The diagnostics of colorectal cancer

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Colorectal cancer (CRC) is one of the most frequent human malignant neoplasms. CRC has an estimated incidence of more than 1,000,000 new cases annually worldwide. Approximately one out of three people who develop CRC dies from the disease. Furthermore, CRC often affects inhabitants of industrialized countries in comparison to less developed countries. Several markers of colon cancer, including CEA, CA-19-9, TPS, TAG-72 and lysosomal hydrolases, have been identified and are now being adopted in routine clinical practice. Increased values of these markers are often the first signal of recurrence or metastases, which is useful in prediction and prognosis of clinical outcome of patients with CRC. Determination of the activity of lysosomal exoglycosidases in body fluids may bring some hope of improving diagnosis of colorectal cancer. However, it has to be remembered that currently the most effective diagnostic method of CRC is endoscopy.

Key words: colorectal cancer, tumor markers, endoscopy, lysosomal exoglycosidases.

Introduction

In 2009, in Poland colorectal cancer (CRC) was the third most commonly observed (after lung cancer and prostate cancer in men and breast cancer and lung cancer in women) neoplasm in both genders [1, 2]. Approximately, one in three people with colorectal cancer dies as a result of the disease, which is a significant proportion (7.1% of men and 7.9% of women) of all deaths connected with neoplasm [1]. CRC more frequently affects citizens of well-developed countries in comparison to poorly-developed countries. The higher number of patients with colorectal cancer in well-developed countries is connected with predisposition to carcinogenesis: low physical activity, high calorie and fat diet, obesity and a sedentary lifestyle [3]. In Poland, 11 thousand new cases of CRC are noted annually and approximately 8 thousand patients die [4, 5].

In Western Europe, a decrease in mortality rate connected with CRC is observed and the percentage of total recoveries is 65%, which is connected with early diagnostics and treatment of CRC [6]. In Poland, the percentage of total recoveries is approximately 30–35%. The reason for such huge differences is late recognition, frequently in the stage when metastases to the distant organs (mainly liver) are noticed [8, 9].

Despite genetic changes in CRC, epigenetic disorders are also observed which influence the response to treatment. It has been proved that in some groups of patients carcinogenesis is a result of DNA methylation and covalent modification of histones [4, 10].

Current oncological diagnostics emphasizes the necessity of early recognition of neoplasms, even in an asymptomatic or pre-cancerous stage [11]. Early recognition of CRC is extremely important in patients with acute symptoms and adverse course of the disease (approx. 155), due to the fact that CRC may cause severe intestinal perforation as a result of obstructive ileus [12]. It is estimated that > 50% of patients will develop colonic polyps; in 6% of them increased risk of CRC is noted [13]. Screening examinations, in order to recognize and remove adenomatous polyps, are extremely important in prevention of colorectal cancer [14].

Invasive examination

The simplest method of CRC recognition, along with the case history, is *per rectum* examination. During this examination, 70% of rectal cancers and

30% of CRCs are recognized. The accuracy of the examination increases with the experience of the doctor [15, 16].

Endoscopy

The most commonly applied and the most efficient method in diagnostics of CRC is endoscopy [6,7]. It includes sigmoidoscopy and colonoscopy. These examinations allow one to localize the tumor and take part of the large intestine for histological examination. Sensitivity and specificity of sigmoidoscopy for polyps and extended CRCs recognition is 92–97%. Sigmoidoscopy allows one to see only the lower part of the colon and rectum. Colonoscopy allows one to obtain an image of the whole intestine with similar sensitivity and specificity [15, 16]. In comparison to other screening examinations, colonoscopy has many more advantages: it is performed in less distant time points and increases the acceptance and tolerance of recent sedative techniques [17]. The research which included patients with average risk of CRC after colonoscopy showed a 67% decrease in the morbidity rate and a 65% decrease in the mortality rate in comparison to the control group. Endoscopy (sigmoidoscopy) may be applied in diagnostics of CRC as well as in palliative procedures in patients disqualified from surgical treatment (according to severity of the tumor or co-morbidities). Such procedures include methods enabling clearing of the obstruction connected with cancer [18]. Despite many advantages, endoscopy is an invasive method and often causes discomfort in patients [19]. What is more, colonoscopy brings the risk of perforation or bleeding from the large intestine. The risk of complications leads to patients avoiding and postponing the examination [20]. Recently, noninvasive virtual colonoscopy is more frequently applied. It allows one to obtain a 3D image of the large intestine with simultaneous application of computed tomography. Application of virtual colonoscopy decreases the risk of complications connected with perforation or bleeding from the large intestine [15, 19].

Imaging tests are also helpful in diagnostics of CRC, including roentgenographic examination of the thorax (Rtg), endorectal ultrasonography (USG), abdominal USG, computed tomography (CT) and nuclear magnetic resonance (NMR). However, these methods are efficient only in case of severe focal lesions [4]. What is more, positron emission computed tomography (PET/CT) is applied in colorectal cancer diagnostics. According to the fact that neoplasm may develop along with changes in metabolism of some chemical compounds, such as carbohydrates, in fluor-18-fluorodeoxyglucose positron emission computed tomography (18F-FDG PET/CT) 18F isotope-labeled deoxyglucose is used with the addition of the most common preparation, F18-FDG.

According to researchers, 18F-FDG-PET/CT shows the main prognostic value in response to treatment [46]. It was proved during FDG PET/CT at staging and after neoadjuvant chemoradiotherapy (mean 6.7 weeks) in 69 patients with locally developed rectal cancer that it is possible to stratify patients with rectal cancer before the surgery regardless of the method of image interpretation [42].

Capirci *et al.* [43] also presented the potential role of 18F-FDG-PET in secondary diagnostics after preoperative chemoradiotherapy in patients with locally developed rectal cancer and indicated that RI (response index) seems to be the best index of estimation of response to chemoradiotherapy.

Grasetto *et al.* [44] suggest that FDG-PET/CT along with routine evaluation of patients with colorectal cancer or with different neoplasms with metastases to the liver have a great impact on estimation of the stage of the disease and selection of suitable candidates for solitary liver metastasis resection and outcome.

In the diagnosis of colorectal cancer, 18F-FDG-PET/CT has an established role and an impact on the clinical image of patient.

In 18F-FDG-PET/CT, glucose metabolism does not depend on changes in the size of the tumor and tumor modification before and after the treatment is not connected with its morphological changes. 18F-FDG-PET/CT may be used for monitoring of the response to chemotherapy in patients with advanced colon cancer [46]. The researchers found that 18F-FDG-PET/CT has higher CT sensitivity in the detection of colorectal cancer metastases to the liver [46].

An increasing number of reports in the literature is observed proving that FDG PET is a powerful tool for monitoring the response results in GIST (gastrointestinal stromal tumors) and describing its central role in the evaluation of early response to treatment [45]. Researchers [45] reported a case where 18F-FDG PET/CT showed a very early treatment response in GIST, only 10 days after the beginning of the treatment, and was useful for 18 months follow-up. The examination may be clinically useful in many stages of colorectal cancer development [46].

At the moment, 18F-FDG-PET/CT is used the most frequently to evaluate the response to treatment after radio-chemotherapy in patients with advanced rectal cancer [46].

Noninvasive diagnostic methods

Fecal occult blood test

Fecal occult blood test is a simple, cheap and noninvasive diagnostic examination. The test reveals hemoglobin in feces, which indicates bleeding from the gastrointestinal tract. What is more, blood in feces is an unspecific indicator of CRC because it may derive not only from cancerous changes but also from polyps > 1–2 cm [14]. Repeating the test increases its sensitivity up to 90% [2].

The immunohistochemical fecal occult blood test (FIT) reveals human globin, a protein which builds hemoglobin along with heme and is also applied in CRC diagnostics [14].

Methods of molecular diagnostics are also applied including sDNA, which reveals changes in DNA in colorectal adenocarcinomas. DNA is stable in feces, which allows one to isolate and differentiate it from the DNA of bacteria [14]. Molecular diagnostics of CRC based on genetic and epigenetic tests has limited applicability. It is not commonly available and the expenses are relatively high [21]. Disadvantages of molecular diagnostics of CRC motivate the search for other biomarkers present in available biological material with lower costs.

Non-enzymatic tumor markers

Improvement of current CRC diagnostics is connected with non-enzymatic tumor markers [4]. Tumor markers are substances produced by tumor cells or healthy cells as a response to the tumor [22]. Markers may be applicable in screening tests, differential diagnostics, prognosis and in observation of disease progress. They may differentiate malignant from benign tumor in case of unspecific histological image [23]. Markers may be assayed in blood, urine and other body fluids [22].

Diagnostics and monitoring of CRC use: carcinoembryonic antigen (CEA), cancer antigen (CA) 19-9, tumor antigen of colorectal cancer (tumor-associated glycoprotein, TAG-72), tissue polypeptide specific antigen (TPS) and TAG-72 (Table 1). Increased values of tumor markers evaluate recurrences or metastases, especially to the liver [11]. Unfortunately, tumor markers recently applied in CRC diagnostics have insufficient sensitivity and organic specificity [24]. There is an urgent search for new, more sensitive and specific biomarkers of CRC.

CEA is the most frequently examined marker when gastrointestinal tract tumor is suspected [22]. The concentration over 5 µg/l is established as high [25]. CEA is a glycoprotein produced by cells of the large intestine. An increased level of CEA in serum may be connected with carcinogenesis. In 50% of patients it is an indicator of tumor recurrence after resection of the tumor. Unfortunately, increase of CEA concentration rarely occurs in early stages of the disease; usually it is observed in severe tumors [11, 22]. An increased level of CEA (> 5 µg/ml) before the operation may correlate with adverse prognosis [25]. According to Locker *et al.* [25], a sustained higher level of CEA in patients with metastases but without clinical symptoms allows one to qualify patients for more intense treatment, which in consequence extends the time of survival. Locker *et al.* [25] suggest estimation of CEA in treated patients with CRC every 1–3 months. Recent research showed that 15% of large intestine tumors do not reveal a higher level of CEA or release small amounts of CEA [11]. Increased concentration of CEA in serum may also occur in inflammatory conditions including hepatitis, inflammatory bowel disease (IBD), pancreatitis or obstructive pulmonary disease. CEA is highly specific in CRC but its sensitivity and validity are not sufficient for early cancer recognition [25, 26]. It has to be mentioned that results of CEA level estimation in the same portion of serum and in different laboratories may differ significantly [25].

CA 19-9 (carbohydrate antigen) is observed in gastrointestinal tract tumors. It is a glycoprotein with high molecular weight which may be released to the blood [27]. CA 19-9 is used in diagnostics of pancreatic, gastric and colorectal cancer [11]. According to the increase of CA 19-9 concentration in pancreatitis and liver disorders, the specificity of tests based on this antigen is limited [22]. CA 19-9 is applied in diagnostics and monitoring of CRC [14]. Similarly to CEA, it is not specific for a particular histological type of neoplasm and the organ which it derives from [27]. CA 19-9 is less sensitive than CEA [14]. Simultaneous estimation of CA 19-9 and CEA may increase diagnostic sensitivity in CRC recognition [27]. Simultaneous estimation of CA 19-9 and CEA is applied as a preoperative prognostic factor in evaluation of tumor stage and survival rate [11].

TPS (tissue polypeptide specific antigen) is a single conjugated polypeptide chain. It is formed in S and G2 phase of the molecular cycle and released to cells after mitosis [27]. TPS is applied in diagnostics and monitoring of chemotherapy in gastrointestinal tract tumors (mainly pancreatic and colorectal) and bronchial tumors [11]. Concentration of TPS in serum, closely connected with neoplasm cell proliferation, is a function of the cell division rate [27]. The upper limit of TPS in the physiological environment is 90 U/l. An increased level of TPS in neoplasms indicates hyperplasia of the tumor preceding the growth of its mass [28]. Estimation of TPS may be especially applicable in early stages of tumors. Increased concentration of TPS occurs in 60–80% of patients with CRC [26]. Michaeli *et al.* [29] observed an increased level of TPS in 75% of patients with histologically confirmed colon and rectal cancer. The time of survival was significantly shorter in patients with an initially high level of TPS. What is more, Michaeli *et al.* [29] suggest that TPS was more applicable than CEA in CRC

Table 1. Non-enzymatic tumor markers of colorectal cancer applied in routine clinical diagnostics

Marker	Full name	Application in the diagnosis of CRC	Elevated values apart from CRC	References
CEA	carcino-embryonic antigen	the highest diagnostic value in CRC; in 50% of patients increase of CEA is a signal of recurrence after tumor resection; 15% of colorectal tumors do not release CEA	inflammation: liver, intestines and pancreas; obstructive pulmonary disease and breast cancer	[20, 23, 29]
CA 19-9	carbohydrate antigen	prognostic factor in evaluation of the severity of the tumor and survival rate of patients with colorectal cancer	pancreatic and gastric tumor, pancreatitis	[11, 20, 27]
TPS	tissue polypeptide specific antigen	diagnostics and monitoring of chemotherapy in CRC; predicts the growth of the tumor, precede the growth of tumor mass	pancreatic and bronchial tumor	[27, 28]
TAG-72	tumor-associated glycoprotein-72	diagnostic sensitivity in CRC (28–67%)	gastritis and cholangitis	[11]

Table 2. Comparison of the accuracy of different diagnostic methods

Diagnostic method	Description of the approach	Sensitivity and specificity	Application in the diagnostics of CRC	Disadvantages	References
Per rectum examination	the simplest method	70% of rectal cancers and 30% of CRCs are recognized	while visiting primary care physician (early and late diagnosis CRC)	medical expertise required	[15, 16]
Colonoscopy	common, efficient method, the location of the tumor allows one to withdraw part of the colon for histology	similar, high sensitivity and specificity	early and late diagnosis CRC	invasive	[15, 17]
Sigmoidoscopy	efficient method, exposes only the lower part of the colon and rectum	polyps and extended CRCs recognition is 92–97%	early and late diagnosis of CRC	invasive	[6, 15, 16]
Computed tomography (CT)	reduces detailed cross-sectional images of body	depends on the size of the tumor	preoperative assessment, postoperative surveillance for recurrence	drinking a contrast solution can cause some flushing, some people are allergic	[4, 44]
FDG PET/CT	prognostics in multiple solid tumors, measurement of viable tumor diameter on contrast-enhanced CT and evaluation of tumor density	sensitivity 84.5%, specificity 80%	detection, predictor of response to therapies, pre-operative staging, radiotherapy planning	there were no side effects of its use	[44]
sDNA	DNA is stable in stool, only one commercially available sDNA test	sensitivity 52% to 91%, specificity 93% to 97%	late: advanced colorectal cancer	applied in late diagnosis, more sensitive for cancer than for advanced adenomas	[14]
Fecal occult blood test (FOBT)	noninvasive, simple, detect the presence of occult blood in stool	variable, varies based on the brand or variant of the test range from 37.1% to 79.4%	detection	repeat the test at regular intervals	[14]
Immunohistochemical fecal occult blood test – FIT	noninvasive, reveals human globin, requires less blood of patients	about 81.8% and 64.3%	detection	relatively expensive, repeat the test at regular intervals	[14]
Tumor markers: CEA, CA 19-9, TPS, TAG-72	noninvasive test, performed using blood, urine and body fluids	sensitivity and specificity increase along with the simultaneous assessment of several markers	detection, monitoring treatment, detection of recurrences	non-invasive test	[4, 22]

monitoring. During estimation of TPS in patients with CRC increased levels of TPS may also be observed in autoimmune diseases and inflammatory lesions and the highest values occur in post-alcoholic hepatitis [27, 29].

TAG-72 (tumor-associated glycoprotein) is a glycoprotein produced by endothelium cells, renal pelvis cells, gastric epithelium and bile ducts. Diagnostic sensitivity, as a CRC marker, is 28–67%. It is recommended to estimate TAG-72 along with other markers, especially CEA [11].

Studies have been conducted on application in CRC diagnostics of other potential markers: protein mutation p-53, *ras* index, thymidine synthesis (TS), dihydropyrimi-

dine dehydrogenase (DPD) and thymidine phosphatase (TP). These tissue markers have been used in prognosis of CRC treatment results, but they are useless in screening tests. It seems that the aforementioned potential markers may be applicable in CRC prognosis; however, there is no reason to apply them in CRC diagnostics [25].

Lysosomal exoglycosidases as potential CRC markers

Recently, studies have been conducted on application of lysosomal exoglycosidases as CRC markers including α-mannosidase, β-galactosidase and *N*-acetyl-β-D-hexo-saminidase, its isoenzymes A and B and cathepsin D [30–32].

In the development of CRC, macrophages, mastocytes and neutrophils take part through the transformation of tumor cells. Development of CRC and its metastases may be supported by exoglycosidases released by macrophages [33, 34]. Szajda *et al.* [30, 31, 35] noted significant increase of HEX, HEX A, HEX B, GAL and FUC activity in serum and urine of patients with CRC. Waszkiewicz *et al.* [36] observed that the increase of lysosomal exoglycosidases and cathepsin D activity is connected with increased degradation and restoration of glycoconjugates in colorectal adenocarcinoma. A correlation has been observed between cathepsin D activity and HEX, HEX A, FUC and MAN activity in tumor tissue and urine along with a correlation between cathepsin D and GAL in urine [36]. Moreover, GAL in CRC participates in degradation of glycoconjugate oligosaccharide chains of colonic mucosa. Szajda *et al.* [30, 31] reported a significant increase of GAL activity in serum and urine of patients with CRC [31, 33]. Estimation of lysosomal exoglycosidases may be conducted in the majority of laboratories. The advantage of the test is its low cost as well as simplicity and repeatability. The disadvantage of lysosomal exoglycosidases is their unspecificity. Lysosomal exoglycosidase activity also increases in other neoplasms – pancreatic, thyroid, renal, ovarian and leukemias [37–39] – as well as such diseases as glomerulonephritis, hypertension, Sjögren syndrome, idiopathic arthritis and after liver transplantation [40].

In summary, it may be stated that analysis of single genes and tumor markers in prognostics of the disease is applicable, but frequently connected with insufficient sensitivity and specificity in routine clinical practice. Application of invasive examinations resolves almost all of the problems. However, it has to be mentioned that some patients do not want or cannot undergo the test due to its local unavailability. Gathering of feces samples for the blood occult test or DNA test may be conducted at home without bowel preparation. Patients should understand limitations and requirements of noninvasive tests – which are less efficient in prevention of the disease in comparison to invasive tests, should be conducted regularly and if the result is abnormal, invasive test will be necessary. What is more, for patients who are not able to repeat the test, noninvasive examinations should not be recommended due to their insufficiency [14]. The best solution seems to be estimation of at least two or three markers at the same time in order to increase their diagnostic applicability. It has to be mentioned that noninvasive tests should be conducted only during screening examination and therapy monitoring [41]. Estimation of lysosomal exoglycosidase activity should be considered as an additional CRC marker. Screening examination using tumor markers and intervention in early stages of CRC may significantly decrease the mortality rate connected with CRC. However, endoscopy is the most precise diagnostic method.

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