CIRCULATING TUMOR CELLS IN RECTAL CANCER PATIENTS – A REVIEW OF THE LITERATURE

D. V. Scripcariu1,2, C. D. Lupașcu1,3
“Grigore T. Popa” University of Medicine and Pharmacy Iasi
Faculty of Medicine
1. Department of Surgery (I)
Regional Institute of Oncology Iasi
2. First Surgical Unit
“Sf. Spiridon” County Clinical Emergency Hospital Iasi
3. Second Surgical Unit

CIRCULATING TUMOR CELLS IN RECTAL CANCER PATIENTS - A REVIEW OF THE LITERATURE (Abstract): Rectal cancer treatment has been continuously improving in the past decades and research has rightly been directed for this purpose, because this is a major public health issue. On the other hand, circulating tumor cells have emerged in the past two decades as an important element in oncology, because of the large potential they offer. They have been studied mostly in breast cancer and have already been implemented as a tool in certain strategies in this field. Thus, circulating tumor cells in rectal cancer may have the same important applicability, with a wide range of usages, from screening to diagnostics, guidance of the treatment and even follow-up. This article aims to review the literature and exhibit data about the pathophysiology, utility and methods of identification of circulating tumor cells with focus on their link to rectal cancer. Keywords: RECTAL CANCER, CIRCULATING TUMOR CELLS.

The importance of rectal cancer resides both in its high incidence, as well as in the complexity of the treatment, that relies in most cases on a multidisciplinary team, being a multimodal approach.

Circulating tumor cells (CTCs) are an innovative field in oncology. This subject is widely investigated in the recent years and is of great interest in the research field due to the various advantages of studying the tumor cells in the blood stream. These cells can derive either from the primary tumor, or from its metastases, thus being an important marker in evaluating the prognostic of the disease, monitoring the response to certain therapies, or selecting patients as targets for certain therapies (1).

Rectal cancer, an important public health issue and its connection to circulating tumor cells

According to the Globocan 2012 study, colorectal cancer is the second neoplasia in incidence in both sexes, both in Romania and within the European Union. On a global scale, it is situated in incidence after pulmonary and breast cancer. The incidence of 13.1%, the 5-year prevalence of 13.3%, as well as a 11.9% rate of mortality make it an important public health issue, especially in developed countries.

With all these aspects aside, the mortality through this type of cancer is on a
descending trend in most developed regions. All this thanks to the increase in national and local screening programs, due to the increase in the efficiency of the diagnostic methods, and because of the improvements brought to treatment methods (2).

Screening in rectal cancer is one method of decreasing mortality, through an early diagnostic and the opportunity yielded by precocious identification of potentially malignant lesions or preneoplastic polyps. The clinical practice guidelines elaborated by the National Comprehensive Cancer Network (NCCN) serve as tools to direct the clinician towards this endpoint. Although not applied on a national level now in Romania, these guidelines setup a consensus between colorectal cancer specialists, that can be taken into consideration when aiming for a precocious diagnosis and a suitable treatment (3).

Bearing this aspect in mind, two categories of screening tests arise: structural tests and stool-based tests.

Structural tests give the possibility of identifying incipient lesions or even polyps that have the potential of malignant transformation. These tests are based on imaging-colonoscopy and radiology. Rigid rectoscopy, rectosigmoidoscopy, colonoscopy, barium enema and computed-tomography (which gives the possibility of obtaining a virtual colonoscopy) are the methods included in this category, which are, of course, invasive, with risks that increase with age and associated morbidities (4).

On the other hand, stool-based tests, consisting of the fecal occult blood test, the fecal immunochemical test and the detection of altered DNA, have the advantage of non-invasiveness, but are, on the other hand, poor in means of specificity; the probability of detection of adenomatous polyps is low. The immunochemical test brings an improvement in the matter of sensitivity (5, 6) and the DNA testing is the best of the non-invasive methods in means of sensitivity but lacking in the matter of cost-effectiveness (7).

All in all, the gold standard for colorectal cancer screening is the colonoscopy, with great advantage of providing a biopsy in given cases. Moreover, a positive non-invasive test will lead to a colonoscopy evaluation (3).

Another factor that aims to improve mortality rates is the treatment and its continuous developments. The multidisciplinary approach, based on the decisions taken by a complex team of specialists aims to individualize the treatment based on the characteristics of each patient. On this subject, a sequential treatment plan is taken into discussion; the decision to apply a neoadjuvant treatment or opt for upfront surgery is based on the extent of the tumor, set up by imaging means. The efficiency of neoadjuvant radio chemotherapy on rectal cancer has been demonstrated through various trials, becoming routine in locally advanced, non-metastatic tumors (8, 9). However, some tumors respond well to neoadjuvant treatment, whereas others do not, with a stationary or even progressive status after the long-term scheme of neoadjuvant radiotherapy, with a considerable amount of time lost in the detriment of the patient. Therefore, additional predictive factors for the neoadjuvant treatment must be developed to reduce this setback. CTCs may play an important role in this matter, providing the examiners with important information.

The adjuvant treatment in rectal cancer is based on the prognostic value of the
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Circulating tumor cells

Circulating tumor cells (CTCs) are cells of tumor origin that are released into the bloodstream. Even if they were identified and described over one century ago, in 1869, they became a domain of interest approximately one decade ago, when studies performed on CTCs, methods of identifying and counting them began to flourish in oncology (10). They are considered the main mechanism of metastasis, thus studying them contributing to a better understanding of the metastasis cascade and helping better the treatment of the oncologic disease (11). Moreover, there is the notion of disseminated tumor cells (DTCs), that result from the embedding of CTCs in a metastasis site (12).

CTCs are rare in the patient’s bloodstream - 1 to every $10^6/10^7$ circulating mononuclear cells. Studies from 1975 mention a quantity of one million tumor cells released per each gram of the tumor (13). However, this number is overrated, bearing in mind the conditions in which CTCs are released into the bloodstream and the half-life of these cells within the bloodstream.

The modality in which they leave the tumor site varies: either single or in clusters - clusters can be homotypic or heterotypic (14, 15). Depending on their properties, there are different characteristics specific to each category. Thus, single CTCs are more frequent in the bloodstream, with a good chance of penetrating small vessels, but have the downside of having a high risk of apoptosis, with low chances of metastasis formation. On the other hand, clusters are rarer, but have higher chances of metastasizing due to the lower risk of apoptosis and the fact that they already have their own formed microclimate. Although many aspects about CTCs and DTCs are yet unknown, one particularity is clear: the phenotypic plasticity, manifested in the high possibility of phenotypic changes to “survive” (15).

**Fig. 1.** Epithelial-mesenchymal transition (reproduction after Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *JCI* 2009; 119(6): 1420-1428)
The efficiency of a CTC or DTC is given by its capacity to metastasize. For this event to take place, these cells need to undergo changes that increase their invasive capacity (16, 17).

Carcinomas are the most frequent type of neoplasia, deriving from epithelial cells from different organs. Under certain mutagen factors, the cells comprising the normal tissue lose their apico-basal polarity along other phenotypes, such as growth control and become adenomas. In time, these abnormal cells become in situ carcinoma, with properties that make it malignant (16).

Up next, these malignant cells undergo changes that are essential for obtaining the ability of invasion and metastasizing. Normally, cells are linked to the basement membrane by molecules such as E-cadherin, plakoglobin and integrins. In special situations, such as organogenesis and tissue repair, these cells obtain the capability of detaching from the basement membrane. Oncogenesis and metastasis also imply the occurrence of this event, in which the above-mentioned proteins are replaced by vimentin and N-cadherin; it is this change that determines the exposure of detached cells to the stromal fibroblasts, leucocytes, extracellular matrix and neoformation vessels. This event is called epithelial-mesenchymal transition (EMT) (fig. 4) (18).

The primary tumor may contain cells in different steps of this transition, thus expressing different characteristics; they may be included in two categories (12):

1. A mesenchymal-like form, with receptors to adhere to the extracellular matrix. These cells produce enzymes such as metalo-proteinase that permit the progression of the tumor through: degradation of the extracellular matrix and of the intercellular junctions, stimulation of cell motility and EMT. These cells may be thought as the promoters of cellular invasion, by creating an adequate microenvironment for this event.

2. An amoeboid form, with cells with high plasticity, that can easily pass through the gaps in the basal membrane made by the above-mentioned cells.

On the other hand, once released in the bloodstream, the tumor cells can have three types of evolution (19):

1. The first situation is when cells are released into the bloodstream, once detached from the extracellular matrix and, in the absence of a propitious microenvironment, they either suffer anoikis, apoptosis or necrosis, being annihilated, finally, by the immune system. Obviously, this is unproductive from the metastasis point of view.

2. The second type of evolution is represented by cells that have reached a metastatic target and remain in this site in a G0 phase of the cell cycle, dormant, with an equilibrium between proliferation and apoptosis, thus being a temporary status, that is partially unproductive from a metastatic point of view.

3. The third category is that of cells that have reached the metastatic site and launch an efficient proliferation process, that allows the cells to grow in number and to invade the metastatic site. These cells can be either from the bloodstream, or dormant cells, that have been locked in the G0 phase for a certain amount of time.

The phenomenon of “dormancy” of cells situated in metastatic sites has been studied in patients with breast cancer, where CTCs have been identified if 22
years distance from the radical intervention (20). However, the clinical implications of this event are still unclear. Moreover, these sites with dormant tumor cells have been found to be capable of releasing CTCs in the bloodstream, which is why it is important to identify and characterize these cells in the patient’s circulation (21).

The utility of identifying and counting the CTCs

Circulating tumor cells may be identified and counted in the patient’s blood, with methods that have seen great improvements over the last couple of decades. The utility of examining CTCs in rectal cancer patients may be seen from several points of view:

1. as a screening instrument
2. for diagnostics
3. as predictive factors for orienting the treatment
4. as prognostic factors
5. for guiding and assessing the treatment
6. as a follow-up tool.

1. CTCs could become primary or complimentary identification markers for rectal cancer, with the possibility of detecting tumor cells that are in the patient’s bloodstream.

Studies from 2008 on breast cancer patients (22) and from 2012 on pancreatic cancer (23) support the idea that tumor cells enter the circulation even before the formation of the main tumor, thus concluding that the identification of CTCs in peripheral blood may be used for screening purposes.

2. Liquid biopsy is a popular subject in recent years. CTCs represent an important source of information in advanced neoplasia, in which they are more abundant in blood. However, the main goals are to identify them when they are less abundant in the patient’s bloodstream, in early-stage neoplasia (which hinders them from being an adequate screening tool now) and to study them in their own tumor microenvironment (which may be solved by examining the heterotypical clusters of CTCs (24)).

3. Prediction of the response to treatment for breast cancer has been studied in a phase III clinical trial – the SWOG S0500 trial, that was based on evaluating the number of CTCs at the beginning of the treatment and at 21 days after commencing chemotherapy; it aimed to observe the opportuneness of changing the line of chemotherapy after 21 days of chemotherapy based on the variation of CTCs. The trial demonstrates that a large number of cells at the beginning and at 21 days is a negative prognostic factor for disease free survival and overall survival. All in all, no clinical predictive utility of CTCs in adjuvant therapy was demonstrated through this trial (25).

However, this domain is promising and could show of great utility in locally advanced rectal cancer patients that must receive neoadjuvant therapy and develop metastases during this period of at least 12 weeks between diagnosis and the surgical sequence. CTC dosage during neoadjuvant therapy may prove to be of interest in this eventuality. No studies can be found on this subject now in literature.

4. An accepted and validated clinical usage of the CTCs is the relationship between the number of CTCs at the beginning of the treatment and the overall and disease-free survival. Thus, it has been
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demonstrated that a number greater than 5 cells per 7.5 mL of peripheral blood is correlated with a worse survival (11). Moreover, this important aspect has been included, for breast cancer for the time being, in the last TNM classification (8th edition-2017): CTCs present in blood classify the disease as cM0(i+) (26).

This aspect has been initially studied in patients with breast cancer (27), but afterwards other types of cancer have been studied; in colorectal cancer, the cut-off value for CTC number per 7.5 mL of blood is 3, which is significant both for overall, as well as disease free survival (28).

5. The term of liquid biopsy has been widely discussed in the past few years and is a significant step forward for oncology. CTCs may prove to be an important marker both to be taken into consideration within the multidisciplinary team to decide upon the treatment, and for obtaining information about the tumor cell’s phenotype and using this information to decide upon the elected treatment by each healthcare professional in part (29).

6. CTCs may be a precocious marker for recurrence or metastatic disease in rectal neoplasia, which is why a sensitive and accessible, cheap identification would be an important follow up tool.

All in all, CTCs are useful in all phases of the neoplastic disease, from screening to follow up. Nevertheless, their usage is subject to the refinement of identification and count methods. This domain is under development, but more trials are necessary to find and apply utilities of this resource in cancer treatment.

Methods of identification of CTCs

Because they are rare cells in the circulation, these cells’ separation from leukocytes, red blood cells and other circulating cells is necessary. There are multiple methods of identifying these cells in the patient’s bloodstream. A classification of these methods taking into consideration the modality used for selection divides them into (30, 31):

1. Affinity-based evaluation
2. Non-affinity-based evaluation (based on physical properties)
3. Molecular techniques of evaluation.

1. Affinity-based techniques are the most widely used modalities of evaluation of CTCs. These techniques are based on ligation to different antigens expressed by tumor cells, that differentiate them from other cells in the bloodstream. One of these molecules is EpCAM (Epithelial Cell Adhesion Molecule), that is expressed by tumor cells originating in epithelial tissue but is absent from other cells in the bloodstream. Thus, identification and ligation of this glycoprotein permits the selection of the targeted cells.

CellSearch is the most utilized method and is considered gold standard for the identification of CTCs. It is the only method approved by the US FDA for identifying and counting CTCs in breast, prostate and colorectal cancer. This method is based on ligation of EpCAM by iron-fluid nanoparticles and attachment of these functionalized cells in a magnetic field. This platform has been validated through multiple studies (30).

Other methods include Mag Sweeper (an automatic immunomagnetic separation technology), CTC-Chip (a chip that uses functionalized micro posts with anti-EpCAM antibodies), GEDI (geometrically
enhanced differential immunocapture. More recently, there have been described technologies that combine affinity-based methods with other physical techniques, thus emerging: Nano-Velcro (a device with a patterned silicon nanowire substrate coated with anti-EpCAM antibodies), Graphene Oxide Nanosheets with EpCAM antibodies, Immuno-microbubbles (perfluorocarbon gas filled microbubbles conjugated with anti-EpCAM antibodies) and GILUPI Cell Collector (a functionalized medical wire that detects CTCs in circulation, being inserted for 30 minutes in a peripheral vein) (30).

The disadvantages posted by these methods are the fact that tumor cells may present phenotypic variations, which may impede their detection by affinity alone, with less cells identified. This setback can be addressed by identifying and ligation of more than one type of CTC receptor.

2. These disadvantages led to the development of other methods of identifying and counting CTCs: methods based on size and electrical properties of CTCs. This is because tumor cells differ from other blood cells by size, density and polarity, alongside the molecules expressed on their surface.

The simplest, but the least effective method is centrifugation, which uses the cells’ density to separate them from other blood components. Through centrifugation, CTCs are separated, along other nucleated cells in the blood, thus being identified afterwards by microscopic examination.

Based on the larger dimension of CTCs, compared to other cells in the blood, micro-filtering may be used, alone or in combination with other affinity-based or non-affinity-based methods. The filtrating material may be obtained through different modalities.

There are other methods to separate CTCs, such as microfluidics or Nano roughened surfaces, but these are still in the testing stage to evaluate their efficiency.

Dielectrophoresis is the modality of separating CTCs in an electrical field. This technique has been patented by Herbert Pohl in 1950 and was observed as a modality of moving particles using their volume and form. Several studies have used dielectrophoresis in separating CTCs for studying them (31).

All in all, we can see that the methods are somewhat complimentary, and a method that combines them would be the most preferred, provided that is cost-efficient as well. GEDI (geometrically enhanced differential immunocapture) has been proven by Gleghorn to do this but has so far been used only in prostate cancer (32).

CONCLUSIONS

The emergence of the subject of CTCs has been, throughout history, a tough one, because in 1869, when they were first described, the medical world was not evolved enough to see the advantages and begin research; only after more than a century were CTCs seen as a domain of great potential.

We can conclude that the subject of CTCs is an emerging one, of great possibilities, with utilities that are reachable through research and refinement of the isolation techniques. The fact that CTCs are useful both in the field of oncology and in surgery is one more argument in favor of the studies and extensive research that are being put into this field.
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