff. The DC Cell Collector® was inserted through a 20 G catheter into a peripheral vein, thus being able to collect elements directly from the patient's blood stream. The procedure was performed at well-defined moments during the patient's treatment sequence. After insertion, the medical device was left on site for a period of exactly 30 minutes. After extraction, the catheter was washed of any blood residue and fixed for a period of 10 minutes, followed by a 5-minute dry up stage. Afterwards, the used device was shipped to the pathological staff, where the count was performed (Figure 2).

In order to explore the CTC count in dynamic, cells were collected at three time points: in the preoperative period, at 24 hours before surgery (with at least 48 hours after a digital rectal examination); during surgery, after the abdominal dissection of the mesorectum was completed; in the postoperative period, at seven days after surgery.

Results

The 20 patients included in this pilot study had a mean age of 62.4 years, ranging between 43 years and 74 years.

Pre therapeutic staging was performed in all patients by pelvic MRI. All patients had stage III rectal cancers, with staging that can be seen in (Table I). In all cases pre therapeutic long course radio chemotherapy was applied, with a total dose of 50.4 Gy, in 28 fractions, over 5.5 weeks. The surgical sequence of the treatment was applied at a mean distance of 72 days after the end of the neoadjuvant treatment (range 62-102).

As seen in (Table II), as well as (Figures 3 and 4), the number of CTCs harvested using a 4 cm harvester tip is higher than by using a 2 cm tip.

When viewing data in dynamics, we see that the mean number of CTCs increases after full surgical dissection, with a mean of 2 (0-4) and 4.5 (0-12), as compared to preoperative values of 1.8 (0-3) and 3.1 (0-7) respectively. However, no statistical significance is reached. Seven days postoperatively, the CTC values drop to levels similar or below the preoperative range in all but one case, in which the values increased to 22, with no clinical significance in the early postoperative evolution.

Discussion

The subject of CTCs has been studied as early as 1869 [8]. Until recent years, precise identification and characterization of these cells could not be achieved. However, modern techniques have allowed the detection and characterization of even rare CTCs in peripheral blood [7,9,10]. The research of CTCs has blossomed and this field has been included in over 400 clinical trials related to cancer [11].

CTCs have been detected in variable solid tumors, such as breast, prostate, gastric, colorectal cancers and melanoma [7].

There are multiple methods of determining CTCs; most studies in the literature use the blood samples in which different techniques are performed in order to isolate CTCs (quantitative real-time PCR – qRT-PCR, immune magnetics combined with qRT-PCR, CellSearch system – the only FDA-approved method) [12]. The method used in this study is innovative, by the fact that it harvests CTCs directly from the blood stream of the patient, thus being able to analyze a larger "sample" from the patient. The method of CTC detection in this assay uses antigens expressed by CTCs of epithelial origin (EpCAM). In order to distinguish epithelial cells from leukocytes, fluorescent-labeled monoclonal antibodies are used (anti-CD45-Allophycocyanin vs anti cytokeratins 8,18,19 – phycoerythrin) [13].

According to a meta-analysis from 2013, by Akagi et al. [14] the prognostic utility of circulating tumor cells in several compartments (lymph nodes, peritoneal cavity, peripheral blood, drainage veins) has been demonstrated, with a significant difference both for morbidity and mortality. However, the high variability in blood CTC counts and the significant differences in disease free survival and overall survival rates between patients, not always correlated with CTC status, make the CTC count a relative factor of prognostic in CRCs [13].

The model of the metastatic process implies the existence of local invasion at the primary site, vascular invasion, dissemination and circulation, attraction to specific organs, active extravasation, mesenchymal-epitelial transition and proliferation into the metastasis [15]. Although the presence of high numbers of CTCs in the CRC patient's blood has been associated to higher rates of metastatic disease [14], these CTCs are widely heterogeneous and, up to this date, no specific characteristic has been defined to distinguish these populations, so we can say that CTC detection today remains unspecific

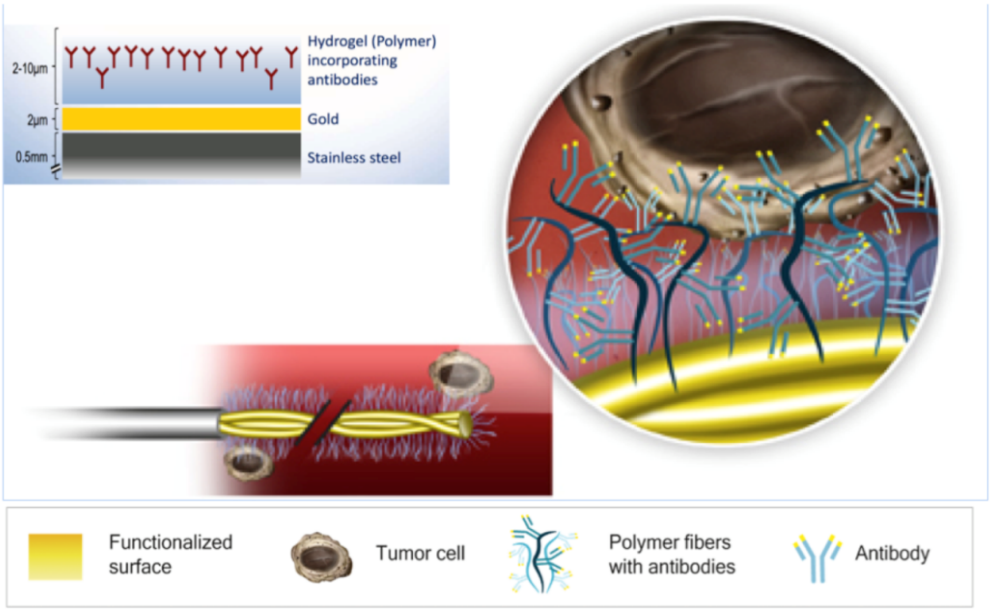

Figure 1: Schematic representation of the harvesting device, as depicted by the manufacturer (Gilupi).

and biased. There is a need for further studies to consider epithelial as well as mesenchymal marker panels, in order to investigate different subpopulations of CTCs [12].

Another potential use of CTC count is to identify subpopulations of patients that would benefit from cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in patients with peritoneal carcinomatosis resulting from CRC [16].

In the study we have performed, although there is an increase of CTCs in the bloodstream during surgical manipulation and a decrease in CTC counts postoperatively, the data is not statistically significant. The reason may be the small number of patients included in the study, as well as an insuficient cell selection of the harvesting tool. The harvest and the CTC count were performed by the same teams, so there is no risk of bias from this point of view.

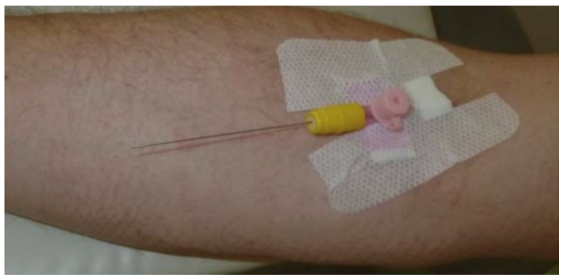

Figure 2: Harvesting sequence.

Table I: The general characteristics of the 20 studied patients.

Characteristic	Number (%)
Tumor location	
Mid rectum	9 (45%)
Low rectum	11 (55%)
Clinical staging	
cT	
2	2 (10%)
3	14 (70%)
4	4 (20%)
cN	
0	1 (5%)
1	13 (65%)
2	6 (30%)
cM	
0	20 (100%)
Pathological staging	
pT	
0	1 (5%)
2	8 (40%)
3	11 (55%)
pN	
0	12 (60%)
1a	3 (15%)
1b	1 (5%)
2a	4 (20%)
Dworak tumor regression grade	
0	1 (5%)
1	11 (55%)
2	3 (15%)
3	4 (20%)
4	1 (5%)
Surgical procedure	
LAR	8 (40%)
ELAPER	12 (60%)

Table II: Data collected through CTC count using the two models of cell collectors.

Characteristic	2 cm harvester tip Number (range)	4 cm harvester tip Number (range)
Number of CTCs		
Maximum number of CTCs harvested	4	22
Mean CTCs 1st count	1.8 (0-3)	3.1 (0-7)
Mean CTCs 2nd count	2 (0-4)	4.5 (0-12)
Mean CTCs 3rd count	1.8 (0-4)	3(0-22)
Mean increase after dissection	1 (0-3)	2 (0-12)
Mean drop 7 days postoperatively	0.3 (0-1)	1.5 (-21-12)

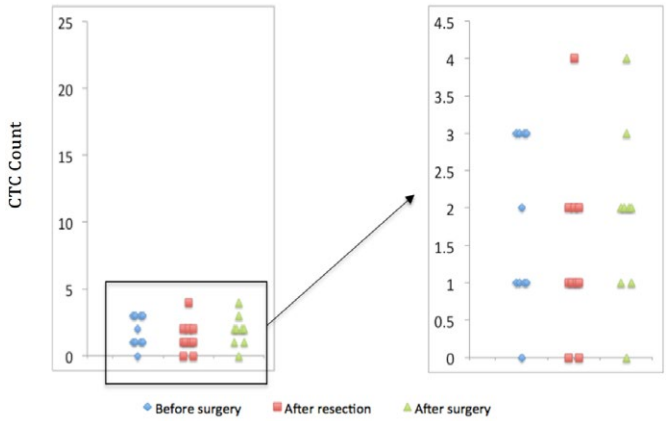

Figure 3: CTC count before surgery, during surgery (after resection) and seven days after surgery, performed with the "Detektor Cancer" GILUPI Cell Collector® - DC01- with a 2 cm long harvesting tip.

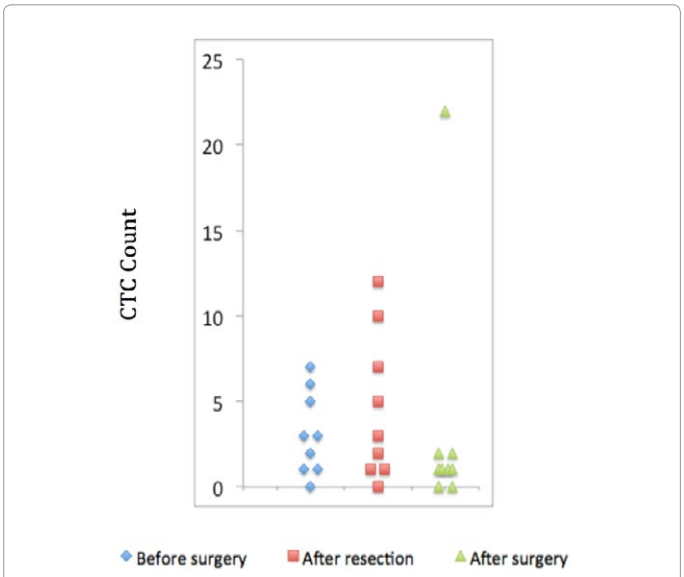

Figure 4: CTC count before surgery, during surgery (after resection) and seven days after surgery, performed with the "Detektor Cancer" GILUPI Cell Collector® - DC02- with a 4 cm long harvesting tip.

Conclusion

The circulating tumor cell number is a useful biomarker for the prognosis of the patients with colorectal cancer, demonstrated through several studies. However, there is need for standardization in this field of research. Our study, although with visible differences between the preoperative, intraoperative and postoperative values, showed no statistical significance.

Conflict of Interest

Authors have no conflict of interest to disclose.

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