

UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

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**CLINICAL, PATHOLOGICAL AND
PROGNOSTIC FACTORS IN
COLORECTAL CANCER**

Ph. D. Thesis Abstract

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STATE OF KNOWLEDGE

1. Introduction

Colorectal cancer (CRC) represents one of the most encountered malignancies, being the second most common cancer type in women and third in men. Although many progresses have been made in both surgical and oncologic therapies, or by implementing screening programs, CRC is still among the first causes of deaths worldwide. Epidemiologic studies have proven the existence of a major variation in geographic distribution of CRC incidence, with a major risk in developed countries, this being associated with alimentary habits and lifestyle changes.

This disease has a slow evolution, with most of the cases being related to premalignant lesions. Most of these situations are polyp based lesions located on the epithelium of the colon or rectum, but there are also other diseases that may evolve to CRC such as inflammatory bowel disease or genetic mutations. Even though most of the cases have a family history of the disease, external environmental factors, intestinal microbiota, or an inflammatory process may assure a high risk in CRC development.

2. CRC carcinogenesis process

CRC may appear through one or a combination of three mechanisms such as chromosomal instability (CIN), metilating phenotype of CpG island (CIMP) and microsatellite instability (MSI). The CIN path begins with mutation acquisition in adenomatous polyposis followed by mutational activation of KRAS oncogene and inactivation of tumor suppression gene TP53.

The cells have thousands of surface receptors, from which the most important seems to be the growth ones. A growth receptor factor is based on at least one protein, which is the product of different proto-oncogenes. EGFR receptors are located on the cellular surface, and the signaling from this level to the nuclei is activated by connecting specific ligands. The main ligands are the epidermal growth factor (EGF) and TGF-alfa. The high signaling through VEGFR was observed in CRC, and monoclonal therapy is capable of enhancing the survival rate without progressing to metastatic disease.

3. Positive Diagnosis

CRC evolves on a long period of time and may present even with 5 years earlier before it has symptoms. Patients present most of the times occult bleeding from the tumor site, and the

bleeding rate grows along with tumor development and degree of ulceration. Clinical aspects are influenced by the lesion location so the proximal colon tumors grow larger when compared with the ones on the right colon. Colonoscopy represents the main imaging investigation for colon exploration, even though there are not many prospective studies that highlight this aspect. First data regarding CRC incidence through early detection and diagnostic with colonoscopy were among the first case-control types, which focused on lower CRC incidence.

4. Pathologic Diagnosis

More than 90% of colorectal carcinomas are adenocarcinomas with origin in the epithelial cells of the mucosa. Others types of colorectal carcinomas include neuro-endocrine tumors with squamous cell carcinoma, adenosquamos and undifferentiated carcinomas. The typical adenocarcinoma is characterized by the glandular form, which is the basis for pathologic assessment and tumor staging. In well differentiated adenocarcinoma we can find 50-95% glandular differentiation, while the least differentiated is most of the times solid, with less than 50% glandular tissue. In practice most of the colorectal adenocarcinoma (almost 70%) are diagnosed as moderate differentiated carcinomas. Well-differentiated adenocarcinomas represent 10% and the undifferentiated carcinomas are 20%.

5. Prognostic Factors

Detecting CRC in an early stage along with a surgical intervention may lead to patients healing. However, for this to be possible there is a continuous need in developing new revolutionary therapeutic methods, either of new screening programs which may result in a larger number of early diagnosed cases and also to identify the patients that may benefit of adjuvant treatment in an intensive program of post-treatment surveillance. Thus, new specific biomarkers should be studied that may identify patients were CRC is most probably to evolve, progress or even metastasize. Until this day, the pathologic examination remains the most important method of assessing the disease prognostic after surgical resection (in I and II stage). Even though TNM staging remains the most capable predictive marker, other pathologic and non-pathologic determinants should be consider most of the times when curative therapy does not represent an option or because tumor extension does not reflect its biology.

For a long time there has been discussed about a link between inflammation and cancer, and a connection was only recently discovered. Cytokines and chemokines produced by tumor

cells, but mostly by the tumor environment are linked to tumor development by interfering in cellular differentiation and as support for the cancer cell survival.

Personal Contribution

Objectives

These studies followed the patient's characteristics with colorectal tumors, from a clinical, imagistic and pathologic point of view as well as the use of new ground breaking non-invasive techniques that may be used in early or evolutionary diagnosis. All data have proposed a new patient's profile that may develop CRC while using diagnosing methods focusing on morphologic assessment and new developed prognostic factors that may be used in disease progression, thus changing the therapeutic management. Implementing new methods in disease prevention as well as in grim prognosis of the disease offers both the patient and the physician a new window for understanding and treating CRC.

- We assessed CRC patients based on clinical, paraclinical and pathologic criteria after surgical intervention which was performed either for curative or paliative purposes;
- Morphological criteria assessment of colorectal tumors encountered with impact on patients disease evolution;
- CRC angiogenesis assesment by characterising vascular microdensity as prediction factors of CRC evolution;
- To identify and corelate with early and advanced stage new diagnosis and prognosis biomarkers;

1. Materials and Methods

This was a prospective study on 228 patients diagnosed within the Gastroenterology Clinic and Ist Surgery Clinic of the Emergency County Hospital of Craiova Romania, as well as from the Surgery Clinic of the Emergency County Hospital of Ploiesti, Prahova. Patients were included for a period of three years between 1st of January 2013 and 31 December 2015.

. CRC diagnosis was made by imagistic methods, the reference method being colonoscopy or after complications which required emergency intervention.

1.1 FOBT Protocol

Within these 3 years, in the Gastroenterology Clinic and the Surgical clinic from Ploiesti, 32 patients were diagnosed with CRC through colonoscopy with biopsy exam, all of these after taking a positive fecal occult stool test. However the number of patients that followed colonoscopy due to FOBT was 52, but the majority of lesions were either polyps, angiodisplasias, diverticulums, or inflammatory bowel disease.

1.2 Colonoscopy

Preparing for colonoscopy required food restriction and the use of polyetilenglicol – PEG solutions (Fortrans) by two-stroke administration, and in some cases only enemas were used. The solutions were divided in three litres of solution that were drunk on a 6 hour period of time and 1 litre in two hours, for a better preparation of the colon.

After imaging investigation and pathologic confirmation, tissue analysis was made with TNM staging and surgery was provided at the right time for either curative or paliative purposes based on disease evolution.

1.3 Vascular Microdensity Study

72 of 288 patients were selected and three tissue samples were harvested from each of them. A sample was selected for immunochemistry and was carefully extracted so that it may have all the colon and rectum layers. For gene expression, the first sample was from epithelial tissue, avoiding necrosis, and the second sample was from normal tissue from the resection margins.

1.5 Serologic study

Of all the 288 patients diagnosed with CRC, 35 were selected after being diagnosed. They were from different evolutive stages of the disease, so after imaging explorations 15 patients were diagnosed in an early stage whereas 20 were included and considered to have distant metastases. No patients with autoimmune disease or oncologic treatment were included.

For the control patients 32 patients were included after performing a colonoscopy in the Gastroenterology Clinic with a diagnosis of adenomatous colonic polyps after histopathologic examination.

Fresh blood was harvested stored at -80°C or -20°C till the measurements were made. For VEGF and IL-6 analysis a special kit was used based on ELISA sandwich technique which was used according to the distributor indications. Optic density was measured on a microtitrate plate reader of 450 nm.

IL-8 and IL-17 also followed the sandwich ELISA technique using a Human IL-8 kit (Krishgen BioSystem Spain), respectively a IL-17 Elisa kit (R&D Systems Minneapolis, MN). VEGF, IL-6, iL-8, IL-17 values were transpose in pg/ml.

2. Results and Discussions

This study was a prospective study, taken place on a three years period and followed aspects of CRC which may influence it's evolution and therapy. Starting from screening methods, to highlighting a risk population of developing CRC, testing prognostic noninvasive factors, or imunohistochemistry and genetic criteria, we tried to emphasize the need of developing a profile for diagnostic and therapy CRC.

While introduced in different countries, screening opportunities based on low costs occult bleeding testing offers a series of possibilities for patients to be investigated with meaningful efforts. Using these techniques for early diagnosis, either if we are talking of FOBT, FIT, DNA-based test, markers which use proteins such as fecal calprotectin or the new introduced markers for fecal microbiota CRC presence might be indicated.

This study, included patients diagnosed with colonic tumors using FOBT. The 32 cases that had a diagnosis of CRC had the FOBT test after visiting a physician or due to symptomatology. All patients that followed this test were from the urban area and had over 50 years, which is screening age for colonoscopy in the USA. Thus, this emphasize the necessity of implementing screening programs over the world.

The CRC carcinogenesis is a sequentially process that takes place thanks to a genetic mutation, followed by cellular proliferation until the development of small adenomas with low grade dysplasia, high grade dysplasia and adenocarcinoma. Using colonoscopy as a screening method but also as a therapeutic option allowed CRC incidence to decrease. This study proved that colonoscopy remains the reference method for imaging diagnosis and has the possibility of harvesting tissue for pathologic analysis. Also by using the standards of the European Society of Digestive Endoscopy which require identifying the appendicular aperture and passing into the terminal ileum, has allowed to confirm a larger number of tumors located within the right colon than the left colon or rectum.

CRC incidence increases with age. This study points out the fact that the mean age of patients with CRC was over 55 years old, which may suggest that the etiological factors may have a cumulative effect. It seems that along with age the risk of developing CRC especially in the

urban areas may be related to lifestyle changes. Only 28 patients were diagnosed with colonic or rectal tumors before 50 years old when compared with other areas of the world where the incidence is over 90% after the age of 40. However, we did not include patients with inflammatory bowel disease or genetic polyposis syndromes.

Regarding the patient's gender we observed that men had a higher risk of developing CRC than women with a ratio ranging from 1,2/1 to 1,7/1. We confirmed that more than half of the patients diagnosed with CRC were men, and this might be due to the fact that women have a higher level of female hormones which are associated with a lower risk of developing CRC and may also have a more favorable evolution.

A negative prognostic factor in disease evolution is the diagnosis made when complications already appear and require immediate surgery. The most encountered complication was tumor stenosis, found especially within the left colon, but we also found perforations and arterial thrombosis. Patients that require immediate surgery for a complication of colonic tumor have a low survival rate, thus these situations are associated not only with a local advanced stage but also with distant metastasis, which enhances the relapse rate. A comparative study over 5 years comparing emergency surgery with selected cases pointed out a mean survival period of 59 months to 82 months.

Symptomatology was also associated with a negative evolution of CRC, mostly when is ignored for a long period. The presented cases had as main symptom abdominal pain for all tumor locations, followed by transit disorders. We observed a high incidence of diagnosed cases in advanced stages either locoregional or with distant metastasis. Besides these, another factor in disease evolution was tumor dimension, which showed that larger tumors are most of the time related to the presence of distant metastasis.

The use of CEA and CA 19-9 in CRC diagnosis is not well established. These are considered loyal biomarkers for disease relapse after oncologic or surgical treatment and may only be suggested for diagnosis when they have high values. CEA is currently the most important biomarker for disease relapse after tumor resection. Our study emphasized the fact that CA 19-9 and CEA testing for these biomarkers in a diagnostic setting are valuable most of the time when is an advanced disease associated with liver, pulmonary, cerebral metastasis.

TNM staging and pathology type are the most useful prognostic factors in CRC evolution. We confirmed that most of the cases were diagnosed in an advanced stage which may be related to the patients level of education and lack of information, followed by a late visit to the physician.

Tumor development requires the development of new neoformation vessels. The metastasis process is correlated with a high vascular density, thus a potential inhibition of the angiogenesis process may lead to stop tumor growth and also limit the metastasis dissemination.

Angiogenesis assessment represents an important target for current diagnostic and therapy strategies because is considered the reference for future oncologic therapeutic options which may control the development of vessels distribution. New formed vessels may be expressed through endothelial cells such as CD 31, CD 34 and CD105. However, vascular microdensity may be assessed and used as a prognostic factor for disease evolution to a metastatic stage.

Chronic inflammation offers a high risk for CRC development. Proinflammatory cytokines and tumor necrosis factor were suggested to be connected with tumor carcinogenesis process. Our study included immunologic evaluation of some specific biomarkers for the diagnosis and evolution of CRC. We tested IL-6, IL-8, IL-17, VEGF on specific group of patients diagnosed with CRC and compared with a control group so that we may highlight their use in early diagnosis process as well as in disease evolution in advanced cases. We observed a correlation between these biomarkers in patients with colorectal tumors that suggests their use in CRC prognosis.

3. Conclusions

- CRC represents the third most diagnosed type of cancer worldwide, however the death rate is still high, especially in industrialized countries;
- The study results confirmed based on the number of patients included as well as the standardized methods the necessity of creating a profile for people that have a risk of developing CRC while using novel noninvasive techniques;
- We pointed out that there is a higher incidence of developing CRC after the age of 60;
- An important aspect of diagnosis was the presence of metastasis with more than 90 patients having liver, pulmonary or cerebral metastasis, which indicates a poor prognosis;
- FOBT may be a valid option for early diagnosis of CRC or may recommend patients for colonoscopy;
- Performing a colonoscopy and reaching the caecum with samples for pathologic examination represents the standard for CRC diagnosis;

- Before surgery tumor markers CEA and CA 19-9 were harvested, which correlated with disease extension, with high values encountered in both situations $p < 0.05$;
- Regarding the tumor stage we concluded that most of the cases were diagnosed in an advanced stage – T3. Among these patients, 93 were men and 62 women. T4 stage disease consisted of 40 patients of which 22 were men and 16 women. There were 2 cases of *in situ* tumors, and 6 patients diagnosed with T1 stage;
- After surgical excision, besides the cases where palliative treatment was instated with surgical derivation, we also assessed tumor dimension. Most of the cases were T3 with a dimension under 55 cm (88), followed by 55 cases with a range between 5 to 10 cm. Moreover, the majority of cases had a tumor under 5 cm, whereas the ones over 10 cm were considered T3 and T4.
- This study also highlighted that gene expression of CD34 as a probabilistic factor in CRC. We found a link between CD34 and CD34 RNA in harvested tissues. The results showed the importance of vascular microdensity of CD34 and gene expression in tumors located within the rectum when compared with the ones colon located;
- The immunologic study revealed that new potential biomarkers (VEGF, IL-6, IL-8, IL-17) are efficient and might be successfully used for early diagnosis and disease evolution;
- As a general conclusion we suggest that a single marker is currently not efficient for assessing CRC prognosis and there is a continuous need of creating a profile or a risk panel to bring forward the result of combined factors such as pathologic examination, inflammatory, molecular, angiogenetic factors so that a better diagnosis and therapy might be provided.

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