Colorectal cancer – clinical and morphological aspects

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Abstract

Introduction: Colorectal cancer (CRC) is one of the most common and most serious malignancies worldwide. Its incidence has increased by more than 200 000 in 2008, both in men and in women. Although CRC is a well-studied malignancy which has a slow progression, known risk factors and pre-neoplastic lesions that can be detected and treated, its incidence is on the rise. In our study, we clinically, histologically and immunohistochemically evaluated a group of 317 patients with colorectal cancer who underwent surgery. Patients and Methods: The trial included 317 colorectal cancer patients hospitalized in the second Department of General Surgery within the Emergency County Hospital of Craiova, Romania, between January 2005 and December 2009, aged between 18 and 89 years. After undergoing clinical and laboratory work-up, all patients were submitted to surgery with curative intent. Resection specimens were collected and histological slides were produced from the biological material. Usual histological staining, Hematoxylin–Eosin and trichromic Goldner–Szekely, as well as immunohistochemistry with anti-Ki67, anti-caspase 3, anti-p53 and anti E-cadherin antibodies was also performed. Results: Regarding gender distribution of cases in the study group was a slightly higher incidence in the number of women affected by neoplasia (n=166, 52.35%) than males (n=151, 47.65%). Analysis of incidence per decade of age showed highest figures between 61–70 years, but we also observed comparable values for the decade 51–60 years. Analysis of topography showed higher incidence of cancer of the rectum (119 cases, representing 37.53%) compared to individual segments of the colon. Average size determined for all locations was 5.05 cm, with a median located at 4.82 and a standard deviation of 1.99. Regarding the degree of cell differentiation, out of the total of 245 adenocarcinomas, a number of 87 (35.52%) were well-differentiated adenocarcinomas, 127 (51.83%) were moderately differentiated, and 31 (12.65%) cases were poorly differentiated adenocarcinomas. Index of cell proliferation, Ki67, had a moderate and intense reaction to all the cases studied. Very few tumor cells were immunohistochemically positive for caspase 3, regardless of the degree of tumor differentiation. E-cadherin was intense in well-differentiated adenocarcinomas. In our study, of 42 colon adenocarcinomas, 29 (69%) were highly positive to anti-p53 antibody, the remaining being negative. Conclusions: Colorectal cancer was diagnosed especially in people over 50 years. Analysis of topography showed higher incidence of cancer of the rectum compared to segments of the colon. More than half were presented in stages III and IV. The histopathological study showed that about 98% of colorectal neoplasms were adenocarcinomas, frequently with moderate differentiation. Keywords: colorectal cancer, adenocarcinoma of the colon, carcinoid, tumor proliferative factor, vegetated tumor.

Introduction

Colorectal cancer (CRC) is one of the most common and most serious malignancies. Relatively recent data have shown that worldwide in 2008 there were over 1.23 million new cases and over 608 000 patients died of colorectal cancer [1]. The incidence of colorectal cancer has seen a slight increase in both men and women. If in 2002 there were about one million new cases [2], their number increased by more than 200 000 in 2008. Clinical and statistical data show that in the last five years colorectal cancer is the 3rd most commonly diagnosed cancer in the world, occupying the 3rd frequency in men (after lung cancer and prostate cancer) and 2nd place in women (after breast cancer) [3, 4]. Approximately 60% of colorectal cancers occur in developing countries. Five-year survival rate in colorectal cancer is about 60–95% in the initial stages and decreases dramatically to 35% in stages where lymph node metastases are detected [5]. These statistics are grim because cancer is a well-studied malignancy, which has a slow progression, known risk factors and pre-neoplastic lesions that can be detected and treated [6]. Because the warning symptoms and signs are belated colorectal neoplasm is found most often in the later stages, which drastically reduces the chances of applying radical curative treatment. Hence, the need is to introduce measures for colorectal cancer screening [7] and a better management of patients with this type of neoplasia [8].

In our study, we clinically, histologically and immunohistochemically evaluated a group of 317 patients with colorectal cancer who underwent surgery.

Patients and Methods

The trial included 317 colorectal cancer patients hospitalized in the second Department of General Surgery within the Emergency County Hospital of Craiova, Romania, between January 2005 and December 2009. Age of patients in the study group was between 18 and 89 years. Of the 317 patients, 102 were admitted in emergency room with acute complications, the remaining 215 patients were admitted through the outpatient specialty clinic. All these patients were performed pre-
operative chest X-ray and an electrocardiogram (EKG), abdominal ultrasound, rectoscopy and/or colonoscopy, computed tomography (CT) or MRI of the thorax, abdomen and pelvis, standard biochemical tests (complete blood count, glucose, transaminases, bilirubin, proteinemia, urea, creatinine, blood group and Rh, urinalysis, carcinoembryonic antigen. For patients with complications was performed in addition an intravenous urography and a cystoscopy. These data were recorded in clinical observation sheet of each patient. Clinical and laboratory data were entered into a Microsoft Excel database for statistical processing. With the program functions or averaged variables, confidence intervals, standard derivatives and significance tests were performed comparing the data series. The statistical significance of the values was considered as \( p < 0.05 \), for a confidence interval CI 95%.

For the histopathology study, there were surgically taken fragments of tumor tissue and locoregional peritumoral lymph nodes, which were fixed in 10% neutral formalin for 48–72 hours and processed for histological techniques including the classic paraffin. Paraffin-included samples were cut using the microtome in 4-μm sections and then stained with Hematoxylin–Eosin and trichromic Goldner–Szekely.

Immunohistochemical study aimed to highlight tumor proliferation markers, intercellular adhesion, apoptosis of tumor cells and synthesis of angiogenic factors. For immunohistochemical study of paraffin-included material, we selected a total of 42 cases of colorectal adenocarcinomas. Since the histological sections were made of 4-μm thickness, biological material was collected on slides coated with poly-L-Lysine, after which they were kept in thermostat at 37°C for 24 hours to increase the adhesion of biological material. Following deparaffinization and hydration of the histological sections, the biological material was incubated for 30 minutes in a solution of 1% hydrogen peroxide. The sections were then washed in tap water before being cooked in citrate pH 6 solution for 20 minutes to expose the antigen. After boiling, the solution was washed using phosphate-buffered saline (PBS), followed by the step of endogenous peroxidase blocking with 2% skim milk for 30 minutes. Then, the sections were incubated with primary antibodies overnight, at 4°C, and the next day the signal was amplified for 30 minutes using the secondary antibody peroxidase polymer-based detection system (EnVision, Dako). The signal was detected with 3,3′-Diaminobenzidine peroxidase polymer-based detection system (EnVision, Dako). The signal was amplified for 30 minutes using the secondary antibody peroxidase polymer-based detection system (EnVision, Dako). The signal was detected with 3,3′-Diaminobenzidine peroxidase polymer-based detection system (EnVision, Dako).

Results

In our study, the incidence of colorectal cancer increased with age. The youngest patient in the group was 18 years (diagnosed with appendix carcinoid associated with phlegmonous acute appendicitis), and the oldest was 89 years (diagnosed with hemorrhagic lower rectal carcinoma). The average age of the batch in the study ranged from 63.84 years with a standard deviation of 11.24 years. Analysis of incidence per decade of age showed highest figures between 61–70 years, but we also observed comparable values for the decade 51–60 years(Figure 1), that seems increasing if we report it to regional previous studies (unpublished results from the 1980–1990).

In the decade 71–80 years and above 80 years, a reduction in the number of cases of colorectal cancer, but this descendant of incidence can be also influenced by the fact that many people die before 80 years of other causes. Incidence in age groups was highly statistically significant \( (p < 0.001) \). Decades 61–80 years had a total number of 207 patients, representing 65.30% of all cases admitted and treated. We can state that the age groups 61–70 and 71–80 years represent significant risk groups for the development of colorectal malignancies. Given time for the pre-neoplastic lesions to become symptomatic neoplasm is estimated between 2 and 10 years, it is clear that colorectal cancer screening should start in the general population at the age of 50 years.

Regarding gender distribution of cases in the study group was a slightly higher incidence in the number of women affected by neoplasia (n=166, 52.35%) than males (n=151, 47.65%), without statistically significant differences. The same aspects were recorded in terms of disease distribution by area of origin, with a slight predominance of the urban environment with 169 cases compared to only 148 in rural areas.

Analysis of topography showed higher incidence of cancer of the rectum (119 cases, representing 37.53%) compared to segments of the colon (Figure 2). Four of the cases we classified as undefined as it extends to more than two segments (in one case the lesion contained transverse colon and descending colon with free splenic angle and in three cases rectosigmoidian junction without being able to specify the exact origin). It should be noted the diagnosis of seven synchronous carcinomas (Figure 3). Two cases presented with acute surgical abdomen in the form of intestinal occlusion, the remaining five cases were detected by colonoscopy or intraoperatively. Therefore, we believe that the intraoperative exploration of the entire colic frame is essential to capture any synchronous neoplasia, regardless of the location of clinically and laboratory identified neoplasm.

Colorectal cancer can represent a surgical emergency. In our study, of 317 patients admitted with colorectal cancer and surgically treated, 102 patients, representing 32.33%, were emergency surgically treated, being hospitalized with a diagnosis of acute surgical abdomen. In their case, corroborating clinical and laboratory data allowed us to establish the following diagnoses of acute syndrome: intestinal obstruction in 89 (87.26%) cases, acute peritonitis in seven (6.86%) cases and lower gastrointestinal bleeding in six (5.88%) patients (Figure 4).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Producer</th>
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<tr>
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<td>MIB-1</td>
<td>Ms/Hu</td>
<td>EDTA, pH 9 1:50</td>
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<td>Anti-caspase 3</td>
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<td>Polyclonal</td>
<td>Sodium citrate, pH 6</td>
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<td>Anti-E-cadherin</td>
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<td>NCH-38</td>
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Ms – Mouse; Hu – Human.
The size of the tumor was another parameter analyzed in our study; the tumors were postoperatively measured in all diameters, taking into account the maximum diameter. Average size determined for all locations was 5.05 cm, with a median located at 4.82 and a standard deviation of 1.99. Depending on location, average size ranged from 5.43 cm in the right colon, 4.89 cm on the transverse colon, 3.54 cm on the left colon and 3.97 cm in the rectum.

In terms of anatomo-clinical form, analyzing tumor type in patients of our group, we found that the most frequent tumor was the vegetant one, present in 220 (69.40%) cases. However, in 134 (61%) patients with vegetant tumor, on the tumor surface were identified ulcerated and/or bleeding areas as well as adjacent infiltrates more or less extensive over the colonic wall, which allows us to say that most forms of tumor were vegetant. Basically, pure vegetant form was present in 66 patients, representing only 30% of cases. Also, in 26 cases, accounting for 11.81% of vegetant tumors, clinical examination of surgically-removed piece allowed identification of macroscopic abscesses.

Pure infiltrative neoplasms were found in 36 (11.35%) cases, being particularly distributed in the left colon; ulcerative lesions were easily identified in 30 (9.46%) patients. In 31 (9.79%) cases, macroscopic appearance of the tumor was polymorphic, showing areas of ulceration and infiltration, and a polypoid or diffuse aspect as in the case of cancer arising in ulcerative colitis lesions.

Regarding the evolutionary stage of studied colorectal cancers, clinical, laboratory and intraoperative data allowed us to note that out of the 317 tumors, 34 (10.73%) have been diagnosed in stage I, 98 (30.91%) in stage II to stage III, 124 (39.12%), and 61 (19.24%) in stage IV.

Histopathological study showed that out of 317 cases of colorectal cancer, 311 (98.11%) cases were adenocarcinomas, and six (1.89%) cases were carcinoid. Regarding the adenocarcinomas, 245 (77.29%) cases were pure adenocarcinomas, 61 (19.34%) cases were diagnosed as mucinous adenocarcinomas and 11 (3.46%) patients were represented by the “signet ring cell” carcinomas. Mucinous adenocarcinomas were more frequent in the left colon (42 cases, accounting for 68.85% of all mucinous adenocarcinomas), especially in the sigmoid colon and rectum, while carcinoids occurred more commonly in the right colon (five out of six cases), especially in the appendix, caecum and ascending colon.

Regarding the degree of cell differentiation, out of the 245 adenocarcinomas, a number of 87 (35.52%) were well-differentiated adenocarcinomas, 127 (51.83%) were moderately differentiated, and 31 (12.65%) cases were poorly differentiated adenocarcinomas.

For histopathological and immunohistochemical study we’ve selected from the archives of the Laboratory of Pathology within Emergency County Hospital of Craiova a number of 42 specimens of pure adenocarcinomas of the colon and locoregional lymph nodes processed to paraffin from as many patients, that were operated in the General Surgery Clinic II of the Emergency County...
Hospital of Craiova between January 2005–December 2009. Based on standard pathological report, we selected cases of pure adenocarcinoma because they represented more than 3/4 of operated colorectal tumors. Histopathological and immunohistochemical aspects varied greatly from one tumor to another. The well-differentiated adenocarcinomas appeared composed of tall columnar cells that delimited glandular structures of various shapes and sizes, all arranged disorderly invading the submucosa or muscular tunic. Glandular epithelium of adenocarcinomas had a heterogeneous character, meaning that sometimes appeared as a monolayer cylindrical epithelium, pseudostratified cylindrical epithelium, sometimes as even the appearance cubic epithelium with marked cellular atypia. In the lumen of the large glands, have been identified cell debris, partially lysed cells, involution of cancer and inflammatory cells (Figures 5 and 6). In moderately differentiated adenocarcinoma, glandular structures were more rare, and cellular and nuclear atypia were more numerous (Figure 7). The most extensive cellular changes occurred in poorly differentiated adenocarcinomas (Figure 8).

In all tumor types, regardless of the degree of differentiation we have observed many mitoses, including atypical mitotic figures. The stroma of all tumors highlighted the presence of an inflammatory reaction, more or less intense, consisting mainly of polymorphonuclear neutrophils in ulcerated tumors and lymphocytes and plasma cells in infiltrating or vegetant tumors. Inflammatory reactions were very intense and have been associated with cell and stromal necrosis spread, in tumors with macroscopic abscesses or associated with peritonitis.

By immunohistochemical study, we investigated:

- The capacity of proliferation of tumor cells, using the Ki67 antibody;
- Tumor apoptosis with caspase 3 antibody;
- Presence of genetic alterations using p53 antibody;
- Adhesion of cancer cells by reacting E-cadherin.

Interpretation of immunohistochemical reactions focused primarily over highlighting the chromogen on the antigenic targets and the number of positive tumor cells. Thus, if no cell has been immunohistochemically labeled, we considered an absent reaction; if the reaction was positive in less than 10% of examined cells with
a microscope objective of 20×, we considered a poor response; if they were positive in 10 to 25% of the cells, we considered a moderate reaction, and if it was positive in more than 25%, we have found that the reaction was intense.

Index of cell proliferation, Ki67, had a moderate and intense reaction in all the studied cases, both in well-differentiated adenocarcinomas but also in the moderately or poorly differentiated, indicating that these tumors have a high growth rate (Figures 9 and 10).

As for the process of cell apoptosis, evidenced by anti-caspase 3 antibody, it was noted that very few tumor cells were immunohistochemically positive for this marker, regardless of the degree of tumor differentiation (Figure 11).

Between genetic modifications, we were interested in p53 protein. This protein is encoded by TP53 gene located on the short arm of chromosome 17. The p53 protein regulates the cell cycle by activating DNA repair mechanisms when needed, stops cells during phases G1/S and G2, when genetic lesions are large and initiating cellular apoptosis if damage cannot be repaired. Therefore, p53 is often appointed as guardian of the genome, having a key role in maintaining genomic stability. In our study, of 42 colon adenocarcinomas, 29 (69%) were highly positive to anti-p53 antibody (Figure 12), the remaining being negative.

Intercellular adhesion study using E-cadherin marker, allowed us to note that immunohistochemical reaction was intense in well-differentiated adenocarcinomas, but decreased in poorly differentiated adenocarcinomas (Figures 13 and 14).

**Discussion**

Numerous clinical and epidemiological studies have pointed out that the colorectal neoplasm has become in recent years a global medical problem with social importance. Although medical imaging techniques, molecular and cellular biology techniques, genetic and biochemistry brought a significant contribution to the early diagnosis of colorectal cancer, its incidence is increasing [9]. The highest incidence rates are found in Australia, New Zealand, Western Europe and North America, while the lowest rates are found in Africa and Asia [10]. The incidence rate of colorectal cancer is increasing rapidly in countries known of having reduced risk. Such countries include Spain, countries in Eastern Europe or Asia [11–13]. This is due to changes in lifestyle and diet [14], with increased consumption of red meat and sugar, increased consumption of animal fat and reduced high fiber diet [15, 16], reduce physical exercises, increased alcohol consumption and smoking [17, 18].
Figure 13 – Colon adenocarcinoma with intense reaction to E-cadherin. Anti E-cadherin immunostaining, ×200.

Figure 14 – Poorly differentiated adenocarcinoma with weak reaction to E-cadherin. Anti E-cadherin immunostaining, ×200.

The incidence of colorectal cancer varies greatly by age. In our study, the incidence rate of CRC has increased explosively in people over 50 years, something which suggests that the action of etiopathogenic factors is cumulative. According to some authors [19], overall cancer incidence rates increase dramatically after 40 years in patients in urban areas and those in rural areas. Similar studies have shown that the incidence rate of colorectal cancer increases with age [20]. In our study, 90.51% of colorectal cancer cases occurred in people over 50 years. In Malaysia, more than 90% of colorectal cancer cases occur after the age of 40 [21].

Cellular and molecular mechanisms that cause colon cancer are not fully understood. We consider that etiopathogenic factors involved in the occurrence of colon cancer act chronically and produce molecular and genetic changes in the lining of the colon, get to persist, are amplified and transmitted to progeny cells. When these molecular and genetic changes go beyond the capacity of epithelial cells to restore and repair the cellular genome, it triggers neoplastic processes. Some authors [22, 23] showed that molecular changes that occur over the life are progressively altering the homeostasis of epithelial cells, leading to neoplasia. DNA damage increases progressively with age causing stochastic cellular insults [24]. In addition, it appears that aging is associated with an increase in cell proliferation of epithelial cells lining of the colon [25]. All these data highlights the complex nature of malignant degeneration in the colon and the importance of age as a risk factor in the development of this cancer.

Regarding the sex of patients with colorectal cancer, in our study we found no statistically significant differences. Of 317 patients with colorectal cancer, 166 (52.35%) were females and 151 (47.65%) males. While most studies indicate that neoplasms of the colon and rectum is more common in men [11, 21, 26–28], male/female ratio ranging from 1.2:1 to 1.7:1 [26], in our study most cases of colorectal cancer occurred in women, male/female ratio being from 1:1.09. This particular issue could be caused by some dietary habits or certain genetic features of patients in the south of Romania. Some epidemiological studies have shown that genetic factors play a role in colon cancer. Ollberding et al. [29] showed that in the U.S. the colorectal cancer incidence rates are significantly different by ethnicity or race. Similar data were reported in Malaysia [21]. Factors increasingly relied on the occurrence of malignancies in the south of Romania could be radioactive pollution from nuclear power plant at Kozloduy (Bulgaria). To date there are no reliable data available to the public on radioactive pollution in the area.

The dimensions of tumor formation are about 5 cm in average. Tumors larger sized plant and occurred more commonly in the right colon. According to some authors, the size of tumor formation at the time of resection is an independent prognostic factor with a significant value for colon cancer and rectal cancer [30, 31] and correlates relatively well with the five-year survival rate.

In our study, tumor localization was predominant in the rectum if we compare it with the segments of colon. According to some authors [23], westernization of lifestyle has increased the incidence of colorectal cancer and the occurrence of tumors mainly in the left colon.

Regarding tumor stage, we found that over 58% of patients in our group were diagnosed with stage III and IV tumors. These issues are due to the lack of information and health education, which makes the patient seek medical specialist only very late, when the prognosis is less favorable. Other authors have reported similar data. In a study in California, Pollack et al. [32] analyzing a number of 59,076 colorectal cancers diagnosed between 1996 and 2000, found that 57% of patients were found in a late stage. Most of these patients had a poor socio-economic status, which emphasizes the importance of social conditions in the detection and diagnosis of colorectal tumors.

Lack of medical culture, reduced symptoms and social status were responsible for the fact that almost one third of cancers of the colon and rectum in our study to be complicated with intestinal obstruction, peritonitis or gastrointestinal bleeding and require emergency surgery. Similar data on emergency interventions for colorectal neoplasm were reported by other authors [33, 34], which shows the insidious evolution of the tumor and its ability to give symptomatology of acute surgical abdomen. Other
authors have noted that colorectal neoplasm was associated with peritonitis and occlusions more frequently in the elderly, in which the prognosis is reserved [35].

The incidence and prevalence of colorectal cancer may be modified by therapeutic measures. In a randomized trial in the UK, a single sigmoidoscopy in a number of 170 432 people aged between 55 and 64 reduced the incidence of colorectal cancer by 33% and mortality by 43% [36].

Histopathological study allowed us to note that colon adenocarcinomas are very heterogeneous. We believe that this occurs due to multiple genetic mutations that tumor cells acquire during neoplastic transformation. Sometimes it was hard to fit a specific microscopic appearance as the same section histopathological appearance was highly variable, from well-differentiated adenocarcinoma to poorly differentiated adenocarcinoma.

In patients our group, more than half of the tumors were classified as moderately differentiated adenocarcinoma. According to some authors, moderately differentiated adenocarcinoma of the colon and rectum cancers can reach up to 70% [4]. The degree of tumor differentiation is a prognostic factor, the degree of differentiation is low, the prognosis is worse [37, 38]. Sometimes, tumor histology has identical microscopic aspects, may have prognostic and/or response to treatment totally different, allowing to hypothesize that colorectal neoplasm is not a simple neoplasia but rather a heterogeneous multifactorial disease [37]. Currently, it is recognized by everyone that malignancy develops through multiple and sequential genetic changes [39, 40], and some patients may have specific synchronous changes for two or three different paths of evolution [41]. Through clonal selection, cancer cell “chooses” the most favorable genetic changes that allow growth [42]. Therefore, microscopic appearance of colon tumors can sometimes be highly polymorphic.

In our study, we evaluated the ability of the antigen Ki67 proliferation of colon adenocarcinoma cell. All tumors that we investigated had a moderate or intense reaction to the Ki67 antigen, which leads us to believe that colon adenocarcinomas have an increased proliferation rate. Although Ki67 has no relation with patient survival [43, 44] and therefore cannot be considered as a prognostic factor, Ki67 is associated with the ability of colorectal tumors to metastasize to lymph regional and remote locations.

Investigation of p53 protein allowed us to conclude that more than two thirds of adenocarcinomas were strongly positive to the p53 antibody. According to some authors [45, 46], colorectal carcinoma with overexpression of p53 protein has a five-year survival rate of 32.5% compared to 71.7% in p53 negative colorectal cancer, although immunohistochemical expression of p53 does not correlate with the degree of differentiation, tumor stage or recurrence, sex and age. Thus, p53 is an independent prognostic indicator of the relative survival correlated with the presence of the metastasis and the later stages of the disease. Other studies have shown that inactivation of TP53 gene is an essential step in the development of colorectal cancer [47] and it occurs in 50–75% of colorectal cancers [48].

E-cadherin is a glycoprotein involved in intercellular and cell–matrix interactions as well as cell growth and differentiation. In our study, we observed that E-cadherin expression diminish with decreasing degree of tumor differentiation. With the differentiating of the tumor cells decreases the expression intensity. Elzagheid et al. [49] estimated that the loss of E-cadherin expression correlates inversely with survival, predicting tumor recurrence and metastasis.

Conclusions

Colorectal cancer was diagnosed especially in people over 50 years, affecting both women and men in relatively equal proportions. Approximately one third of colorectal tumors presented as acute surgical abdomen requiring emergency surgery. Most cancers were recorded in the rectum and left colon. More than two thirds of colorectal tumors have shown as vegetant tumors. More than half of the patients were staged III and IV at first admission. The histopathological study showed that about 98% of colorectal neoplasms were adenocarcinomas, frequently with moderate differentiation.

Acknowledgments

All authors have contributed equally in preparing this manuscript and thus share first authorship.

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