CIRCULATING TUMOR CELLS IN COLORECTAL CANCER*

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Colorectal tumors are considered as the third most common neoplasms in the male population and second in case of female patients (1). The five-year survival of patients with colorectal cancer depends on the clinical stage of the disease, amounting to 90% in case of stage I and 11% in case of stage IV (2).

According to The National Cancer Registry the incidence of colorectal carcinoma in Poland (2010) amounted to 12% considering the male population, and 10% in case of female patients (total of 15,800 patients) (3).

Available imaging diagnostic methods (CT, MRI) only allow to determine the local advancement of the disease, as well as already existing metastatic foci, which are a significant prognostic factor and main cause of death. Other confirmed prognostic factors of colorectal carcinoma include:

– depth of tumor infiltration,
– stage of the disease, according to the TNM classification,
– histological differentiation,
– infiltration of vascular and lymph vessels,
– infiltration of local lymph nodes (4, 5).

Surgery is the basic therapeutic method in case of colorectal carcinoma, and if necessary, adjuvant chemotherapy. In reality, despite the implementation of all possible therapeutic methods, 25-50% of patients with colorectal cancer stage I and II present with cancer recurrence (6). Therefore, the search for prognostic factors which would accurately determine the likelihood of recurrence. Circulating tumor cells (CTCs) seem to pose such an opportunity.

Circulating tumor cells

Circulating tumor cells are present in the peripheral blood, possessing specific antigens and genetic features of a given type of tumor (7). They were described for the very first time by Thomas Ashworth in 1869, who observed the presence of vascular neoplastic cells in a patient with disseminated cancer (8). It is now believed that CTCs are either derived from primary cancer or already existing metastatic lesions. Recent studies indicate that circulating tumor cells play a significant role in the development of metastatic lesions (9). Metastasis is initiated by the invasion of tumor cells to the peripheral circulation by means of already existing vessels, located in the vicinity of the tumor, or through newly formed vessels (angiogenesis) (10).

Tumor cells located in the bone marrow are referred to as disseminated tumor cells (DTC). Evidence suggests that the bone marrow is a common organ to which cancer cells migrate from many different tumors. One may speculate, that the bone marrow is a significant CTC reservoir from which cells can migrate to other distant organs, where one may observe better growth conditions (liver, lungs) (11, 12).

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Improvement of molecular and genetic techniques facilitates identification, enumeration, and characterization of circulating cells (12). The ability to easily and reliably detect their presence can have a significant impact on their early diagnosis, monitoring the course of treatment, and development of cancer recurrence. The therapeutic goal should aim at eliminating the presence of circulating tumor cells as a potential source of cancer metastasis.

CTCs in patients with colorectal cancer

The number of circulating tumor cells in the bloodstream is very limited \((1/10^6–10^7\) mononuclear cells) (13, 14). Therefore, only very sensitive techniques enable to determine the presence and enumerate the number of CTCs generated by different tumors, including colorectal cancer.

The detection of CTC in colorectal cancer (similarly to esophageal and gastric carcinoma) is lower, as compared to such tumors as breast and prostatic cancer (15). Using immunomagnetic techniques it is possible to isolate circulating cells (defined as the presence of 2 cells in 7.5 ml of peripheral blood) in only 30-40% of patients with metastatic Colorectal Cancer (mCRC) (13). The amount of detectable circulating cells is still lower, as compared to the earlier stages of the disease. These differences might be associated with the high surface of cellular expression of adhesion molecules to CTC in patients with colorectal cancer, which might lead to their occurrence in the peripheral blood as cell clusters (16).

In order to identify and isolate CTC from other circulating mononuclear cells different methods and techniques are used. Thus far recognized, include the following:

1. Identification based on physico-chemical properties
   These properties include size, density, and presence (expression) of characteristic proteins.
   - Isolation based on size (ISET – isolation by size of epithelial tumor cells) allows to isolate all cells larger than \(8 \mu m\) (11, 17).
   - Isolation in the density gradient (tumor cells are less dense than cells of the hematopoietic system).
   However, these methods lack the desired sensitivity, and many CTC cells are lost during the isolation process.

2. Immunomagnetic isolation
   The most commonly used immunomagnetic methods include MACS (Milteni Biotech, Bergisch Gladbach, Niemcy), and RARE™ (StemCell Technologies, Vancouver, BC, Canada). Isolation is carried out by means of iron particles, which on their surface possess epithelial cell adhesion molecules (EpCAM). The simultaneous use of various antibodies allows for a more accurate cellular identification.

3. Identification technique based on the isolation of nucleic acids
   RT-PCR – the most common method used for the identification of RNA tumor cells.

4. Combined methods
   They combine several methods of isolation, increasing sensitivity and specificity. One may distinguish the following:
   - CellSearch Test (Varidex LLC, San Diego, CA) allows to precisely enumerate the number of circulating tumor cells in the peripheral blood within 72 h since sample collection. The CellSearch Test is the only method approved by the FDA (18).
   - CTC-chip (flow platform).
   - AdnaTest is the combination of the immunomagnetic method and genetic identification of CTCs using a three-gene panel and RT-PCR.

Furthermore, the difference in the detection of the number of CTCs also depends on the location of the sample collection. Negin and Cohen (15) observed the occurrence of a greater amount of CTCs in the mesenteric vessels, as compared to central circulation. This might be evidence of the role of the liver as a potential filter, which eliminates a part of the CTCs from the circulation.

Wong et al. (19) postulated that the use of specific markers towards a given tumor (anti-CK20 in case of gastrointestinal tumors) increases the detection of circulating cells in the peripheral blood and may be more effective, as compared to standard markers used in the detection of epithelial cells. Welinder et al. (20) came to the same conclusions.

CTCs in the early stages of cancer

Due to the difficulties in the detection and continuous improvement of CTC isolation
techniques in patients with low-grade cancer, only a few studies evaluated their clinical usefulness (21-32). However, meta-analyses have shown that the presence of CTCs in case of colorectal cancer after radical surgery was associated with worse prognosis (27, 33).

A similarly worse prognosis was observed in case of patients diagnosed with cancer stage I and II, and presence of CTCs in lymph nodes. The presence of circulating tumor cells significantly correlated with increased risk of recurrence and low overall survival (OS) (34-37).

CTCs in patients with metastatic colorectal cancer

So far, only a few studies showed the potential role of CTCs in predicting survival, considering patients diagnosed with colorectal cancer (38, 39).

While the importance of peripheral CTCs was less clear than the presence of DTCs in the bone marrow of patients with breast cancer (12), in case of colorectal cancer the situation is reverse. The meta-analysis undertaken by Rahbari et al. (40) demonstrated that the detection of CTCs in the peripheral blood is more important than their presence in the bone marrow. Additionally, bone marrow biopsy is an invasive procedure and time-consuming. Another limitation of the bone marrow biopsy is the difficulty to perform it in outpatient care.

Hiraiwa et al. (41), using the Cell-Search system™ isolated circulating tumor cells in case of gastrointestinal carcinomas (esophagus, stomach, colorectum). They considered the early and distant stages of the disease (presence of distant metastases). The Authors demonstrated that the amount of CTCs in case of patients with distant metastases was significantly greater, as compared to those with only locally advanced disease. This suggests that the enumeration of CTCs in case of patients with gastrointestinal carcinomas (including the colon) might be useful when determining the stage of the disease, as well as when monitoring the response to the proposed adjuvant therapy.

Cohen et al. (38) conducted a pilot study indicating that CTCs may be isolated by means of immunomagnetic separation in patients with mCRC. The study demonstrated the independent prognostic value of CTCs in patients beginning chemotherapy with mCRC. The study group comprised 460 patients. Patients were divided into unfavorable and favorable prognostic groups, based on the presence of a given amount of CTCs (>3 and <3/7.5 ml of blood). Blood was collected before treatment and 1-2, 3-5, 6-12 and 13-20 weeks after implementation of adjuvant therapy. Mean observation period amounted to 11 months. Initially, 26% of patients presented with an unfavorable CTC level. A shorter period free-of-recurrence (progression free survival- PFS (4.5 vs 7.9 months; p = 0.0002) and OS (9.4 vs 18.5 months; p < 0.0001) were observed in patients from the unfavorable group.

Moreover, the transition from the unfavorable to the favorable group (reduction in the initial CTC level) between the third and fifth week correlated with prolonged PFS and OS, as compared to patients where the CTC level was not subject to reduction following treatment implementation (PFS- 6.2 vs 1.6 months, p = 0.02; OS – 11 vs 3.7 months; p = 0.0002). Considering the group with a favorable CTC level, patients lived longer (18.8 vs 7.1 months; p < 0.0001).

The prognostic value of CTCs has been confirmed in a multicenter, phase III study, on a group of 755 patients with advanced colorectal cancer (mCRC) who received first-line treatment with capecitabine, oxaliplatin, and bevacizumab (CAIRO2) (42). Considering the amount of CTCs, patients were divided into the low (CTC <3/7.5 ml of blood) or high CTC groups (>3/7.5 ml of blood). Cells were isolated by means of the CellSearch System™. The study group comprised 467 patients. The median observation period was 16.8 months. Both PFS and OS in patients with the low CTCs level was greater as compared to the latter group (10.5 vs 8.1 months; HR = 1.5; p = 0.0003; 22 vs 13.7 months; HR = 2.2; p < 0.0001).

In a recently published study, Gazzaniga et al. (43) concentrated on the presence of CTCs in metastatic colorectal cancer. To test whether the presence of CTCs, regardless their number is a worse prognostic factor the authors compared patients with 0, 1-2 and 3 CTCs / 7.5 ml of blood. PFS considering specific patient groups amounted to 8, 4 and 5 months (p = 0.059). Gazzaniga et al. postu-
lated that patients with 1-2 CTCs be transferred from a favorable to an unfavorable group, considering prognosis (PFS and OS were similar to patients with CTC >3).

Almost all studies have shown that the detection of CTCs is closely correlated with the stage of the disease, independently of the method or markers used for their detection (44, 45). Although the detection of circulating tumor cells in the peripheral blood is not evidence of distant metastases, these cells play an important role in their development (10).

Particular advantage considering enumeration and detection of CTCs is observed in case of patients with diagnosed stage I and II cancer, and presence of micrometastases to lymph nodes. In such patients adjuvant chemotherapy would probably improve distant treatment results. Unfortunately, due to the high costs of the procedure and equipment limitations CTC evaluation is not a standard.

In conclusion, the identification of circulating tumor cells in the peripheral blood, especially during adjuvant chemotherapy may play a significant role when monitoring treatment, as well as when choosing individual therapy in case of patients with colorectal cancer. If as a result of the implemented treatment the CTC level is not reduced, it may indicate its ineffectiveness (46).

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