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THE PREDICTIVE ROLE OF MICROSATELITE INSTABILITY IN RESPONSE TO FLOUROPYRIMIDINE TREATMENT FOR STAGE II COLON CANCER PATIENTS

PHD THESIS SUMMARY

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THESIS SUMMARY

Colorectal cancer is a major cause of death and morbidity worldwide, being the fourth leading cause of death worldwide and the second leading cause of cancer death in Europe [1]. The risk of colorectal cancer (CRC) increases after the age of 40, 90% of cases being reported after the age of 50, with a maximum incidence between 60 and 79 years [2].

Several risk factors involved in colorectal cancer have been described, divided into non-modifiable risk factors and modifiable risk factors.

Given that the vast majority of colorectal cancers are sporadic, it is considered that environmental factors (cultural, social, educational, occupational) are those that lead to this type of cancer, but which could be modified through population education programs. About 75-80% of all cancers are found in this category. [3]

The rest of the colorectal neoplasms, up to 25-30% of them, are diagnosed in people with a family history. [4]

Family syndromes represent 5-10% of colon cancer cases with a genetic component [5]:

- Familial adenomatous polyposis (<1%) (PAF) with its attenuated forms: attenuated familial adenomatous polyposis (PAFA), Gardner syndrome and Turcot syndrome
- Hereditary non polyposis colorectal cancers (HNPCC) or Lynch Syndrome (2% -5%)
- Polyposis associated with the MYH gene (<1%) (MAP)
- Peutz-Jeghers Syndrome (PJS)

Also, when talking about colon cancer risk factors, cases of personal history of inflammatory bowel disease, namely ulcerative colitis and Crohn's disease, cannot be omitted. [6]

From all these data, it can be concluded that colorectal neoplasia is a heterogeneous disease, which occurs as a result of the interaction of environmental factors with genetic ones. The precursor lesion may be a tubular or tubulovillous adenoma or a serous polyp. [7] Following genetic and epigenetic changes, the transition from adenoma to carcinoma can be made.

Serum neoplasms are more commonly located in the right colon and occur as a result of microsatellite instability, BRAF V600E gene mutations, or changes in DNA methylation in the CpG islets [8]. In contrast, tubular adenomas occur as a result of inactivation of the APC tumour suppressor gene and the association of chromosome instability. [9]

Thus, three major pathways leading to colon carcinogenesis are described:

- Chromosomal instability
- Microsatellite instability
- Epigenetic instability - the methylation phenotype of CpG islands and DNA hypomethylation.

Chromosomal instability is the most common form of genomic instability, characterised by changes in the number of chromosomes or by numerous structural deviations. It is found in approximately 85% of colorectal cancers. [10]

Microsatellite instability occurs in those tumours that do not have chromosome instability. It is the main feature of Lynch syndrome, being found in about 15-20% of sporadic colorectal cancers. [11]

Regarding the instability of microsatellites, the mechanism of emergence of MSI is by inactivation of DNA repair genes (MMR), namely - MLH1, MSH2, MSH6 and PMS2, with the appearance of aberrant DNA fragments. Analysing the number of areas of instability, tumours can be divided into tumours with low instability (10-30% areas of instability), the so-called MSI-low, tumours with high instability (MSI-high) (at least 30% areas of instability) and stable tumours (MSS). [12]

In sporadic cancers, an aberrant DNA methylation process can occur that leads to the inactivation of the MLH1 gene, which subsequently leads to the inactivation of DNA repair genes. Sporadic tumours with microsatellite instability develop a close link to the malignant pathway of serous polyps and carry the BRAF V600E gene mutation. [13] The important conclusion is that the presence of the BRAF V600E mutation in tumours associated with microsatellite instability excludes Lynch syndrome.

Regarding the correlations between the existence of tumours with microsatellite instability and clinical and histological manifestations, it has been shown that these tumours are present in the proximal colon and especially in the elderly, especially women [12]; histologically, they have a rich lymphocytic infiltrate (Crohn-like), are usually mucinous tumours, poorly differentiated. [13]

Epigenetic instability includes both the aberrant hypermethylation reaction of promoters and the hypomethylation of DNA. CIMP tumours derive from sessile adenomas, are frequently diagnosed in elderly patients, mostly women, are tumours located distal to the splenic flexure and do not respond to 5FU treatment. Histologically, tumours are poorly differentiated, mucinous, with signet ring cells. [14]

The vast majority of colorectal cancers are adenocarcinomas, in a proportion of over 90%, originating in the glandular epithelium of the lining of the colon and rectum. [15] MSI status changes tumour behaviour, so some tumours, although high-grade tumours, may have low-grade behaviour. Grading is useful in establishing the prognosis and correlates with histological features and molecular changes. [16]

Mucinous adenocarcinoma is specific to hereditary nonpolyposic cancers, ie tumours with microsatellite instability (MSI-H). These tumours will have a low-grade behaviour. In contrast, stable microsatellite (MSS) tumours, but with a mucinous component, will have a high-grade behavior. [17]

Unlike gastric cancers, signet ring cell tumors (<1%) are rare in the colon. The prognosis of these tumours is unfavourable, having a high-grade behaviour. However, if the tumours show microsatellite instability (MSI-H), then the behaviour will be low-grade. [18]

Medullary carcinoma is an extremely rare form of colon cancer. It is present in MSI-H tumours, thus changing their behaviour and prognosis, becoming tumours with a favourable prognosis, despite undifferentiated histology. [17]

Immunohistochemically, colorectal cancer can be correctly diagnosed by labeling with cytokeratins (CK) 7, 20 and cytokeratin CDX2. [19] Immunohistochemical testing is needed to differentiate between sporadic colon cancer and colon cancer developed in a family syndrome, such as Lynch syndrome.

However, there are situations when the instability status of microsatellites cannot be established by immunohistochemistry, a situation that must be confirmed by PCR analysis. Chain polymerization reaction (PCR) analysis compares DNA extracted from tumour tissue with that extracted from normal colonic tissue, in terms of the presence of microsatellites, through a process of capillary electrophoresis.

The diagnosis in colon cancer is established: clinical, biological and imaging. Colonoscopy remains the most important test for diagnosing colorectal cancer. Sigmoidoscopy also remains a viable option. Computed tomography analysis is a useful and recommended method of staging the disease.

The staging of colon cancer has the role in determining the prognosis, but also in achieving the treatment and monitoring plan. The current staging in colorectal cancer is done according to the AJCC Cancer Staging Manual, 8th edition.

One aspect that cannot be overlooked when discussing colorectal cancer is screening. It is recommended that screening should be initiated at the age of 45 and maintained until the age of 75 for people without a personal or family history of cancers. [20]

Colonoscopy remains the most important test for colorectal cancer screening. Sigmoidoscopy remains the valid option especially for people who refuse colonoscopy. The recommendation, in this case, is to repeat the procedure every 5 years, accompanied by the test for occult bleeding, if no tumours have been detected. [21]

Occult stool bleeding can be detected by two types of tests: immunochromatographic (FOBT) or immunohistochemical (FIT). In the case of a test with a positive result, colonoscopy is recommended.

Fecal DNA analysis (mt-sDNA) has a sensitivity of over 90% in the determination of colorectal cancer. It is recommended to repeat the test every 3 years. Positive testing requires a colonoscopy.

The method of choice for screening patients with inflammatory bowel disease is colonoscopy, which should be performed during periods of remission of the disease, preferably chromomoendoscopy with confocal endomicroscopy or autoflowering. [22] Recommendations for the interval between colonoscopes are 1-2 years depending on the presence of dysplastic lesions. [23]

Hereditary polyposis syndromes recognised for the increased risk of colorectal cancer in young patients are: familial adenomatous polyposis (FAP), attenuated familial adenomatous polyposis (AFAP), and MUTYH mutation-associated polyposis (MAP). Colonoscopy is the only screening option for these patients. Endoscopic evaluation is recommended every 2 years. [24]

The treatment of colon cancer is complex, requiring the presence of a multidisciplinary team consisting of surgeon, pathologist, medical oncologist, radiologist. However, the first step is the surgical resection within oncological limits of the tumour formation and of the regional lymph nodes, with curative visa, in the localised disease.

The principles of curative surgical treatment for cancer are:

- complete resection of the tumour formation within oncological limits
- evaluation of possible routes of loco-regional invasion (lymphatic, venous, intramural or by local direct extension) and their complete excision; regional lymphadenectomy
- prevention of intraoperative dissemination of tumour cells - isolate tumours that exceed the serosa, ligate the vascular pedicle - valid for the left colon and proximal and distal ligation of the tumour

- restoring digestive tract continuity (when possible) [25]

The “no- touch isolation technique” technique, which involves first ligating the vessels that serve the affected colon segment. Thus, the incidence of both liver metastases and local dissemination of neoplastic cells has been shown to decrease. [26]

The laparoscopic technique is non-inferior to the classical resection with general, recurrent survival rates or survival without similar signs of disease. In addition, in groups of patients operated on laparoscopically, postoperative complications and the period spent in hospital were lower. [27]

Radiation therapy can be used in conjunction with concomitant 5FU chemotherapy in advanced T4 colon cancer or recurrent tumours that cannot be re-operated surgically or in patients inoperable due to co-morbidities.

Neoadjuvant chemotherapy is recommended for the conversion to resectability of large T4b tumours. Preferred regimens are FOLFOX or CAPEOX.

Adjuvant chemotherapy is established postoperatively depending on the stage of the disease and the patient's comorbidities. The chemotherapeutics used are flouropyrimidines and platinum salts (Oxaliplatin).

For stage I colon cancers, routine adjuvant chemotherapy is not recommended, in which case surgical resection within oncological limits is considered curative.

For stage II colorectal cancer there is no consensus on adjuvant treatment. Patients with stage II colon cancer, MSS tumours and at least one risk factor for recurrence are considered to receive chemotherapy:

- T4 tumours, especially those that have produced obstruction or perforation
- Poorly differentiated tumours (not valid for MSI-H tumours)
- Lymphovascular or perineural invasion
- Less than 12 lymph nodes examined [28]

Regarding the chemotherapy regimen, the recommendation is for the use of flouropyrimidines. Treatment for patients with stage II colon cancer with MSI-H tumours does not benefit, but neither does it negatively impact DFS and OS, the benefit being obvious in patients with MSS tumours. [29]

The treatment of stage III colon cancer is based on the combination of flouropyrimidine and platinum salts. The duration of treatment is between 3 and 6 months depending on the T stage.

The recommendations for the supervision of the colon cancer patient are based on the observations that the risk of recurrence is maximum in the first 3 years after treatment. Thus, the

proposed methods for patient surveillance are: clinical examination, tumour marker dosage - CEA, colonoscopy and computed tomographic imaging examination.

In the patient with metastatic disease, two situations must be considered:

- metastatic disease that can be resected per primam (resection R0)
- non-resectable metastatic disease for which palliative chemotherapy will be initiated

For patients with metastatic disease, but with potential for curability, it is recommended to evaluate the case in the multidisciplinary commission and surgery if it is considered that R0 resection can be performed. For this purpose, it is recommended to initiate neoadjuvant chemotherapy based on 5FU, irinotecan or oxaliplatin to which biological therapy can be added. Liver metastasectomy should be performed as soon as possible after initiating neoadjuvant therapy. Adjuvant treatment does not bring a benefit on survival, but only on the disease-free interval. [30]

If liver metastases cannot be resected, local methods may be tried: stereotactic radiotherapy (SBRT), transhepatic arterial chemoembolization (TACE), radioembolization, radiofrequency or microwave ablation, cryoablation, percutaneous alcohol injection or electrocoagulation.

Non-resectable metastatic disease requires the initiation of chemotherapy with known FOLFOX or FOLFIRI regimens to which biological therapy is added depending on the presence or absence of RAS gene mutations.

Immunotherapy has been approved starting with the second line of treatment, for patients with metastatic colorectal cancer with unstable microsatellite tumours (MSI-H) or tumours with deficiency of DNA repair genes (dMMR). These tumours have been shown to have an important mutational load (TMB), which causes the formation of neoantigens, leading to increased tumour immunogenicity. Approximately 5% of metastatic tumours have dMMR and are susceptible to immunotherapy. [31]

The monitoring of patients with metastatic disease is done according to the recommendations of specialised guides and consists of clinical examination, computer tomographic imaging evaluation and dosing of tumour markers.

Chemotherapy is known to have many side effects. These are divided into 5 degrees according to CTCAE, grade 5 being represented by the death of the patient due to the adverse reaction.

The aim of the current research paper is to evaluate the response to fluoropyrimidine treatment of patients diagnosed with stage II colon cancer depending on the status of microsatellite instability (microsatellite unstable tumors - MSI-H or microsatellite stable tumors - MSS).

The present study had the following main objectives:

- establishing disease-free survival at 3 years (DFS = disease free survival)
- overall survival assessment at 3 years (OS = overall survival)

The secondary objectives were:

- evaluation of unfavourable prognostic factors for patients with colon cancer TNM stage II
- establishing the role and importance of immunohistochemical testing to assess the instability status of microsatellites for stage II colon tumours and individualising treatment in order to apply the concept of personalised medicine
- evaluation and comparison of therapeutic efficacy according to genetic / epigenetic changes
- evaluation of patients in terms of survival indicators to avoid over-treatment

The current research work was carried out on the basis of case studies from the Department of Medical Oncology, Internal Medicine III and General Surgery within the Bucharest University Emergency Hospital. A prospective, interventional, two-arm study was designed. In the first arm were included 74 patients with colorectal cancer stage II TNM. In the second arm, the control group of 56 patients with stage II colorectal cancer TNM previously monitored in the Oncology Department was found.

The study received the approval of the Ethics Commission of the Bucharest University Emergency Hospital. The considered population was represented by patients diagnosed with colon cancer who presented to the Medical Oncology Department of SUUB between October 1, 2014 and October 1, 2016.

In order to initiate the study, a useful list was developed for the selection of patients, which included both inclusion criteria (age over 18 years, diagnosis of stage II colon adenocarcinoma (according to TNM) operated, R0 resection, immunohistochemical analysis, cooperating patient, status good performance (ECOG 0-1)), as well as exclusion (ECOG 3 performance status, other associated neoplasms, HIV / AIDS or active / chronic active hepatitis, active pulmonary tuberculosis, decompensated heart failure, myocardial infarction or stroke ischemic / hemorrhagic in the last 6 months, patients with familial adenomatous polyposis, allergic reaction to fluoropyrimidines, dihydropyrimidine dehydrogenase (DPD) enzyme deficiency)

Patients with good performance status, ECOG score 0-1 were included.

R0 resection was defined according to the recommendations of the American Joint Committee on Cancer in 1977 due to the lack of residual tumor on microscopic analysis. Resection margins and associated lymph nodes are included in the R0 resection.

According to the TNM classification, stage II colorectal cancer is characterised by the presence of tumour formation that invades the colonic wall, without the involvement of lymph nodes. Stage II is subdivided into IIA (T3N0), IIB (T4aN0) and IIC (T4bN0)

Lymphovascular invasion is defined as the presence of tumour cells in the endothelial space - lymphatic vessels or blood vessels. Perineural invasion refers to the presence of tumour cells in the space around the nerve fibers that are around the tumour.

The paraffin block resulting from the processing of the excised tumour material was used for immunohistochemical analysis. Tumour material was analysed for the presence or absence of DNA repair gene proteins.

After obtaining the opinion of the Ethics Commission, each patient who met the criteria for inclusion in the study, signed the informed consent to participate in the study, in two copies.

The data were collected at the time of the patient's presentation in the Department of Medical Oncology, after surgery and the release of the anatomopathological result and were subsequently entered into a database necessary for statistical analysis.

Patients were initiated on chemotherapy with Capecitabine (5-FU as oral tablets) or 5-FU as an intravenous infusion.

Both patients included in the treatment and those in the control group were followed according to the monitoring protocols approved by the European Society of Medical Oncology.

Laboratory investigations were performed at the beginning of treatment, but also before each treatment cycle. After completing the chemotherapy treatment, the patients were called for monitoring visits according to the schedule of visits as follows: at 3 months, at 6 months, at 12 months, at 24 months.

Patients were monitored for both local and systemic recurrence.

Patients were monitored for adverse effects of chemotherapy both during and after treatment according to the CTCAE (Common Terminology Criteria for Adverse Events) 2009 edition.

Data collected from patients included in the study were entered into a database using the Microsoft Office Excel program. Starting from this database, statistical analysis was performed using STATA 13 / MP software (StataCorp LLC US). The Chi square test was used to compare the qualitative quantities. For the comparison of several groups, the ANOVA analysis was used. The statistical significance threshold was $p < 0.05$. Survival data were plotted using the Kaplan-Meier curve.

Among the 74 patients enrolled in the study, the predominance of males was highlighted as follows: 54.05% men and 45.95% women. A similar ratio was recorded in the control patient group - 55.36% men versus 44.64% women.

Regarding the age of the patients, the youngest patient enrolled in the study was 37 years old, and the oldest was 76 years old, with an average age of 56.5 years.

In the group of patients treated with chemotherapy, 11 patients had MSI-H tumours (14.86%) and 63 had MSS tumours (85.13%). In the control group, of the 56 patients, 16% were diagnosed with MSI-H tumours and 83.93% with MSS tumours.

Of the 34 cases of colon cancer diagnosed in women, 7 cases were MSI-H tumours (20.58%), and the rest were MSS tumours - 27 cases (79. 42%). Of the tumours diagnosed in men, only 10% (4 cases) were MSI-H, and the remaining 90% (36 cases) were MSS-p tumours without statistical significance. ($p = 0.290$)

In the control group, among women, 6 cases of MSI-H tumours and 19 MSS tumours were registered. Among men, 3 MSI-H tumours and 28 MSS tumours were diagnosed.

Regarding the location of the tumour, in the study group, 31 tumour developed (41.89%) in the right colon and 43 (58.11%) in the left colon. The same share was maintained in the control group, where tumours of the left colon predominated - 57.14%, compared to tumours located in the right colon - 42.86%.

Of the tumours located in the right colon, 8 patients had MSI-H tumours and 23 had MSS tumours. The percentage of MSI-H tumours in the right colon is higher than in the left colon (72.73% vs 27.27%). At the same time, the percentage of MSS tumours in the left colon is higher than in the right colon (63.49% vs 36.51%), but with a p without statistical significance ($p = 0.057$)

From the point of view of tumour staging, the study group was dominated by patients with T4a tumours (51.34%), followed by patients with staged T3 tumours (28.38%) and then T4b tumours (20.27%). Approximately the same share was recorded in the control group, where 50% of patients were diagnosed with T4a tumours, 28.57% with T3 tumours and 21.43% T4b tumours. In the control group, among patients with MSI-H tumours, an equal number of locally advanced tumours (T4a and T4b) were registered, namely 44.44%. In the control group, with MSS tumours, T4a tumours predominated (51.06%), then T3 tumours (31.91%).

Patients with MSI-H status had mainly locally advanced tumours - T4b (54.55%) or T4a tumours (27.27%). Only 18.18% of T3 tumours were diagnosed. In the group of patients with stable microsatellite tumours, T4a (55.56%) and T3 (30.16%) tumours predominated, less T4b tumours

(14.29%). Data related to the correlation between tumour stage and microsatellite stability status recorded a statistically significant p ($p < 0.05$)

Among patients with MSI-H tumours, 27.27% were diagnosed with mucinous type adenocarcinoma, and 72.73% of patients with MSI-H tumours presented conventional adenocarcinoma tumours on histopathological analysis. By comparison, 6.35% of patients with MSS tumours had the histopathological diagnosis of mucinous adenocarcinoma, in which the predominant colonic tumours of conventional adenocarcinoma type - 93.65%. The histopathological type was correlated with the status of microsatellites, the correlation having statistical significance ($p = 0.029$) .

In patients with unstable microsatellite tumours, G2 (54.55%) and G3 (36.35%) tumours predominated. The percentage of G1 tumours was only 9.09%.

Stable microsatellite tumours were well or moderately differentiated tumours, the percentage of poorly differentiated being lower. Thus, 69.84% were G2 type tumours, and 15.87% were G1 type tumours. As a percentage, 14.29% of G3 tumours were registered. The data recorded from the point of view of grading are without statistical significance ($p = 0.156$).

Of the patients with unstable microsatellite tumours who received treatment, 54.55% had less than 12 excised lymph nodes, and 45.45% had more than 12 excised / analysed lymph nodes. In the control group, the situation with over 12 examined lymph nodes predominated (in 63.49% of patients). Data were not statistically significant ($p = 0.606$)

22.97% of patients treated with fluoropyrimidines had lymphovascular or perineural invasion. Approximately the same percentage was recorded in patients in the control group (23.21%). Of the patients with MSI-H tumours treated with fluoropyrimidines, 36.36% had lymphovascular invasion. Approximately the same percentage was recorded in patients with MSI-H tumours in the control group (33.33%).

Among patients with stable microsatellite tumours, among patients treated with 5FU, 20.63% had lymphovascular invasion and 21.28% of patients in control group.

Regarding colorectal cancer recurrence in the study group, 22 patients (29.73%) had locoregional or remote recurrence documented during the follow-up period. The percentage registered in the control group was 33.93%, without p with statistical significance. From the point of view of the data of survival without signs of disease, the average among the treated patients was 28.54 months, and among the witnesses, 27.96 months, with a p without statistical significance ($p = 0.7859$)

During the follow-up period, 21% of the patients in the group of patients who received treatment and 28.57% of the patients in the control group died. In terms of overall survival, the Kaplan Meier curve shows a discrete survival benefit for patients treated with fluoropyrimidines compared to patients in the control group, but with p without statistical significance ($p = 0.43$). The benefit for the disease-free interval appears to be in favor of young patients in the first two years of follow-up, and then the curve is reversed in favour of patients with a mean age over 62 years.

Among male patients, the benefit of relapse-free survival appears to be for male patients in the control group, but without a statistically significant p ($p = 0.1996$). The unfavourable prognosis was registered for the patients from the control group, observing from the analysis of the Kaplan-Meier curve a rapid recurrence, in the first 10 months of follow-up.

The results of the analysis showed that patients with tumours located in the left colon, both those treated and those who did not receive adjuvant chemotherapy, have a better prognosis. Of the 31 patients with tumours located in the right colon, 12 had progressive disease (38.7%), compared to 10 patients with left colon adenocarcinoma, in which the recurrence of the disease was highlighted (23.25%).

Of the total number of patients included in the study, 7 patients were diagnosed with mucinous adenocarcinoma (9.46%). Of these, 3 patients had progressive disease. The result is not statistically significant due to the small number of patients.

Of the G3 tumours, 9 tumours were microsatellite stable and 4 were associated with microsatellite instability. Among the poorly differentiated tumours, progression was recorded in 5 patients (38.46%).The degree of tumour differentiation is statistically significantly correlated both with survival without signs of disease and with overall survival ($p < 0.05$).

In the current study, patients with G1 or G2 tumours who received chemotherapy performed well. The degree of differentiation correlates with the status of microsatellite instability and survival without resumption of disease evolution. Thus, of the 13 G3 tumours, no progression was recorded in patients with MSI-H tumours. MSS G3 tumours have the highest risk of recurrence, which is also confirmed by the analysis of current data.

Among the patients included in the study who progressed to metastatic disease, it was found only in 2 of the 21 patients with T3 tumours (9.52%) and in 10 of those with T4a tumours (26.31%). The highest rate of disease progression was recorded in patients with T4b tumours - 66.6%. Patients with T3 tumours, both those treated with 5FU and those who did not receive treatment, had the best survival. The results were statistically significant with a $p < 0.0001$.

In the study, 22.97% of patients presented with the histopathological analysis of the resection piece lymphovascular or perineural invasion (17 patients). Of these, 9 patients (52.94%) progressed to metastatic disease. (statistically significant $p < 0.0001$).

In the current study, the low number of excised lymph nodes (less than 12 lymph nodes) correlated with a higher recurrence rate (statistically significant p $p < 0.05$). Of the 74 patients, 39.19% had less than 12 lymph nodes analysed in the histopathological report. Of these, 15 patients progressed to metastatic disease.

Of the 22 patients with relapsed disease, 3 patients had MSI-H tumours and 19 MSS tumours. The median survival without disease recurrence for patients treated and diagnosed with MSS tumours was 28.37 months, and for patients with MSI-H tumours was 29.55 months. In the control group, the median DFS for patients with MSS tumours was 27.13 months, and for patients with MSI-H tumours was of 32.33 months.

Patients with MSI-H tumours had a minimum overall survival of 16 months and those with MSS tumours 10 months. Data were different in the control group where OS was higher for patients with MSS tumours than for those with MSI-H tumours (18 months vs. 15 months) but without a statistically significant p ($p = 0.9194$).

In the case of metastases, survival was much reduced for patients with significant tumour load, data of statistical significance ($p < 0.0001$).

In the group of patients with dMMR tumours treated with 5FU, 72.73% were not diagnosed with resumption of evolution, unlike the group of patients with pMMR tumours, where the percentage was 69.84%. The highest percentage of distant recurrence was recorded in the group of patients without fluoropyrimidine treatment (26.98%), with 8 percent higher than in the group of patients with unstable microsatellite tumours treated (18.18%)

Discussions required at the end of this study are related to the implications of adjuvant fluoropyrimidine treatment depending on microsatellite instability status, reported by disease-free interval (DFS) and overall survival (OS) values at 3 years, compared between the group of patients treated with 5FU and the control group. Also, the analysis and validation for the two groups of patients of some prognostic factors highlighted in the literature and specialised studies was taken into account.

Of the 74 patients included in the study, 14.86% had MSI-H tumours (11 patients) and 85.14% (63 patients) had MSS tumours. In the control group, there were 16% MSI-H tumours (9 patients) and 83.93% MSS tumours (47 patients). Indeed, with regard to sporadic colon cancers,

numerous data from the literature and clinical studies show that approximately 15% of tumours express microsatellite instability, the rest being stable microsatellite tumours.

Regarding the sex of the patients, men predominated in the study population, being 1.17 times more than women in the study group. In the control group it was about the same ratio (1.24 times more men than women). In the group of patients with MSI-H tumours there were 1.75 times more women than men, thus confirming the data showing that MSI-H colon tumours are more common among women [32]. The same situation remained in the control group.

Regarding the location of colonic tumours, those located in the left colon predominated (58.11% compared to 41.89%). dMMR tumours are more common in the right colon than in the left colon where MSS tumours are more common (72.72% compared to 27.27%). The same report was maintained in the control group. The recurrence rate was higher for tumours located in the right colon than in tumours located in the left colon (54.54% vs 45.45%), and it was reported that distal tumours, according to the Treitz angle, have a lower recurrence rate and a better survival than tumours located in the right colon [33], a situation that is also found in our study.

Tumour stage (T) is recognised as a prognostic factor independent of microsatellite instability status. Thus, T3 tumours have the best prognosis, while T4b-type tumours have an unfavourable prognosis, despite a correct therapy. The current study was dominated by T4a tumours (38%), followed by T3 tumours (21%) and T4b tumours (15%). In the group of patients with unstable microsatellite tumours, T4 tumours (either T4a or T4b) and only two cases of T3 tumours, given statistically significant, were more common ($p < 0.0001$).

Analysing the correlation between the progression of the disease and the stage of the T tumour, 9.52% of the patients with T3 tumours progressed, 26.31% of the patients with T4a tumours and 66.6% of the patients with T4b tumours. Of the patients with MSI-H tumours, only 3 patients progressed, two patients with T4b tumours and one patient with T4a tumour. It should be noted that they also had other unfavourable prognostic factors, such as lymphovascular / perineural invasion or less than 12 excised lymph nodes. Statistical analysis showed that these data have statistical significance with $p < 0.05$.

In the group of patients, moderately differentiated tumours predominated, in proportion of 67.57%, followed in frequency by G3 tumours (17.57%) and then G1 tumours (14.86%). In the group of control patients, G2 tumours were common (64.29%) followed by G1 tumours (23.21%) and then G3 tumours (12.5%).

Well-differentiated tumours were rarely diagnosed in the group of patients with unstable microsatellite tumours (9%). In the group of patients with stable microsatellite tumours, the

percentage was higher in favor of the group with well-differentiated tumours (15.87% patients with G1 tumours and 14.29% patients with G3 tumours). The same percentage ratio was in the control group.

The degree of tumour differentiation is a prognostic factor in survival without signs of disease, which is confirmed by statistical analysis, with a $p = 0.0353$. Thus, OS was better for patients with G1 or G2 tumours and unfavourable for patients with G3 tumours. At the same time, the degree of differentiation G is correlated with the MSI status, not being an independent factor such as the stage of the tumour. In the group of treated patients no patient with MSI-H tumour and G3 tumour progressed. It is not known exactly why this is happening. The assumption is that in the case of these tumours, modified proteins are synthesised that are recognised by the body and lead to the initiation of an important immune reaction by the body, which would explain the appearance of inflammatory infiltrate. [34]

From the point of view of histopathological analysis of resected tumour specimens, 90.54% of tumours were of conventional adenocarcinoma type, and 9.46% (7 tumours) were of mucinous adenocarcinoma type, correlated with the degree of G3 differentiation (at 5 of the 7 mucinous tumours).

When mucinous adenocarcinoma correlated with MSI-H tumours (in the present study 27.7% of patients with MSI-H tumours had mucinous adenocarcinoma), the prognosis was favourable, with no local or distant progression, with significant statistics ($p = 0.029$). Among patients with pMMR tumours, 6.35% had mucinous adenocarcinoma, 50% of which then progressed at a distance.

A predictive factor for patient survival is lymphovascular and perineural invasion, proven to be a predictive factor independent of microsatellite instability status. In the study, 17 patients in the group of treated patients (22.97%) and 13 patients in the control group (23.31%) had lymphovascular or perineural invasion. The percentage of invasion was higher among patients with MSI-H tumours, but without statistical significance. Regarding the progression of the disease, they were in favor of patients without detectable lymphovascular or perineural invasion, with a statistically significant $p < 0.0001$. Survival data were unfavourable for patients in the control group.

As for excised lymph nodes, 60.81% of patients receiving treatment, had more than 12 excised lymph nodes, a percentage consistent with data from other clinical trials [35]. The percentage in the control group was 57.81%. Of the 22 patients who reported recurrence of the disease, 15 of them had a low number of excised lymph nodes (<12 lymph nodes) (68% of cases).

Thus, both the disease-free interval and the overall survival were reduced for patients with a small number of excised lymph nodes, with or without adjuvant treatment, statistically significant data ($p < 0.05$).

There are two important issues: the experience of the surgeon, essential in performing surgery as carefully as possible and in accordance with the recommendations of specialised guides and the problem of substadialization of these patients, who in these conditions do not receive adequate treatment, thus having an increased risk of local or remote recurrence.

Recurrence of the disease occurred in 29.73% of patients. The occurrence of recurrence was correlated with the previously mentioned and validated risk factors: lymphovascular and perineural invasion, number of excised lymph nodes, histopathological type of tumour, degree of tumour differentiation, stage T of the tumour. Of the 22 patients who progressed, only 3 were patients with MSI-H tumours and 19 with MSS tumours. The minimum survival for patients with MSI-H tumours was 16 months, and for patients with MSS tumours, 10 months.

Regarding the survival data, it is worth mentioning that 72.7% of the study patients, treated with fluoropyrimidines, with MSI-H tumours survived at 3 years without signs of disease. 90.9% of patients with MSI-H tumours lived at the end of the follow-up period. In the control group, 88.8% of patients with MSI-H tumours had no signs of recurrence at 3 years, and 100% of them were alive.

Among patients with MSS tumours, 70% of patients survived without signs of recurrence at 3 years, and 74.6% were alive at 3 years. In the control group, 60.71% of patients with MSS tumours survived 3 years without signs of disease, and in life they were 68%.

The limitations of this study come primarily from the fact that this study was conducted over a short period of time in terms of overall survival data, namely 3 years. The information obtained cannot be generalised considering that all the patients included in the study come from a single center - Bucharest University Emergency Hospital.

Given the fact that the study was conducted in an emergency hospital, it is possible that this limited the number of patients with early-stage colon cancer (stage II), locally advanced tumours predominate, or stage III or IV colon cancer patients predominate. Another limitation of the study comes from the relatively small number of MSI-H stage II colon cancer patients (11 patients vs. 63 MSS stage II colon cancer patients) enrolled in this study. Obviously, the numerical differences come from the low percentage of MSI-H tumour cases reported in the literature, but also diagnosed in this study - about 15%.

The aim is to deepen these data by continuing clinical trials with this topic, increasing the number of stage II colon cancer patients enrolled in studies and the inclusion of several surgery clinics, from several hospitals, over a longer period of time and with longer tracking, which would allow generalisation and reporting of data.

The conclusions of the study result from the statistical analysis of the data of the patient group.

Some results obtained had statistical value, which validated the importance of the data obtained.

- The study group included a number of 74 patients with stage II colon cancer, of which 14.86% had dMMR tumours, this percentage being consistent with reports from other clinical trials and literature.
- The number of cases of colon cancer MSI-H is higher in women than in men, but with p without statistical significance in the present study. ($p = 0.290$)
- In the present study, MSI-H tumours predominated in the right colon (72.73%), compared to MSS tumours (27.27%). Also, MSS tumours predominated in the left colon (63.49%) compared to MSI-H tumours (36.51%).
- In the case of patients with MSI-H tumours, locally advanced tumours predominate (T4a and T4b), and in the group of patients with MSS tumours, T4a and T3 tumours predominate. Data were validated by reporting a statistically significant p ($p <0.05$).
- Patients with T3 tumours had the best survival. The results were statistically significant with a $p <0.0001$. Patients with advanced locoregional tumours (T4b) with or without treatment had the highest recurrence rate, with the specification that patients with T4b tumours without treatment had the most unfavourable prognosis.
- Patients with T4a or T4b tumours but with microsatellite instability have a better prognosis and a lower recurrence rate compared to patients with MSS T4a or T4b tumours
- In the study group, the correlation between the status of microsatellites and the histopathological type of tumours was established, the data obtained being of statistical significance ($p = 0.029$). Thus, MSI-H tumours are associated with the presence of mucinous adenocarcinoma, while MSS tumours are associated with conventional adenocarcinoma-type tumours.
- From the histopathological analysis of colon tumours and tumour grading, it is concluded that patients with dMMR tumours, more frequently present G2 and G3 tumours. pMMR tumours

were well or moderately differentiated tumours, the percentage of poorly differentiated being lower, but without statistical significance ($p = 0.156$).

• The degree of tumour differentiation correlates statistically significantly both with survival without signs of disease and with overall survival ($p < 0.05$).

• The number of excised lymph nodes correlates statistically significantly with survival data ($p = 0.0292$)

• The presence of lymphovascular and perineural invasion correlates statistically significantly with the occurrence of recurrence, with a statistically significant p ($p < 0.0001$) Survival data demonstrated the benefit of fluoropyrimidine treatment in patients with lymphovascular and perineural invasion

• Analysing the data of DFS and OS the two groups of patients according to the sex of the patients, it was observed that there was a benefit for the female population that received treatment with fluoropyrimidines. Among male patients, the survival benefit was recorded for male patients in the control group.

• Survival-free survival data demonstrate a relapse-free survival benefit for 5FU-treated patients with stable microsatellite tumours compared to patients treated with 5FU but with unstable microsatellite tumours (data of no statistical significance)

• The results of the analysis showed that patients with tumours located in the left colon have a better prognosis, regardless of the presence or absence of treatment.

• The prognosis from the point of view of OS and DFS is favorable for patients with right colon cancer who have received treatment with fluoropyrimidines

• In the control group, there was a survival benefit for patients with unstable microsatellite tumors compared to patients with stable microsatellite tumours.

• In the absence of 5FU treatment, DFS data are favourable for patients with microsatellite instability (dMMR tumours)

• In the presence of 5FU treatment, survival data are favourable for patients with stable microsatellite tumors (pMMR), without benefiting patients with microsatellite instability.

• Analysing the data from the point of view of microsatellite status, regardless of the presence or absence of fluoropyrimidine treatment, the conclusion is that microsatellite instability offers a small survival benefit even if no p with statistical significance was reported ($p = 0.1308$)

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