UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA
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PhD THESIS

-*SUMMARY*-

CLINICAL,ENDOSCOPIC AND HISTOPATHOLOGIC ASPECTS OF PREMALIGNANT AND MALIGNANT COLONIC LESIONS

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INTRODUCTION

Colorectal cancer is an important health problem worldwide, mainly in developed countries, due to the high number of cases diagnosed annually but also due to the difficulty of early diagnosis of this neoplasm, becoming an important cause of mortality worldwide.

Approximately 80% of colorectal cancers have as precursor the adenomatous colorectal polyp (1), thus demonstrating the enormous benefit of colonoscopy in screening patients for the early detection and removal of lesions that present high risk.

Although attempts to overcome the current limitations of colon cancer are numerous, this being one of the topics of great interest to researchers and clinicians alike, there are still numerous mechanisms involved in the rapid progression of pathology that have not been fully understood.

The purpose of this paper is to bring additional information to the literature on the negative prognostic factors of colorectal cancer and the importance of their knowledge for an effective personalized treatment.

GENERAL PART

ANATOMY AND HISTOLOGY OF THE BIG INTESTIN

The last portion of the gastrointestinal tract is represented by the colon, extending from the ileo-cecal valve to the anus, having the following component parts: check, ascending colon, liver flexion, transverse colon, splenic flexure, descending colon, sigmoid colon and rectum. The main features that distinguish it from the small intestine are: increased diameter, the three longitudinal muscle bands (taenia coli), saciform dilatations (haustrations) and the presence of epiploic appendages (2).

The colon differs from the small intestine by several features, one of them being the size of the colon which is considerably larger than that of the small intestine. The largest caliber is measured at the level of the check, gradually decreasing to the sigmoid colon, so that it then increases in size at the level of the rectum, where the lower third is dilated and forms the rectal ampule. The average internal diameter of the large intestine is 4.8 cm, the descending colon having the smallest colonic dimensions (3).

Although there are some structural differences between the right colon and the left colon, the miscroscopic aspect is similar throughout its length. The digestive wall consists of four distinct tunics: mucous, submucosal, muscular and serous.

The colonic mucosa is the most metabolically and immunologically active compartment, as it differs from the small intestine mucosa by the absence of intestinal villi and circular envelopes.

Submucosa is composed of bundles of smooth muscle tissue, fibroelastic and adipose tissue, among which are the enteric nervous system, vascular and lymphatic system. The muscular layer of the colon is composed of an internal circular muscular layer and an external longitudinal layer, differing from the muscle of the small intestine by the three thick longitudinal bands formed by the muscular fibers of the longitudinal layer, called the tapeworm. The serous layer presents in the intraperitoneal portions of the colon, at the level of the antimesenteric surface, saciform dilations with adipose content, called epiploic appendages (4).

ETIOLOGY AND DIAGNOSIS OF COLO-RECTAL CANCER

Etiology

The data provided by GLOBOCAN 2018 show a worrying increase in mortality, with colorectal cancer taking second place in terms of mortality. The distribution of colorectal cancer worldwide has a marked variability.

This variability may be due to both genetic factors but, according to new studies, environmental factors play a much more important role than genetic ones. Exogenous factors in the development of colorectal cancer have been shown to have an important role: the dietary intake of red meat and processed meat, smoking and alcohol consumption.

Currently, there is a high public interest in intestinal bacterial flora, which physiologically induces an immunosuppressive response, produces metabolites with role in tumor suppression and detoxifies the substances responsible for carcinogenesis (5).

Detailed knowledge of premalignant lesions is very important as their removal leads to a significant decrease in the risk of developing colorectal cancer.

Adenomatous polyps represent the substrate for 80% of colorectal cancers. The premalignant lesions described above are also found in hereditary syndromes, with the difference that the latter show inherited genetic changes.

About 1% of colorectal cancers are given by hereditary polyposis syndromes and 1-3% by Lynch syndrome, the latter being the most well-known form of hereditary colo-rectal cancer.

Diagnosis of colorectal cancer

Currently, the most commonly used screening methods are evaluation of occult bleeding by fecal immunohistochemical (FIT) or faecal occult bleeding test (FOBT) and imaging evaluation by flexible colonoscopy and sigmoidoscopy (6).

Colonoscopy is still considered to be the gold standard in the diagnosis of both colorectal cancer and premalignant lesions by the ability to biopsy the detected lesions and to excise the detected polyps, thus eliminating the risk of their transformation into neoplasms.

CT colonoscopy, also called virtual colonoscopy, is a noninvasive method that does not require sedation and is much less expensive than colonoscopy, data demonstrated in a large US study (7).

MRI represents the gold standard in the detection and staging of tumor formations located in the rectum, being necessary for establishing the prognosis of the disease according to the local and regional extension and distance of the primary tumor.

TREATMENT OF COLO-RECTAL CANCER

Surgical Treatment

In the case of adenocarcinomas, which represent over 90% of colorectal neoplasms, TNM staging is the one that dictates the subsequent treatment (8).

Thus, clinical stages I-III of operable colonic cancer are indicative of radical surgery per primam, except for the presence of a primary tumor invading other adjacent structures, which will benefit from neoadjuvant treatment of cytoreduction, as well as except for the metastatic stages when the surgery is performed for the prevention or treatment of complications of primary tumors.

Rectal cancer is an entity treated distinctly from colon cancer, thus the degree of recurrence decreasing from 50% to below 10% with the entry into medical practice of excision of the mesorectum and of the preoperative treatment, the rectum being a radiosensitive location.

Systemic therapy

The current worldwide consensus regarding the administration of adjuvant chemotherapy in stage II of the disease includes the following risk factors: pT4 tumors, increased degree of differentiation, perineural or perivascular invasion, perforation or obstruction, as well as suboptimal lymphodissection with low number analysis of lymph nodes.

Microsatellite instability is part of the new stratification methods for the prognosis of colon cancer, currently using international guidelines in stage II of the disease, and recently in stage IV to benefit from immunotherapy treatment. Adjuvant chemotherapy in stage III of the disease has shown its benefits, bringing a benefit of 22% to 32% in overall survival and a reduction of up to 30% in the risk of recurrence of the disease (9). Currently, adjuvant standard chemotherapy in colon cancer is represented by the administration of a platinum salt representative, respectively Oxaliplatin in combination with 5Fluorouracil iv pev or its oral equivalent, Capecitabine.

The first line of treatment in metastatic colorectal cancer is a combination of standard chemotherapy plus a biological agent targeting either angiogenetic vascular growth factor or antibodies targeting the epidermal growth factor receptor. Currently, these targeted molecular therapies are used considering the localization of the primary tumor as well as the mutational status of RAS (10).

A new biomarker has been introduced in medical practice to stratify the risk groups in colorectal cancer, namely mutation in the BRAF V600E gene.Currently, international guidelines recommend that the determination of the BRAF V600E mutation be performed from the first presentation of a patient with metastatic colorectal cancer because this may dictate subsequent therapeutic behavior.

Radiotherapy

The current therapeutic scheme used in advanced loco-regional rectal cancer involves the administration of concomitant neoadjuvant radio-chemotherapy in order to convert to operability the tumors and to achieve an excision within the limits of oncological safety.

PERSONAL CONTRIBUTIONS

CLINICAL AND IMAGISTIC STUDY OF COLO-RECTAL MALIGNE AND PREMALIGE INJURIES

Studied materials

We performed a retrospective and analytical study that included 98 patients, 72 of whom were diagnosed with colo-rectal neoplasm and 26 with precursor lesions of malignancy. These were evaluated during the period January 2013-November 2017 in the Emergency County Clinical Hospital Craiova, the Internal Medicine section and the Renasterea Craiova Medical Center, Romania. All patients were diagnosed by colonoscopy with biopsy sampling.

Results

The group of patients included was characterized according to the following parameters: sex, age, place of origin, symptomatology at onset. Regarding the gender distribution, we recorded a much higher frequency of both the colo-rectal neoplasm and of the premalignant lesions in the male patients compared to the female, with a percentage of 68.37% of the total 98 patients studied., respectively 48 men (66.67%) in the 72 patients diagnosed with colorectal cancer and 19 men (73.08%) of the total 26 patients with premalignant lesions.

The stratification by age group revealed a high incidence in patients aged 60-69 years, the study including 36 (36.73%) of patients in this category out of the total 98 included in the study. The same distribution was identified both in patients with colorectal cancer and in patients with precursor lesions of malignancy. Regarding the distribution according to the environment of origin, a significant difference was observed, being included in the study 62 (63.27%) patients coming from the urban area.

Analyzing the symptomatology reported by the patient at the time of presentation to the doctor, in the group of patients diagnosed with colo-rectal neoplasm, a predominance of the transit disorders was observed, these being reported in 51 patients (70.83%) of the 72 included in the study, followed of the anemic syndrome reported in 42 cases (58.33%).

The same distribution was also registered in the group of patients with precursor lesions of malignancy, 16 (61.54%) patients out of the 26 accusing transit disorders and 6 (23.08%) patients being detected with anemic syndrome.

The lesions evidenced imaging by colonoscopy were divided according to location. Thus, in the case of premalignant lesions as well as in the case of colo-rectal neoplasm, the most frequent localization was recorded in the rectum, with a percentage of 59.18% (58 cases) of the total of 98 patients included in the study, respectively 59.72% (43 cases) in patients diagnosed with colorectal neoplasm and 57.69% (15 cases) in patients with premalignant lesions.

The imaging aspect of the tumor formations was also studied, the tumor formations being included in separate categories. The most frequent macroscopic description was that of the vegetative tumor formations, occupying 54.16% (39 patients) of the studied group of 72 patients with colo-rectal neoplasm.

Our study tried to correlate the imaging aspect of the lesions with their location. Thus, in all anatomical structures of the large intestine, vegetative tumors occupy the leading place.

HISTOPATOLOGICAL STUDY

Materials and methods

There were included 98 patients with lesions identified by colonoscopy in the Internal Medicine section of the Municipal Clinical Hospital Craiova and the private medical center Renasea Craiova. All the samples were subjected to histopathological examination by the technique of inclusion in paraffin and specific coloration with Hematoxylin-Eosin and Tricrom Goldner-Szeckeli, carried out in the Faculty of Medicine Craiova, the Histology department and in the private office of pathological anatomy Elana Med Craiova.

Results

The histopathological analysis of the tumors, besides the confirmation of the malignancy, brings important information for establishing the therapeutic conduct, the integration in a risk group that gives the patient a certain prognosis.

In our study we have shown that the most common histopathological type in patients with colorectal neoplasm is adenocarcinoma in 94.47% (68 cases), according to the data from the literature. The next histopathological type as frequency was 4.16% mucinous adenocarcinoma (3 cases). Also, only one case of signet ring cell carcinoma (1.37%) was reported.

Analyzing the degrees of differentiation of the tumor formations, we found that the most frequently encountered lesions were moderately differentiated (G2) in percentage of 59.72% (43 cases), followed by the well differentiated ones (G1) in proportion of 19.44% (14 cases) , on the last two places being the tumors that have an association between the good and moderate differentiated degree (G1 + G2) in a percentage of 13.89% (10 cases) and the one with the association of the moderate and weakly differentiated areas (G2 + G3) in a percentage of 6.94 % (5 cases).

The correlation of the degree of differentiation with the localization of the primary tumor demonstrated the predominance of the moderately differentiated tumor formations in all the analyzed anatomical segments, namely the right colon, the left colon and the rectum, with a percentage of 50% (3 cases) in the proximal colon, 47.83% (11 cases) in the distal colon and 67.44% (29 cases) in the rectum.

We also analyzed the relationship between the degree of differentiation and the macroscopic aspect of the primary lesion. Moderately differentiated tumors (G2) were reported to be the most common both in vegetative tumors and in ulcero-vegetative and ulcero-infiltrative tumors, with the following percentage values: 61.54% (24 cases) in the vegetated ones , 11 cases (57.89%) ulcer-vegetative and 6 cases (85.71%) ulcer-infiltrative. Pure infiltrative tumors were equally associated with both moderately differentiated (G2) and well-differentiated (G1) tumors, as well as with the association between well and moderately differentiated (G1 + G2) tumor cells, with a percentage of 28,57% (2 cases).

In the case of signet ring cell carcinoma and mucinous adenocarcinomas, these have been described as tumor formations that associate moderately and poorly differentiated areas, according to the data presented in the studies, these being more aggressive tumor formations.

IMMUNOHISTOCHEMICAL STUDY OF MALIGENOUS LESSONS

Materials and Methods

In the immunohistochemical study we included 20 patients diagnosed with colonic or rectal neoplasm in the County Clinical Hospital of Craiova Emergency and in the Clinic Renasea Craiova. They were selected from the group of 72 patients who were imaged with tumor

formations or suspected lesions, afterwards the malignancy was confirmed by the histopathological examination of the tissue fragments collected by biopsy.

Immunohistochemical analysis of tumor fragments obtained by biopsy was performed using the following types of antibodies: anti-p53 (monoclonal mouse anti-human p53 protein, clones DO-7, Dako, 1:50 dilution); anti-CK7 (monoclonal mouse anti-human CK7, clone OV-TL 12/30, Dako, 1:50 dilution); anti-cluster of differentiation (CD) 34 (monoclonal mouse anti-human CD34 Class II, clone QBEnd10, Dako, 1:50 dilution); anti-CK20 (monoclonal mouse anti-human CK20, clone Ks20.8, Dako, 1:50 dilution); anti-Ki67 (monoclonal mouse anti-human Ki67, clone MIB-1, Dako, 1:50 dilution); anti-CK19 (monoclonal mouse anti-human CK19, clone RCK108, Dako, 1:50 dilution); anti-vascular endothelial growth factor (VEGF) -A (monoclonal mouse anti-human VEGF-A, clone VG1, Thermo Fisher Scientific, 1: 200 dilution); anti-VEGF-C (polyclonal anti-human VEGF-C, Thermo Fisher Scientific, 1: 100 dilution).

Results

In our study, the evaluation of the tumor cell multiplication capacity was performed using the anti-Ki67 antibody. A correlation of the proliferation index with the degree of tumor differentiation was observed, the well differentiated tumors having a much lower Ki67 index in comparison with the tumors with poorly differentiated component that present a high proliferation index score, giving them a more aggressive status. .

The immunohistochemical study demonstrated different reactions of the tumors to these markers. As stated in internation data, the tumor cells analyzed in the present study showed a negative reaction when marking with the CK7 antibody. In contrast to this result, anti-CK19 antibody immunostaining showed positive results in all tumor cells regardless of the degree of tumor differentiation. Our study recorded varied reactions of immunolabeling with anti-CK20 antibody, with results from high positive to negative response in tumor cells, without correlating with the degree of tumor differentiation.

In our study, the investigation of the genetic changes of the p53 tumor protein, known as the guardian of the genome, this being involved in the repair of AND lesions, was performed by evaluating the expression of the p53 protein in the tumor cells, with a response that varied from intensely positive to negative for anti-p53 antibody. The variability of TP53 gene

expression in tumor cells was demonstrated by the lack of correlation of the immunohistochemical response with the degree of tumor differentiation.

The anti-CD34 antibody was used in the current study to evaluate the tumor microvascularization, the variability of these tumors being demonstrated, the response being different even from one tumor area to another. However, a correlation between the degree of differentiation and the response to anti-CD34 has been described. The well and moderately differentiated tumor formations showed richer microvascular networks, with an intensely positive expression of the anti-CD34 antibody. In contrast, tumors with a poorly differentiated component showed a negative response.

In our study, we analyzed the ability of the rectal tumor cells to stimulate angiogenesis and lymphangiogenesis through two specific markers, namely VEGF-A and VEGF-C. The VEGF-A marker has been studied for angiogenesis, being involved in the proliferation and migration of endothelial cells, with an important role in mediating the genes responsible for the formation of new vessels. VEGF-C is an indicator of lymphangiogenesis, directly stimulating the proliferation and migration of endothelial lymph cells, thus promoting the invasive character of colorectal cancer by disrupting the endothelial barrier, before the initiation of new lymphatic vessels.

In our study, tumor cells exhibited both positive and negative reactions, depending on the degree of tumor differentiation. The lesions with poorly differentiated component had a negative or low intensity response to VEGF-A and VEGF-C, whereas the well and moderately differentiated cells showed an intensely positive response.

CONCLUSIONS

In contrast to the sustained efforts of medical researchers, the survival curve of patients suffering from colorectal neoplasm is decreasing, demonstrating the need to develop new therapeutic targets that will improve the prognosis of this pathology.

The analysis of the clinical data included the distribution of the study group according to age, sex, place of origin, symptomatology present at the hospital, as well as correlations between these data.

The imaging evaluation followed the determination of the localization of the primary tumor or of the precursor lesions of the malignancy as well as their macroscopic aspect.

The histopathological examination specified the histopathological type of tumors and premalignant lesions, as well as their simultaneous association, evaluating the degree of differentiation in the case of malignant tumors.

In the immunohistochemical study were included 20 patients diagnosed with colonic or rectal neoplasm, with the aim of evaluating the following immunohistochemical markers: Ki67, p53, CK7, CK19, CK20, CD34, VEGF-A, VEGF-C.

Our study concluded that the occurrence of colorectal cancer and premalignant lesions is more common in males, from the urban area, over the age of 60 years, the specific symptomatology being variable depending on the primary location. The most common localization of both malignant tumor formations and precursor lesions of malignancy was recorded in the rectum. The predominant imaging aspect was vegetative tumor formations.

In our study we have shown that the most common histopathological type in patients with colorectal neoplasm is adenocarcinoma.In conclusion, the detailed histopathological analysis can lead to a better understanding of the tumor genesis, being able to classify patients in various risk groups to benefit from individualized, more aggressive or less aggressive treatment, depending on the prognosis provided by the data obtained by histopathological analysis. of the tumor.

Immunohistochemical analysis assumed the characterization of tumor formations using specific immunohistochemical markers.

First, we performed the evaluation of cell proliferation with the help of the anti-Ki67 antibody, establishing a correlation between the tumor grading and the proliferation index, being directly proportional to the increase of the degree of differentiation. In correlation with international immunohistochemical data, the colo-rectal tumor cells did not show a positive reaction on contact with the anti-CK7 antibody. In contrast, tumor cells exhibited varied reactions upon immunoblotting with anti-CK20 antibody. anti CK19 which caused positive reactions in the tumor cells, also without correlating with the degree of differentiation.

The ability of tumor cells to promote angiogenesis and lymphangiogenesis was studied using VEGF-A and VEGF-C markers, respectively. VEGF-A, involved in angiogenesis, showed positive reactions in well and moderately differentiated tumor formations (G1, G2), whereas in cells with poorly differentiated component (G3) they showed negative response. The VEGF-C marker was studied for the analysis of lymphangiogenesis, with results identical to

those reported in the study with VEGF-A, the positive expression correlating with the low tumor grading (G1, G2).

The modernization and emergence of new molecular characterization techniques for the evolution of colorectal cancer has led to the understanding of the hypothesis that individual biomarkers represent the future in diagnosis and treatment.

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