DOCTORAL THESIS ABSTRACT

Early prognostic factors and phase prognostic factors in locally advanced colorectal cancer.

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INTRODUCTION

Colorectal cancer is a problematic pathology in public health systems because, frequently, diagnosis occurs in late stages and is correlated with an increased mortality.

Improvement of the means of diagnosis, improvement of anaesthetic procedures, antalgic procedures and modern surgical procedures, along with the remarkable progress of radiotherapy and especially chemotherapy, are reasons that require continuous updating of the therapeutic strategy in malignant colorectal pathology.

The research area has been expanded with the development of molecular biology, revealing a number of factors involved in the development of malignant colorectal tumors such as oncogenes, tumor suppressor genes, cytokines and monoclonal antibodies.

My purpose was to study the correlations between the measured values of interleukin 8 in the blood and colorectal malignant tumor tissue, as well as between gene expression of interleukin 8 in normal colorectal tissue and tumor tissue.

CHAPTER I. ANATOMY NOTIONS OF COLON AND RECTUM

The large intestine, the last part of the digestive tract, continues caudally the small intestine, from the ileo-cecal valve to the anus, which opens to the outside of the anal margin. It plays a role in the absorption of water in the intestinal content, subsequently transforming indigestible remnants into semisolid feces which will be accumulated and temporarily stored in the rectum [1].

Embryogenesis

All portions of the large intestine are of endodermal origin, with the exception of the anal canal that originates from the proctodeum, an invagination of the cloacal tuber of the ectoderm [2]. The colon and rectum also have different embryological origins. The right colon is formed from the ascending arm of the umbilical cord (mesenteron) and comprises the check, the ascending colon and two thirds of the right transverse colon, vascularized by branches of the superior mesenteric artery. The left colon develops from the terminal intestine and comprises the left third of the transverse colon; the colon and the sigmoid colon, all vascularized from branches of the lower mesenteric artery, while the rectum develops from the cloaca.

Descriptive anatomy

With a mean length of 150 to 180 centimetres, the large intestine creates a frame for the small intestine in the infracolic portion of the abdominal cavity and comprises several segments: the check, the vermiform appendix, the ascending colon, the transverse colon, the descending colon and the sigmoid colon, the rectum, and anal canal [2].

The thick intestine wall is made up of four tunics. On the outside, the serous tunic is represented by the peritoneum at the level of the caecum, the colon, and the first rectal portion, while at the last part of the rectum there is only an adventitia, followed by the muscular tunic formed by an outer layer of longitudinal fibers, and an inner layer with circular fibers. Advancing to the lumen, the penultimate layer, the submucosal, is crossed by the blood vessels, lymphatic vessels and nerves. Mucosal tunic delimits the lumen and it's thicker compared to the small intestine.

Topographic and surgical anatomy

An anatomical-surgical division can be done by reporting fixed and mobile segments. Thus, the fixed portions comprise the ascending colon, the right third of the transverse colon, the colon and the rectum, and the moving portions are the caecum, the two thirds of the transverse colon and the sigmoid. The check is in the proximity of the inguinal ligament, the upward colon projection in the anterior abdominal wall is made at the top of the right gut and right flank, the projection of the transverse colon on the anterior abdominal wall is in the right upper abdominal quadrant. The descending colon is located on the left side of the inframesocolic stage, whose content is made by the sigmoid and rectum terminating at the anorectal flexure localised antero-inferior by the coccyne.

CHAPTER II. COLORECTAL ONCOGENESE

The progression of colorectal cancer from a single crypt to adenomatous polyp is the result of complex genetic changes. This pathology is multifactorial, conditioned by genetic and environmental factors such as obesity, smoking, red meat consumption, excessive alcohol consumption, genetic predisposition, chronic intestinal inflammatory diseases that modulate oncogenesis and disease progression.

Colorectal cancer can develop in the mucosa through the de novo oncogenesis process, the majority of lesions having as their starting point malignant degeneration in the adenomatous polyp. The morphological substrate is an epithelial dysplasia in the colon or rectum, following the adeno-carcinoma sequence, in which, due to a progressive accumulation of cellular mutations, an uncontrolled cell proliferation occurs in the crypts [3].

CHAPTER III. CLINICAL MANIFESTATIONS, DIAGNOSTIC STAGES, THERAPEUTIC ATTITUDE IN COLORECTAL CANCER

As a result of slow tumor growth in the lumen of the large intestine, the neoplasm may slowly evolve for a long time without obvious clinical signs. Clinical manifestations such as persistent transit disorders (constipation / diarrhea), dyspeptic syndrome, interruption of intestinal transit, abdominal pain, presence of blood in the faeces or general non-specific manifestations (such as weight loss, inappetence, asthenia, fever or subfebrility) can help to orientate the diagnosis and the complementary examinations to be performed [4].

The final diagnosis, the stage of the tumor, the degree of cell differentiation, the degree of tumor infiltration beyond the apparent limits and the prognosis of the disease are established after the anatomopathological examination. Immunohistochemistry examines the presence of new antigens or the loss of normal antigens at tumor level.

The indication for chemotherapeutic treatment is mainly based on the stage of the tumor at the time of diagnosis and begins with stage II using various molecules such as oxaliplatin, capecitabine, folinic acid, bevacizumab, etc, in various therapeutic regimens, as adjuvant or neoadjuvant treatment, depending on the stage of neoplasia at diagnosis. Rectal adenocarcinomas are included in the category of moderately radio sensible cancers. Radiotherapy or preoperative radio chemotherapy is preferable to postoperative radiotherapy due to better assessment and reduced toxicity [5-9].

Regarding the surgical treatment of colon cancer, the technique differs depending on the location of the tumor and the stage in which it is operated. It must, however, respect certain limits of oncological safety: distal and proximal excision of at least 2 centimetres, block excision of the mesorectum or mesocolon with the reference of the vascular pedicle to obtain a correct lymphadenectomy.

CHAPTER IV. THE IMPORTANCE OF MARKERS IN COLORECTAL CANCER

A biomarker is defined as a characteristic that can be objectively measured to evaluate a physiological process, a pathological process or a pharmacological response to a therapeutic intervention [10].

The fact that cancer cells express at their surface receptors for mediators of inflammation is already demonstrated in many international studies. Several cytokines, such as macrophage migration inhibitor (MIF), TNF-α, interleukin 6, interleukin 17, interleukin 12, interleukin 23, interleukin 10 and TGF-β were linked to both experimental and human cancer and is considered to promote or inhibit tumor growth.

Interleukin-8 has an important role in the pathogenesis of colorectal cancer. Interleukin-8 is a member of the chemokine family responsible for attracting and activating neutrophils during immune responses [11] and is therefore capable of inducing an inflammatory response [12], being one of the most significantly modified chemokines in colorectal cancer [13]. It is produced by some malignant cells including colorectal cancer cells [227] and is a tumor micro environement regulator that can contribute to tumor progression [221, 228].

PERSONAL RESEARCH

CHAPTER V. THE OBJECTIVES OF THE PAPER. GROUPS OF STUDY

Colorectal cancer, one of the most common malignant tumors, is a problematic pathology in public health systems. The main purpose of this paper is to study prognostic factors that would open the possibility of using new therapeutic targets.

To achieve this, we analysed interleukin 8 - a member of the chemokine family capable of inducing an inflammatory reaction, detecting whether there are correlations between the measured values of interleukin 8 in the blood and colorectal malignant tumor tissue, as well as between the interleukin 8 gene expression in normal colorectal tissue and tumor tissue.

In order to investigate the proposed goal we performed a retrospective study in which we used a group of 108 patients who were admitted between 2012 - 2015 in the Clinic of Surgery of Craiova County Clinical Hospital.

Eligible patients for study were males and females aged 18 to 90 years, diagnosed with colorectal primary tumors without receiving radiotherapy or chemotherapeutic neoadjuvant treatment during this interval.

The proposed scientific research has been structured in several stages:

- the immunoenzymatic study with marked reagents for which we used a first subgroup of 68 patients in which we evaluated the enzymatic values of interleukin 8 in the blood and the respective tumor tissue pairs from the surgical treatment stage..

- the genetic study by which we attempted to evaluate the gene expression of interleukin 8 in tumor and peritumoral tissue, looking for possible clinical-pathological associations using a final group of 19 subgroup 40 patients at an early stage of the colorectal cancer therapeutic regimen.

CHAPTER VI. IMMUNOZENZIMATIC ANALYSIS

A subgroup of 68 patients hospitalized in Surgery Clinic II of the Craiova County Emergency Clinical Hospital in Romania between 2012 and 2013 was used for the marked reagent immunoenzymatic study.

From each patient, two samples were simultaneously collected during the surgery: a peripheral venous blood sample and a tumor tissue sample. The presence of interleukin 8 protein in the supernatant was evaluated using the BRADFORD technique described in the Bio-Rad Protein Assay protocol.

Interleukin 8 values were measured using the Sandwich ELISA technique using the Human IL-8 ELISA kit produced by Krishgen BioSystem in Spain in the presence of standard concentrations. For the interpretation of the results, we used the specialized software for the ELISA

technique, Magellan IVD in version 5.4 produced by DIGIREAD Software, which determined the average absorption for each set of duplicate standards and samples.

Values of interleukin 8 in tumor tissue

The minimal value of interleukin 8 in the tumor supernatant for colorectal cancer TNM stage II patients was 9,200 picograms / milliliter and the maximum value was 60,100 picograms / milliliter. For patients in stage III TNM, the minimum value was 5.300 picograms / ml and the maximum value was 150,000 picograms / milliliter. For TNF stage IV patients, a minimum of 15,900 picograms / millilitre and a peak of 320,000 picograms / milliliter were measured. There is an increasing tendency towards progressive TNM status.

Values of interleukin 8 in the blood

In the group of patients in stage II TNM, the minimum blood interleukin 8 value was 6.900 picograms / ml and the maximum value was 33,600 picograms / milliliter. The minimal interleukin 8 value for TNF stage III TNM patients diagnosed with colorectal cancer was 7,200 picograms / ml and the maximum value was 80,100 picograms / milliliter. Stage IV TNM patients were measured with a minimum of 18,300 picograms / ml and a maximum of 210,000 picograms / milliliter. Also, in the case of IL-8 measured in blood, a progressive trend towards TNM status can be observed.

The mean value of interleukin 8 for colorectal cancer TNF stage II patients was 21.35 μg / ml in serum and 32.05 μg / ml in the tumor supernatant, a statistically significant difference. To compare the results of the serum and tumor supernatant measurements, we used the Wilcoxon assay for the sum of the pair ranks, and we observed statistically significant differences, in all cases the serum values being lower than the tumor tissue values. For the Stage II samples we obtained a probability $p = 0.00129$, for the Stage IV the probability was $p = 0.00252$, and in the Stage IV the statistical significance was even higher with a probability $p = 3.62 \times 10^{-7}$ less than 0.001

CHAPTER VII. GENETICAL ANALYSIS

The purpose of the study was to evaluate the gene expression of interleukin 8 in tumoral and peritumoral tissue, looking for possible clinical-pathological associations. The study was conducted in accordance with the Romanian bioethics legislation.

Initially, 40 patients were diagnosed with primary colorectal tumors following endoscopic and eco-endoscopic assessment at the Center for Research in Gastroenterology and Hepatology at the University of Medicine and Pharmacy Craiova between January 2014 and May 2015. Of the 40 patients initially included, after the RNA integrity assessment, only 19 samples were selected as long as they complied with the inclusion criteria and had a pure amount of RNA.

The samples were processed in two steps: first, reverse transcription beginning with complementary DNA synthesis and second, Real-Time PCR amplification, when the results are quantitatively evaluated from complementary DNA using TaqMan technology.

The qRT-PCR analysis of all samples revealed the gene presence of interleukin 8 in both tumor tissue and normal tissue. Increased values were found in biopsy of non-invaded by the colonic tumoral tissue compared to tumoral biopsies.

Using the Mann-Whitney test, the mean of the relative expression of the interleukin 8 gene from the tumor tissue compared to the corresponding TNM stages is evaluated. The probability obtained, ($p = 0.235$) being statistically significant, allows us to affirm that there is a correlation between the values of the tumor tissue interleukin 8 gene and the corresponding TNM stage.

There is a statistically significant correlation in the values of the gene expression of interleukin 8 in the normal tissue according to each degree of differentiation in part, but this correlation is not maintained for the samples in the tumoral tissue. In the study we highlighted that the gene expression of interleukin 8 is low in tumor tissue and increased in normal tissue.

CHAPTER VIII. CONCLUSIONS

Conclusions immunoenzymatic analysis

Interleukin 8 values measured in the serum as well as those measured in the tumor are increased directly proportional and correlate with the staging of TNM.

For serum interleukin 8 levels, we obtained statistically significant differences between Stage II and IV, as well as between Stage III and IV, but not between Stage II and III. For tumoral interleukin 8 levels, the differences were significant between all stages in the patient group, ie between Stage II and III, between Stage II and IV, and between Stage III and IV.

Interleukin 8 values measured in the tumor supernatant were higher than serum measured interleukin 8 values, suggesting that in the tumor microsphere, interleukin 8 is produced by malignant cells but also by normal cells (endothelial cells, neutrophils and macrophages). Maximal values of interleukin 8 in TNF stage IV colorectal tumors in both serum and tumor supernatant demonstrates that this cytokine correlates with tumor progression and increased metastasis potential.

For colorectal cancer, interleukin 8 is a negative prognostic factor and should be monitored, with elevated levels suggesting an unfavourable progression. Interleukin 8 is a therapeutic target in colorectal cancer, and therapies that reduce its values can improve patient progress.

Reducing interleukin 8 expression in colorectal cancer using therapeutic methods to block its production could improve prognosis and disease progression. As the risks of interacting with patients' immune systems are unpredictable, the nanoparticle variant may reduce interleukin 8 expression in colorectal cancer, thereby reducing the risk of tumor growth.

Conclusions of the genetic analysis

In the study we highlighted that the gene expression of interleukin 8 is low in tumor tissue and increased in normal tissue. The results are focused on a qualitative analysis of interleukin 8

expression in colorectal cancers. The biopsies taken were small and sometimes required their exclusion from the genetic study.

Our study is the first to evaluate the gene expression of interleukin 8 using freshly frozen endoscopic tumor biopsies, a method that allows an early evaluation of the prognostic marker. Theoretically, the biopsy quality-dependent errors were minimal for our study, as we used frozen fresh biopsies. The different values obtained in this study compared to other literature studies can be justified by the different sampling and the type of biological samples used.

The results obtained in this study do not allow us to make statistically significant correlations that transform interleukin 8 into an early prognostic factor. Comparative studies should clarify whether the expression of the interleukin 8 gene should be related to surgical full wall thickness colorectal samples. Other studies are needed to compare the differences between endoscopic biopsy values and intraoperative biopsies.

We mention the low addressability of patients in our country, because although the current technology of diagnostic evaluation, colonoscopy, echo endoscopy or imaging methods would have allowed us to discover tumors in stage I, we have failed to include in the lot any of this case category. A higher and homogeneous study would be necessary for the accuracy of the findings.

Other extensive studies are needed with larger cohorts to clarify the gene expression of interleukin 8 in tumors, current results being contradictory. The results obtained are a starting point for research into new cellular markers that should be included in the treatment assessment protocols for this pathology.

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