UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

DOCTORAL SCHOOL

FACTORS INFLUENCING SURVIVAL TIME IN COLORECTAL CANCER

- summary -

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<p style="text-align:center">KEY WORDS</p>

<p style="text-align:center">colorectal cancer, angiogenesis, immunohistochemistry, cell markers</p>

KNOWLEDGE STAGE

Colorectal cancer

Colorectal cancer (CCR) is globally 15% of all malignancies and is the third leading cause of cancer in men (10% of the total) and the second leading cause of cancer in women after breast cancer (9.4% of total) [1].

In Romania according to Globocan estimated data for 2008, colorectal cancer is the second leading cause of morbidity from cancer in both men (after lung cancer) and in women (after breast cancer).]. In the last two decades, the incidence of colorectal cancer has increased from 13,5/100.000 inhabitants/year, placing Romania among the countries with intermediate risk [9].

The extension study of genetics and molecular medicine, developing study of oncogenesis markers, studying aspects of ontogenetic differences of embryology, histology, histochemical and immunochemically order brought further clarification in CCR oncogenesis. Arguments of epidemiological, histological and experimental order certify that the CCR appears as a cumulative effect of multiple sequential genetic changes responsible for histologic transition from normal mucosa to adenoma and finally to adenocarcinoma.

An important role in the development of screening programs has put the group risk stratification of asymptomatic population depending on age, family history or personal pathology. Screening medium or high-risk population is achieved in the secondary prevention and identifies individuals who are most likely to have CCR or polyps in the group without symptoms or signs of disease [87]. Risk stratification in CCR is presented in Table 1.

Risk	Age	Personal history	Family history
Low	Under 50 years	Without	Without
Medium	Any age	Colorectal tumors (vilous polyps, adenomatous polyps diameter > 1cm) Ovarian or uterine cancer	One or more first degree relatives diagnosed wtih CCR One or more first degree relatives diagnosed with PAF
High	Any age	Chronic inflammatory bowel disease (ulcerative colitis or Crohn's disease)	HNPCC and PAF

Table 1 Risk stratification in CCR [adapted after Levin B]

Tumor angiogenesis

Angiogenesis is the formation of new vessels from existing vessels and plays an important role in both providing nutrients and growth factors and as a means of disseminating tumor cells. The process takes place both during embryogenesis and in vascular remodeling in the postnatal period.

From data obtained was issued assumption that: "*once the tumor was initiated, any subsequent increase in tumor cell population must be preceded by an increase in neovascular tissue that surrounds the tumor*" [126]. According to this concept, a small area of tumor cells can not grow without inducing angiogenesis process.

The development of blood vessels in tumors differ significantly from physiological angiogenesis.

Normal vasculature	Tumor vasculature
• well differentiated	• undifferentiated
• evenly distributed	• unevenly distributed
• structured	• unstructured
• one direction, steady blood flow	• unsteady blood flow
• norma, unchanged permeability	• high permeability
• endothelial cells mitotically inactive	• endothelial cells mitotically active
• continuous basal membrane	• discontinuous basal membrane

Table 1. Normal Vasculature vs. Tumor Vasculature

OWN CONTRIBUTIONS

OBJECTIVES OF STUDY

The research theme chosen is very topical because explores techniques of new histological diagnosis for colorectal cancer, relying in particular on the pathological and immunohistochemical evaluation of biological material using computer assisted morphometry with the application of worldwide vanguard morphometric techniques.

The existence of the Centre for Studies of Microscopic Morphology and Immunology and the Center for Research in Gastroenterology and Hepatology

Craiova of high technology research infrastructure and skilled personnel have paved the successful completion of the following objectives:

1. Making a prospective clinical-statistical study over three years to help identify the main features of a representative group of patients diagnosed with malignancies in the colon and rectum.

2. Study the morphological parameters: Histopathological shape, degree of differentiation, vascular and perineural invasion, nodal involvement, presence or absence of distant metastasis, tumor stage. Establishing statistically significant correlations between clinical and morphological parameters investigated.

3. The phenomenon of angiogenesis in colorectal cancer by immunohistochemical, morphometric analysis and computerized image. Linking microvascular density with different clinical and morphopathological parameters such as histological shape, differentiation grade, nodal involvement, presence or absence of distant metastasis and tumor stage.

4. Histological study results correlated with survival.

CLINICAL AND HISTOLOGICAL STUDY OF COLORECTAL CARCINOMAS

Material and methods

In this prospective study conducted from October 2012 – July 2015 were included patients with malignant tumors at one of the segments in the colon and rectum. Patients were selected from those admitted to the Medical Clinic I – Gastroenterology County Emergency University Hospital Craiova and patients who came in the Center for Research in Gastroenterology and Hepatology (CCGH) Craiova, University of Medicine and Pharmacy (UMF) Craiova. Histological and immunohistochemical study was conducted at the Centre for Studies of Microscopic Morphology and Immunology (CSMMI) of UMF Craiova.

The study group included a total of 118 patients diagnosed with colorectal cancer, from which was taken a fragment of tumor tissue either by colonoscopy with biopsy (75 patients) or from the piece of surgical resection, where possible (43 patients). Where there have been both, we preferred surgical resection piece to the

detriment of the piece obtained by direct endoscopic biopsy. All patients included met the inclusion criteria and received all information about the nature of the study and protection of personal data before signing the consent.

Secondary processing of data – descriptive analysis of the group based on various parameters, calculation of statistical fundamental parameters, mean and standard deviation, its report called the coefficient of variation, their graphic representation and calculation of the regression coefficient – was performed with Excel, using the controls Pivot Tables, Functions-Statistical, Chart and Data Analysis Module.

Results

Most cases with studied pathology were 60-69 years old (almost 40%) and 70-79 years old (30%), less than a quarter of patients were under 60 years old.

Of the patients belonging to the study group, 80 cases were males and 38 cases were females, thus highlighting the greater incidence of the disease in males.

As the population of Dolj county lives in proportion of 46.87% in rural areas and 53.13% in urban areas, we believe that there is no significant difference in terms of area of residence between the study group and the population of the county (p test z for scale = 0.545 > 0.05).

Only for the location of tumors (right colon, left colon and rectum) we have identified three statistically significant results – in terms of platelet count (p = 0.023), Leukocyte count (0.007) and segmented neutrophils percentage (0.002). Comparing the values of these parameters, by ANOVA test, we found a significant difference between the three groups of patients, grouped according to tumor location. Continuing analysis by Fisher LSD post-hoc test, was revealed that differences manifest themselves only with cases of right colon tumors and those with tumors of the rectum, for all three parameters listed above.

IMMUNOHISTOCHEMICAL STUDY OF COLORECTAL CARCINOMAS

Material and methods

Histopathological material used also for immunohistochemical studies were processed within CSMMI of UMF Craiova. Immunohistochemical study was conducted on a group of 65 patients included in the histopathological study. Were

used samples resulting from endoscopic biopsies collected within the Clinic of Gastroenterology or Internal Medicine of SCJUC Craiova or CCGH Craiova, as well as samples obtained from surgical resection pieces obtained from the Department of Surgery of SCJUC Craiova.

For immuno-location of blood vessels, fixed paraffin tissue sections were subjected to immunostaining for CD31, CD34 and CD105. After deparaffination, endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxide in methanol for 30 min. Then sections were incubated with the corresponding antibodies in PBS containing 1% BSA for 16 hours at 4°C. The antibodies were detected using diaminobenzidine (DAB) as a substrate and the sections were counterstained with Mayer's hematoxylin.

Results

Statistical analysis performed in the immunohistochemical study revealed statistically significant differences for CD105 antibody (p = 0.046<0.05), resulting that this antibody is a valuable tool for assessing the grading in colorectal cancer.

CD31 antibody differences depending on tumor grading are not very large; even if CD31 values are slightly lower for G1 than G2 and lower for G2 compared to G3, the variability observed for measurements make this comparison irrelevant (p ANOVA = 0.964>0.05).

Quick Time decreases together with increase of pT stage, the observed differences being close to statistical significance. However, ANOVA test result does not allow us to say that differences can be generalized and are not just a particular result, valid only for the study group, p=0.061>0.05.

There is a difference highly significant between patients with stage T different in terms of value: hemoglobin (p ANOVA<0.001), hematocrit (p ANOVA<0.001), MCV (p ANOVA <0.001), MCH (p ANOVA = 0.002<0.01 – significant difference with 99% confidence).

Neither in the relationship between antibodies CD34 and CD105 is there a statistically significant correlation, although the value of the Pearson correlation coefficient is higher (r=0.130), but not sufficient for the value p to reach below the maximum allowed, which indicates statistical significance (p=367>0.05).

By calculating the Pearson correlation coefficient for CD31-CD105 relationship we obtained a value r = 0.440, which corresponds to p=0.0013<0.05, indicating a

statistically significant direct correlation between the two factors. In conclusion, we can say that CD 31 increases in parallel with CD 105 for cases examined in this study.

HISTOLOGICAL STUDY RESULTS CORRELATED WITH SURVIVAL

Due to the prospective nature and short interval of the study, survival assessment was carried out only up to 36 months of diagnosis.

Of the 118 cases studied, 65 cases were still alive at the end of the study period.

Analysis of estimate curves survival to 1 year depending on location showed that ascending colon tumors have a worse prognosis than the descending colon tumors, both locations have a poor survival rate than the rectum ones (p=0.045<0.05).

Correlation Analysis of survival at 2 years depending on the location revealed that colon tumors have a comparable survival at 2 years, and weaker than the rectum tumors, but no longer existing statistical significance (p=0.099>0.05); and at 3 years there was no significant difference in terms of survival (p=0.291>0.05).

Correlation analysis of survival with histopathological types of cancer revealed that there are no significant differences between adenocarcinoma and mucinous carcinoma:

- survival at 1 year : p=0,270>0,05
- survival at 2 years: p=0,363>0,05
- survival at 3 years : p=0,338>0,05

After histopathological types classification was applied the grading system depending on the degree of differentiation.

The vast majority of cases were classified as moderately differentiated neoplasms, with nearly half of cases (49.15%). Poorly differentiated appearance was the second in frequency, covering a percentage of 40.68%.

Kaplan Meier curves analysis for estimating survival according to the degree of extension of tumors in depth of colorectal wall confirmed that T4 shows a survival weaker than T2 or T3, Wilcoxon test result is near the limit of statistical significance (p=0.052>0,05) at one year. Also at 2 years T4 shows a survival significantly weaker than T2 or T3 (p=0.035), but increases the difference between T2 and T3, without

statistical significance, and at 3 years there are statistically significant differences between T4 and T3, respectively between T3 and T2 (p=0.028<0.05).

Kaplan Meier curves analysis for estimating survival at 1 year depending on the degree of lymph node invasion has identified that there are no significant differences according to N stage (p=0.889>0.05), and at 2 years differences occur, but are not statistically significant (p=0.711>0.05).

As expected survival correlation analysis with the presence of secondary tumor measurements confirmed that after two years of follow up patients survival without metastases is significantly higher than those with secondary tumor measurements (p=0.048<0.05) and at 3 years those without metastasis have superior survival compared to those with metastases, the difference is statistically significant (p=0.046<0.05).

CONCLUSIONS

• Colorectal cancer is the third most common cancer in men (representing 10.0% of all malignancies) and second in women (representing 9.4% of all malignancies) worldwide.

• News of colorectal cancer research is imposed by the continuous increase in the incidence of disease in developed countries, which already record high levels of prevalence, and in countries in which colorectal cancer showed no worrying rate other times.

• Colorectal cancer was more common in males, 80 patients of the 118 investigated being male.

• The tumors included in this study were highly aggressive, only 65 cases were still alive at the end of the study period and 58.47% of the deaths were in the first year of the study.

• There were no significant differences in survival between the sexes in the group studied.

• The rectum was the most affected with 47.46% of cases, followed in order of frequency of right colon and left colon.

• Most cases with studied pathology were 60-69 years old (almost 40%) and 70-79 years old (30%), less than a quarter of patients were under 60 years old.

• No statistically significant difference between urban and rural areas in terms of sex distribution, over 2/3 of patients are males.

• In terms of histopathology 88.14% of colorectal neoplasms were adenocarcinomas and 11.86% epidermoid carcinomas with no significant statistical differences in terms of survival.

• 44.07% of evaluated patients had tumor localizations away and had a lower average survival rate (averaging 19 months).

• Looking at the overall results, we found levels of CD4 to be almost double, compared to CD31 or CD105, average values for CD31 are significantly higher than CD105 values.

• The average values of CD34 for moderately differentiated cases are lower than for well-differentiated cases, which is somewhat unexpected, and lower than the poorly differentiated, none of the differences were however statistically significant.

• Patients with pT4 stage appear to have a significantly higher number of platelets and segmented neutrophils than pT2 and pT3 stages.

• The CD 31 antibody increases in parallel with the CD 105 antibody for cases examined in this study.

• Mean values for CD105 are statistically significant in terms of grading, which demonstrates that CD105 antibody is a valuable tool for assessing grading for colorectal cancer.

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