

A Statistical Analysis of Risk Groups in Colorectal Cancer Patients

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ABSTRACT: Colorectal cancer (CRC) is considered a major global health concern due to an increasing number of new cases and cancer-related deaths each year, strong link to dietary habits prevalent in middle and high-income countries and limited therapeutic options especially in locally-advanced and metastatic settings. To counter this growing problem, the scientific community has strived to underpin the major molecular mechanisms behind the aggressive phenotype displayed by CRC and also develop new agents to selectively target and inhibit these core drivers. This evolution has allowed the separation of patients according to different risk groups in concordance with epidemiological parameters alongside novel biomarkers such as gene alterations, protein overexpression and aberrant signaling pathways. In this study we included 20 patients who underwent colonoscopy and were later received histopathologic confirmation of CRC. The statistical anamnestic data obtained from the patients (age, gender, home distribution, signs and symptoms) was corroborated with the results obtained from the histopathologic and immunohistochemical analysis of the samples obtained via colonoscopy. The average age was 63.8 years, the male: female ratio was 2.33 and the origin of 2/3 of the patients was urban and the most encountered symptoms were transit disorders (75%). In terms of colonoscopy results, the majority of tumors were found on the rectum (85%), 90% of tumors were adenocarcinomas, having a vegetant aspect in 60% of the cases and a moderate degree of differentiation in 50% of situations.

KEYWORDS: Colorectal cancer, incidence, angiogenesis, immunohistochemistry, colonoscopy

Introduction

Colorectal cancer is considered one of the most significant causes of death worldwide, being the third most frequently diagnosed malignancy after lung cancer and breast cancer [1]. More worryingly, a statistical analysis between 2012 and 2018 has shown that the percentage of new colorectal cancer cases has increased from 9.7% in 2012 to 10.2% in 2018 while the percentage of deaths attributed to colorectal cancer has risen to 9.2% in 2018 from 8.5% in 2012. This means that in the span of 6 years, colorectal cancer become the second cause of cancer-related death, making this particular disease a global health crisis [1,2].

In terms of geographical distribution, colorectal cancer has the highest incidence in European countries such as Hungary, Netherlands or Norway, North America and Eastern Asian countries such as Japan or South Korea. A noteworthy mention is that colorectal cancer seems to be three times more likely to be

diagnosed in countries with a high HDI (human development index) in comparison to developing countries. Despite this trend, colorectal cancer mortality is evenly distributed between low and high HDI [1]. This peculiar link between rising incidence in developing countries and decreasing mortality in developed ones is seen as a result of behavioral patterns such as dietary customs around the world, lifestyle choices such as smoking or obesity and implementation of health policies such as screening programs or standard of care diagnostic and treatment procedures.

While colon and rectal cancer have almost been seen as different cancer types, with the advent of genetic and epigenetic profiling and subsequent discoveries regarding the molecular patterns which govern different cancer types, the term colon cancer has become more a historical notion. Nowadays, we regard left colon cancer (LCC) and right colon cancer (RCC) as completely distinct entities with their own unique clinical features, treatment philosophies

and genetic/epigenetic alterations which make them stand out amongst each other [3,4].

In the present study, we corroborated the statistical data obtained from the clinical consultation performed on the patients with the results of the colonoscopy and histopathological/immunohistochemical analysis performed afterwards in order to observe statistical patterns which could help differentiate certain risk groups.

Material and Methods

All the patients included in our study underwent colonoscopy for suspicion of colorectal cancer. The colonoscopies were performed between January 2013 and November 2017 at the “Renașterea” Medical Center in Craiova and at the Internal Medicine Clinic of the Emergency County Hospital, Craiova, Romania using a GVS308389/2009 Pentax® colonoscope. Biopsies were retrieved during each colonoscopy and the tumor samples were fixed in 10% formalin, included in paraffin and stained using Hematoxylin-Eosin (HE) and Goldner-Szekely (GS) trichrome dyes at the “Elenamed” Private Pathology Laboratory in Craiova, Romania. Data regarding the patient’s private information (age, gender, origin) and clinical information (signs and symptoms, blood panels) were extracted from the clinical consultation registries. All the patients signed an informed consent regarding their anonymous participation into this study, and the project has been approved by the Ethics Committee of the UMF Craiova. The data was entered into a Microsoft Office Excel database and processed statistically.

The immunohistochemical analysis of the tumor fragments extracted from the biopsy samples was performed using the following antibodies: anti-p53 (monoclonal mouse anti-human p53 protein, clone 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-MNF116 (monoclonal mouse anti human cytokeratin, clone MNF116, Dako, 1:100 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

This study included 20 patients which underwent colonoscopy for suspicion of colorectal cancer and had their samples analyzed using the immunohistochemistry techniques described in the Materials and Method chapter.

In the first part of the study, we analyzed the data obtained from the patients records combined with the data obtained from the colonoscopy in terms of age, gender, home environment, histological characteristics and localization of the tumors.

Regarding gender distribution, out of the 20 patients, 16 (70%) of the patients were male while only 6 (30%) patients were female (Fig.1).

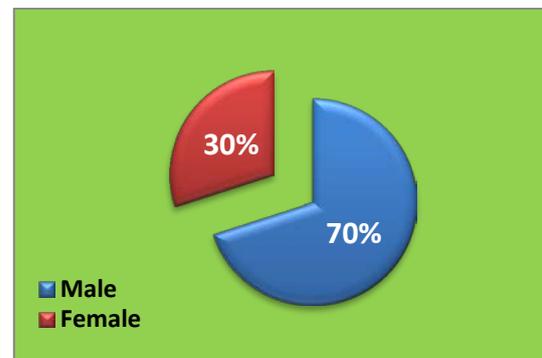


Fig.1. Gender distribution

In terms of age, the median age of the patients in our study was 63.8 years. Of a total of 20, only 1 patient was under 50 years old (5%), 6 patients were between 50-59 years old (30%), 7 patients were between 60-69 years old (35%) and 6 patients were between 70-79 years old (30%) (Fig.2).

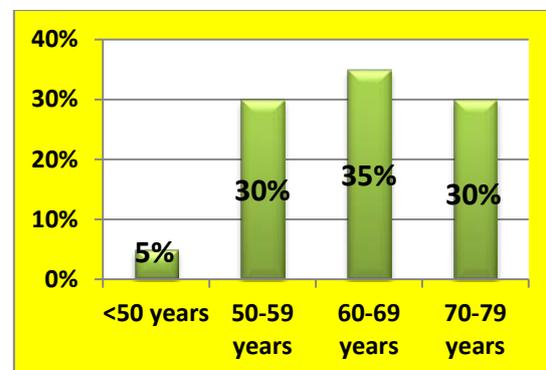


Fig.2. Age distribution

In terms of home distribution, of the 20 patients, 13 (65%) of them originated from an urban environment while only 7 (35%) originated from a rural home (Fig.3).

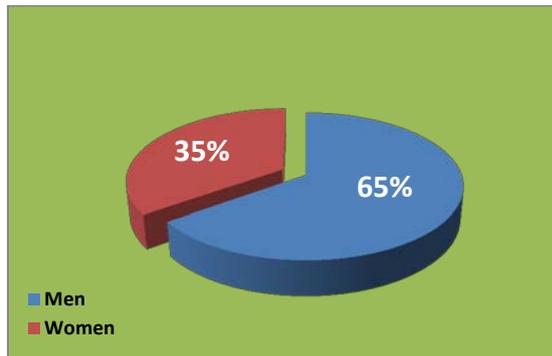


Fig.3. Home environment distribution

The main signs and symptoms which prompted the patients to schedule a doctor’s appointment and undergo a colonoscopy were: anemia, transit disorders, abdominal pain, rectal bleeding and weight loss. Of the 20 patients, 13 (65%) of them presented with anemia, 15 (75%) with transit disorders, 14 (70%) suffered from rectal bleeding, 9 (45%) of them experienced weight loss and only 2 (10%) patients presented with abdominal pain (Fig.4).

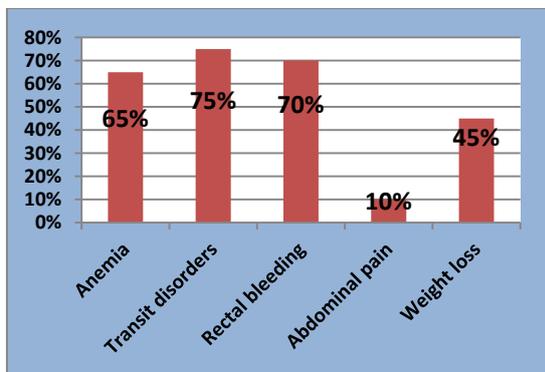


Fig.4. Distribution by symptoms

All the patients in our study underwent a colonoscopy for signs and symptoms of CRC and were confirmed to have this form of cancer. After the histopathological confirmation, a immunohistochemical analysis was performed to assess the level of several biomarkers which are commonly expressed by CRC.

Of the 20 confirmed cases, no patients presented with a right colon cancer, 3 (15%) patients presented with a left colon cancer and 17 (85%) patients were diagnosed with a rectal malignant tumor (Fig.5).

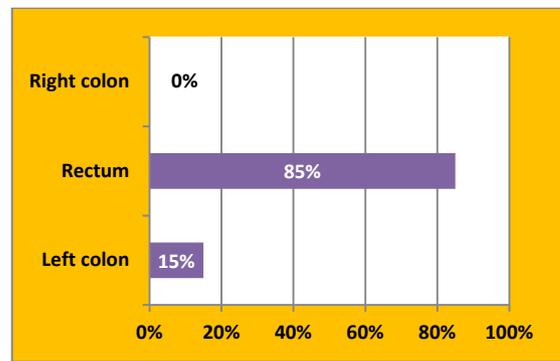


Fig.5. Distribution by tumor site

Upon further inspection, 12 (60%) of the tumors were vegetant, 5 (25%) tumors were vegetant with ulceration and 3 (15%) tumors were described as infiltrative with ulceration (Fig.6).

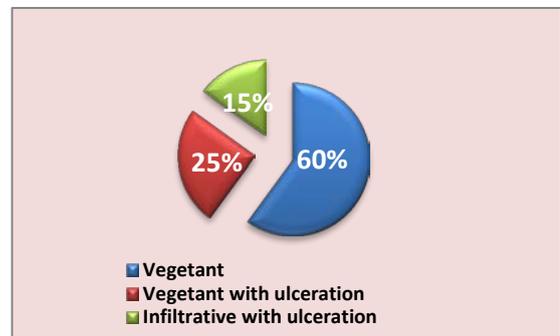


Fig. 6. Distribution according to endoscopic findings

After the histopathology assesment, 18 (90%) patients were diagnosed with adenocarcinomas while only 2 patients were diagnosed with mucinous adenocarcinomas (Fig.7).

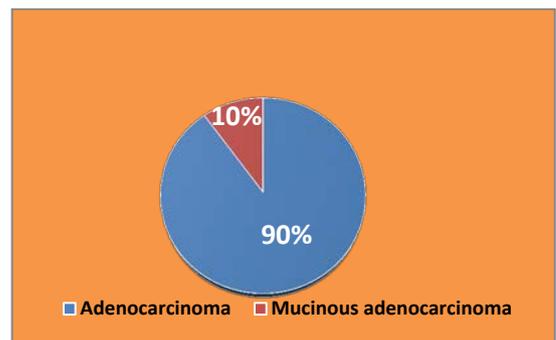


Fig.7. Distribution by histopathological results

After the analysis of the differentiation degree, 4 tumors (20%) were well-differentiated (G1), 2 (10%) tumors were well to moderately-differentiated (G1-G2), 10 (50%) tumors were moderately differentiated and 4 (20%) tumors were moderately to poorly-differentiated (Fig.8).

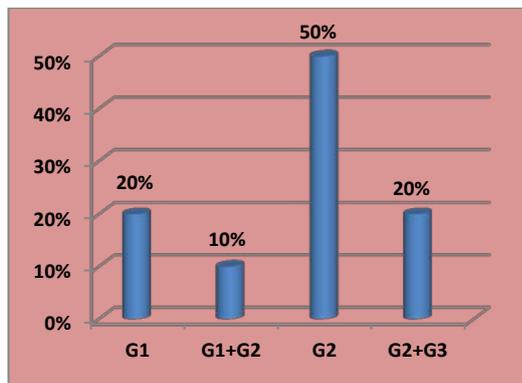


Fig.8. Distribution according to differentiation degree

Two of the immunohistochemical markers studied showed a correlation with the differentiation degree of the carcinoma. The anti CD34 antibody used to evaluate the microvascularization of the tumors showed better expression in well and moderate differentiated tumors, with negative response in poorly differentiated carcinomas. Also, the tumor capacity to stimulate the creation of new blood and lymphatic vessels was studied with the help of two markers. VEGF A and VEGF C were highly positive in G1, G2 tumors with negative results for tumors in the G3 group.

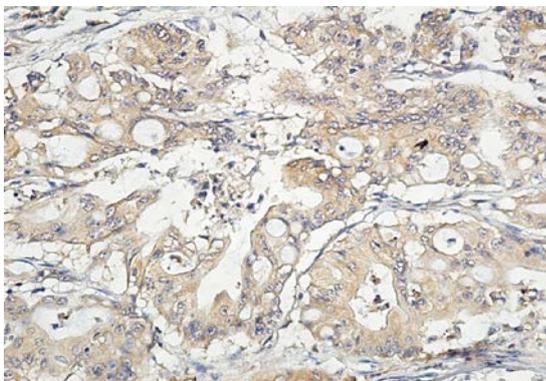


Fig.9. Image of G2 carcinoma with positive reaction of VEGF-A

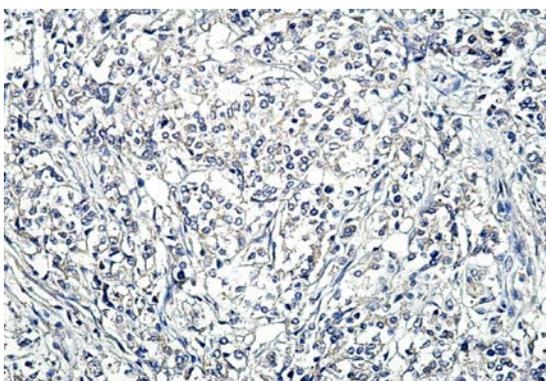


Fig.10. Image of G3 carcinoma with negative expression of VEGF-A

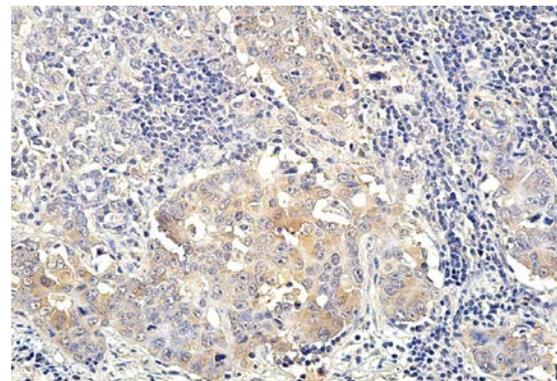


Fig.11. Image of moderately differentiated carcinoma with positive VEGF-C expression

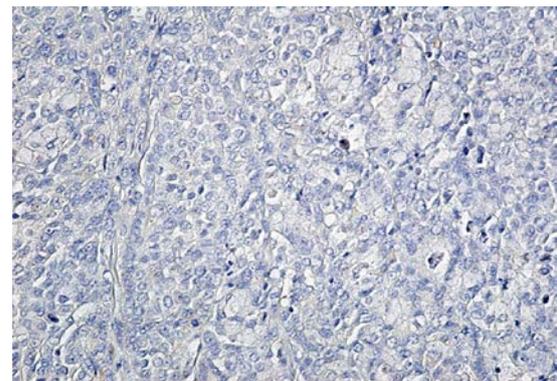


Fig.12. Image of poorly differentiated carcinoma with negative VEGF-C expression

Discussion

In the past several years, CRC has steadily become a global health crisis, with more and more new cases being diagnosed each year. Additionally, despite the new therapeutic options being constantly introduced into current clinical practice, CRC has recently become the second cause of cancer related death, second only to non-small cell lung cancer. With the advent of genomic sequencing and the refinement of existing techniques such as immunohistochemistry we now have a clearer picture of the genotypic and phenotypic characteristics which are quintessential for the development, evolution, invasion and metastasis of CRC. Lately, the great effort invested in understanding the intricate mechanisms which govern CRC have paid off, with discoveries regarding the receptor pathology and aberrant signaling which constitute major drivers behind this form of cancer [5-7].

More so, several key ligand/receptor/ pathways tandems have been discovered as being strongly implicated in the development, invasion, metastasis and treatment resistance of CRC. Of these, the most encountered genomic alteration which plays a major role both in the

prognosis and selection of treatment is the mutation of the RAS genes [8-10].

Other genomic alterations such as the BRAF mutation, Her2 overexpression or MSI high/low-MSS status have also been identified as being involved in the pathology of CRC and as such, have become points of interest in the development of therapeutic agents either in monotherapy or alongside canonical drugs which are already used to treat this form of cancer [11-13].

Our study was conducted on 20 patients diagnosed with CRC. All the patients in our study underwent biopsy colonoscopy with subsequent histopathologic confirmation and immunohistochemical analysis of several markers specific for CRC (p53, Ki67, CK7, CD 34, CK19, CK20, MNF 116, VEGF-A, VEGF-C). This data was centralized and cross-referenced with the anamnestic data obtained during the first consultation (age, gender, home environment and signs and symptoms).

Statistically, in terms of gender distribution, the male/female ratio was 2.33. This is consistent with current literature which shows that males tend to have a higher incidence and mortality from CRC (1). The median age for the patients included in our study was 63.8 years, with the majority of patients being between 60-69 years (35%), followed by the 50-59 and 70-79 age intervals with 30% each and only one patient being under 50 years old. This broadly coincides with data provided by current literature provided by the American Cancer Society which states that the median age for diagnosis for colon cancer is 68 for men and 72 for women while the median age for diagnosis for rectal cancer was 63 for both sexes [14]. In terms of home environment, almost two thirds of the patients were from urban areas (65%) while only 35% belonged to a rural environment. As for the signs and symptoms and subsequent investigations prior to the colonoscopy, the most encountered were transit disorders in 75% of all the cases, followed by rectal bleeding in 70% of the cases and anemia in 65% of the cases. Weight loss was present in 40% of the cases and abdominal pain was described only by 10% of the patients.

After performing the colonoscopy, the information regarding the tumor's localization, macroscopic aspect and details obtained from the histopathological examination were centralized and the data obtained was statistically analyzed. In terms of tumor localization, the vast majority of the tumors

(85%) were found in the rectum, followed by the left colon (15%) while no tumors were found on the right colon. The majority of the tumors also had a vegetant aspect upon endoscopic examination (60%), while only 25% of them had a vegetant with ulceration aspect and 15% had an infiltrative with ulceration aspect. After the histopathological examination, the overwhelming majority of tumors were found to be adenocarcinomas (90%) while only 10% of them were described as being mucinous adenocarcinomas. The differentiation degree of the tumors was dominated by moderately differentiated tumors which made up half of all the samples analyzed, followed by well differentiated and moderately to poorly differentiated tumors with 20% each while only 10% of the tumors were well to moderately differentiated.

In our study, we tried to characterize the microvascularization of the carcinomas VEGF-A is involved in angiogenesis, enhancing the proliferation and migration of the endothelial cells, playing an important role in regulation of the genes that stimulate the formation of new blood vessels [15]. VEGF-C has been studied for lymphangiogenesis, stimulating proliferation and migration of lymphatic endothelial cells, promoting colorectal cancer invasion by disrupting the endothelial barrier before launching of formation of new lymphatic vessels [16]. Both VEGF-A and VEGF-C were found positive in well and moderate differentiated carcinoma with negative results in poorly differentiated ones.

Conclusion

In summary, our study has provided further proof that several risk groups can be identified based on anamnestic data with men, between 60-70 years of age, hailing from an urban environment having the highest risk of developing CRC. More so, the results from the colonoscopy have presented data concordant with current literature with adenocarcinomas of the rectum, having a vegetant aspect and a moderate degree of differentiation being the most encountered type of tumor. In conclusion, further studies are required in order to correlate epidemiological data with histopathologic, immunohistochemical and genomic data in order to refine and better differentiate risk groups. These findings could lead to the discovery of potential new biomarkers which could serve as both diagnostic tools and therapeutic targets.

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