The prognostic role of microsatellite instability in colorectal cancer patients

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The prognostic role of microsatellite instability in colorectal cancer patients

BACKGROUND: *Data from the literature regarding the prognostic role of DNA mismatch repair system (MMR) in colorectal cancer are still controversial.*
AIM: *The aim of the study was to identify the prognostic role of different phenotypic, clinical and pathological characteristics in microsatellite unstable vs. microsatellite stable colorectal cancer in terms of survival and disease free interval.*
METHODS: *We conducted a retrospective study that included a total of 103 patients who underwent curative surgery for colorectal cancer. Immunohistochemistry testing revealed MLH1, MLH2, MLH6, PMS2 genes and mutations of the BRAF gene. We identified three groups of patients: patients with colorectal tumors with MSI produced by hypermethylation, (MLH1/BRAF+) group, patients with microsatellite instable tumours produced by genetic mutations MSI groupb(MLH1, MLH2, MLH6, PMS2) and patients with microsatellite stable tumours (MSS).*
RESULTS: *The study shows that: MSI tumours (MLH1/BRAF+) group occur more frequently in women (p=0.05), on the right side of the colon (p=0.001). The 5-year survival rate was higher in patients with MSI tumours (MLH1/BRAF+) group than in those with microsatellite stable tumours, the differences were not statistically significant ; relapse rate was higher in patients with MSI tumors than in those with MSI tumours (MLH1/BRAF+) group (p=0.03) or with MSS tumors (p=0.004).*
CONCLUSIONS: *The identification of microsatellite unstable colorectal tumours is an important molecular marker with role in recognition subgroups of patients with different phenotypic characteristics, survival and relapse rates.*

KEY WORDS: Colorectal cancer, Mismatch repair genes, Prognostic role

Introduction

Most colorectal cancers arise from chromosomal instability in the adenoma-carcinoma sequence, but about 20% of colorectal cancers develop via an alternative pathway of tumour genesis characterized by microsatellite instability (MSI). Microsatellites are short and repetitive sequences in the DNA structure, prone to mutations. During DNA replication, errors may occur in DNA sequences. DNA mismatch repair (MMR) is a system composed of repair genes of mismatched nitrogenous bases, whose role is to fix errors that occurred during replication. If there is a malfunction in the MMR system, microsatellite errors occur, phenomenon called microsatellite instability, responsible for the accumulation of somatic mutations of the oncogenes or suppressor genes playing an important role in tumour initiation and progression. These tumours are characterized by microsatellite instability (MSI), while those where this

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ABBREVIATIONS

MMR: mismatch repair
MSI: microsatellite instability
MSS: microsatellite stability
MLH1: human mutator L homolog 1
MSH2: human mutator S homolog 2
MLH3: human mutator L homolog 3
MSH3: human mutator S homolog3
MSH6: human mutator S homolog 6
PMS2: human postmeiotic segregation increased 2
GTBP: guanine/thymidine mismatch-binding protein
LNR: lymph node ratio

phenomenon does not occur are characterized by microsatellite stability (MSS) .[1]
There might be a sporadic malfunction of the MMR system triggered by the hypermethylation of the promoter region of the MLH1 repair gene, which causes a reduction or loss of MLH1 expression or a genetic mutation by inactivating one of the genes of the MMR system: MLH1, MSH2, MLH3, MSH3, MSH6, PMS1, PMS2, GTBP [2].
There was great variability in terms of survival and prognosis in the subgroup of patients with colorectal cancer characterized by MSI. Studies investigating the phenotypic differences between different types of colorectal cancers displaying microsatellite instability are controversial [3,4]. The proposed study aims to identify various phenotypic, clinical and pathological characteristics with a prognostic role in sporadic colorectal cancers with microsatellite instability in comparison to inherited cancers or cancers that do not display microsatellite instability, and to determine any differences between the three entities in terms of cancer-specific survival, disease-free interval and the occurrence of relapses.

Materials and Methods

To achieve the objectives we conducted a retrospective study on a total of 103 patients diagnosed with colorectal cancer stage I-III, hospitalized and consecutively undergoing radical surgery between January 2013 and December 2015. The following patients were excluded from the study: patients with distant metastases detected pre- or intraoperative, synchronous primary tumour, inflammatory bowel disease, patients with other histological types of cancer besides adenocarcinoma, patients undergoing emergency surgery, patients receiving preoperative radiotherapy, patients who died in less than 30 days after surgery, patients with missing data and those who did not sign the informed consent form.
A database was created including demographic data (age, gender, area of origin), clinical and anamnestic data (symptoms, duration of symptoms, the main comorbidities), laboratory examinations (WBC count, lymphocyte count, neutrophil count, neutrophil-to-lymphocyte ratio, platelet count, haematocrit, haemoglobin), paraclinical data (tumour location, histological type), intraoperative findings (tumour location, loco-regional extension, distant metastases, type of surgery), pathological findings (tumour size, macroscopic appearance, histological type, tumour grading, tumour stage [5], lymph node stage [5], number of lymph nodes resected, the ratio of involved to the total resected lymph nodes defined as lymph node ratio (NRL), vascular invasion, perineural invasion, presence of necrosis and its quantification, quantification of the mucinous component.
The following were considered: WBC count defined in three ranges (<8500/mm^3; 8500-11000/mm^3; >11000/mm^3) according to Leitch et al. [6], lymphocyte count (<1000/mm^3; 1000-3000/mm^3; >3000/mm^3), neutrophil count (<7500/mm^3; >7500/mm^3), neutrophil-to-lymphocyte ratio (5 being considered as interval value), haemoglobin (11.5 to 15 g/dl), haematocrit (37-47%), platelets (150000-370000/mm^3), according to studies by Sasaki et al. [7], the presence of anaemia (<11 g/dl in men; <10 g/dl in women).
A new anatomopathological microscopic examination was conducted by analysing tumour invasion margins and based on the newly defined TNM staging system according to the latest edition of the AJCC Cancer Staging Manual [5], the seventh, effective from 1 January 2010.
Based on studies by Petersen et al. (8), the Petersen index was calculated, the scores being subdivided into low risk (0-1) and high risk [2-5].
Local inflammatory response was calculated using Klintrup criteria [9], at the edge of the invaded tumour, quantifying the local inflammatory infiltrate. Score 0 was assigned in case of complete absence of inflammatory infiltrate at the edge of the invaded tumour. Score 1 was assigned to a minimum or average infiltrate and score 2 to a prominent inflammatory infiltrate. Score 3 indicates an extremely rich inflammatory infiltrate which occasionally disrupts cellular architecture. Local inflammatory response is considered low for scores 0 and 1, and high for scores 3 and 4.
Tumour necrosis was also quantified [10], assigning score 0 for absence of necrosis, 1 for "focal" necrosis, less than 10%; 2 for "moderate" necrosis, between 10 and 30%; 3 for "extensive" necrosis, more than 30%, according to Richards et al. 2012 [11].
When quantifying mucinosa, 0 score was assigned in case of complete absence of the mucinous component; 1 for minimal mucinous component, less than 10%; 2 for moderate mucinous component, between 10-50%, and 3 for extensive mucinous component, over 50%.

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For patients with stage III cancer, the lymph node ratio (LNR) was calculated by dividing the number of tumor invaded lymph nodes to the total number of resected lymph nodes. Based on this criterion, patients were divided into 5 groups (<0.10, 0.11-0.21, 0.22-0.36, 0.37-0.6 and >0.61). This LNR classification had already been applied in several previous studies [12,13].

The genetic study was performed using immunohistochemistry to determine MLH1, MLH2, MLH6, PMS2 gene expression. In this regard, at least one representative paraffin block was purchased for each patient, as indicated by the pathologist who reviewed the slides with histological specimens, which should contain colon cancer tissue, as well as normal colon tissue adjacent to the tumor. Immunohistochemistry was performed using the streptavidin-biotin complex method (DAKO, Carpinteria, CA) [15].

For samples that showed absence of gene expression for MLH1, considering that it can occur through both promoter hypermethylation of DNA repair gene MLH1 (sporadically) and autosomal dominant inheritance, a second examination was conducted consisting in testing BRAF V600E gene mutation. In patients with the lack of MLH1 expression and the presence of BRAF V600E mutation, the mechanism of carcinogenesis is considered to be determined by MLH1 promoter hypermethylation, which is sporadic.

Patients were followed for a period of 5 years, after 3 and 6 months in the first year after surgery and annually in the coming years, through complete clinical examination, laboratory tests, chest X-ray, abdominal ultrasound, colonoscopy and CT for screening purposes. Patients with stage III cancer received chemotherapy with 5-fluorouracil.

All patients included in the study signed the informed consent form and the study was approved by the Ethics Committee by the board of our institution.

Distant recurrences were identified by general local exam and paraclinical examination (laboratory tests, chest X-ray, general ultrasound, computed tomography, scintigraphy), while local recurrences were detected by abdominal ultrasound and/or lower gastrointestinal endoscopy with anastomotic biopsy. The period (number of months) following surgery until the occurrence of local-regional or distant recurrences was calculated and defined as relapse-free survival.

Statistical analysis was performed using MedCalc Statistical Software version 16.8 (MedCalc Software bvba, Ostend, Belgium, https://www.medcalc.org; 2016). Data were presented as nominal, ordinal or quantitative. Quantitative data were tested for normality using the Kolmogorov-Smirnov test. Quantitative variables were described as median and 25th and 75th percentiles (non-normal distribution). Nominal and ordinal variables were described as frequency and percentage. The comparison between the two groups was performed using the Mann-Whitney test. Differences in frequency of a nominal or ordinal variable between the two groups were tested using the chi-square test or Fisher's test, as appropriate. Differences in survival or relapse between the two groups were checked with the log-rank test and Kaplan-Meier curve. A p value of <0.05 was considered statistically significant.

Results

Immunohistochemistry showed that of the total of 103 patients, 21 (20.3%) had absent expression of MLH1, 2 (1.9%) had absent expression of MLH1 and PMS2, 1 (0.9%) had absent expression of MSH2, 1 (0.9%) had absent expression of MSH2, MSH6 and PMS2, and 9 (8.73%) had absent expression of PMS2. In patients where MLH1 gene expression was absent, a second examination was performed consisting of BRAF V600E gene mutation testing. The presence of BRAF gene mutation was identified in 18 (17.47%) patients, who were categorized as MLH1/BRAF+ group. The other patients who had absent expression of MLH1 associated with absent BRAF mutation, as well as patients who had absent expressions of the genes mentioned above were included in the group of patients with microsatellite instability cancers (MSI group).

Therefore, three groups of patients were produced: one group of 18 patients with microsatellite instability of sporadic colorectal cancers, the MSI (MLH1/BRAF+) group, a second group of 16 patients with microsatellite instability of colorectal cancers with genetic defects of one of the MLH1, MSH2, MSH6, or PMS2 genes, the MSI group, and a third group of 69 (67%) patients with microsatellite stable colorectal cancers, the MSS group.

In terms of age, the patients in the sixth decade were more numerous in the entire study group. This distribution was also maintained within the three groups. The main clinical characteristics of the study group are presented in Table I.

The sex ratio in the entire group was 42.8% women and 57.2% men.

In the MSI (MLH1/BRAF+) group, 2 patients (11.1%) had rectal cancer and 16 (88.9%) colon cancer. In the MSI group, four patients (25%) had rectal cancer and 12 (75%) colon cancer, and in the MSS group, 44 patients (63.7%) had rectal cancer and 25 (36.3%) colon cancer. Differences between the MSI (MLH1/BRAF+) group and the MSS group were highly statistically significant (p<0.001), as well as those between the MSI and the MSS group (p=0.01).

The most frequent location of the tumour in the MSI (MLH1/BRAF+) group was on the right side of the colon. In the MSI (MLH1, MSH2, MSH6, PMS2) group, the predominant location was on the right side of the colon in five (31.2%) patients, on the left side of the colon in four (25%) patients, in the transverse colon in three (18.75%) patients, in the lower bound-

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Table I - *Clinical and pathologic characteristics of patients in the three groups*

Variable	MSI (MLH1/BRAF+)	MSI (MLH1, MLH2, MLH6, PMS2)	MSS	P
Age	61 (55.7; 70.2)	66 (56; 72.7)	67(55;71)	0.3
Gander	12 (66.7%)	5 (31.2%)	27 (39.1%)	0.05
F	6 (33.3%)	11 (68.8%)	42 (60.9%).	NS
M				
urban	15 (83.3%)	12 (75%)	39 (56.5%)	
rural	3 (16.7%)	4 (25%)	30 (43.5%)	0.3
Symptoms				
Pain	13(72.2%)	8 (50%)	20 (29%)	NS
Transit disorders	4 (22.2%)	4 (25%)	26(37.7%)	NS
Haematochezia	1 (5.6%)	3 (18.75)	11 (16%)	NS
Tenesmus	0	1 (6.25%)	12(17.3%)	>0.05
Symptom duration				
< 6 months	16 (88.9%)	9 (61.2%)	41 (58.5%)	NS
> 6 months	2 (11.1%)	7 (38.8%)	28 (40.5)	0.2
Location:				
Right side of the colon	9 (50%)	5 (31.2%)	7 (10.2%)	<0.001
Transverse colon	1 (5.6%)	3 (18.75%)	2 (2.9%)	NS
Left side of the colon	6 (3.33%)	4 (25%)	16 (23.2%)	NS
Rectosigmoid junction	1 (5.6%)	1 (6.3%)	10 (14.5%)	NS
Upper part of the rectum	1 (5.6%)	0	10 (14.5%)	NS
Lower part of the rectum		3 (18.75%)	24 (34.8%)	0.002
WBC COUNT				
<8500/mm^3	0	1 (6.2%)	5 (7.2%)	NS
8500-11000/mm^3	16 (88.9%)	15 (93.8%)	61(88.4%)	0.6
>11000/mm^3	2 (11.1%)	0	3(4.3%)	NS
median	7035 (5500;8997,5)	7900 (6900;9600)	7200(6212;8975)	NS
NEUTROPHIL/LYMPHOCYTE RATIO				
>5	2 (12.5%)	1 (5.6%)	8 (11.6))	NS
<5	14 (85.7%)	17 (94.4%)	61 (88.4%)	NS
median	2,65 (2;3.22)	3.36(2.58;4.9)	2.86 (2.18;3.62)	0.04
Hemoglobin (mg/dl)	10.5 (8.68;12.28)	12.5 (10.3;14)	12.4 (10.6;13.3)	0.08
PLATELET COUNT/mm^3	298500 (251250;444250)	264000 (237000;397000)	289500 (245000;359000)	0.06

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ary of the rectum in three (18.75%) patients, and in the recto sigmoid junction in one patient. In the MSS group, the tumour was located in the rectum in 44 (63.7%) patients. There were statistically significant differences regarding the location of the tumour on the right side of the colon between the MSI (MLH1/BRAF+) group and the MSS group (p<0.001), as well as between the MSI (MLH1, MSH2, MSH6, PMS2) group and the MSS group (p=0.44). There were also statistically significant differences in terms of tumour location in the lower rectum between the MSI (MLH1/BRAF+) group and the MSS group (p=0.002). There were no statistically significant differences between the two MSI groups (p=0.5) regarding tumour location.

Regarding the general inflammatory response, quantified by WBC count, neutrophil-to-lymphocyte ratio, and platelet count, differences between groups were not statistically significant. In contrast, patients with anaemia were more likely to belong to the MSS group (44 (63.8%) than to the MSI (MLH1/BRAF+) group (17 (50%). The main pathological characteristics are shown in Table II.

There were statistically significant differences in terms of vegetant macroscopic tumour appearance between the group of patients with MSI (MLH1/BRAF+) group and the group of patients with microsatellite stable tumours (p=0.05).

Table II - *Pathological characteristics of patients in the three groups*

Variable	**MSI (MLH_1/BRAF+)**	**MSI (MLH1,MLH2,MLH6,PMS2)**	**MSS**	**P**
T<2	5 (27.8%)	4 (25%)	12 (17.4%)	NS
T>2	13 (72.2%)	12 (75%)	57 (82.6%)	0.9
N_0	10 (55.5%)	9 (56.3%)	41 (59.4%)	NS
N_1	6 (33.3%)	3 (18.7%)	19 (27.5%)	0.8
N_2	2 (11.2%)	4 (25%)	9 (13.1%)	NS
TNM				
I	4 (22.2%)	3 (18.75%)	9 (13%)	NS
IIA	4 (22.2%)	5 (31.25%)	23 (33.3%)	NS
IIB	2 (11.1%)	–	7 (10.1%)	NS
IIC	–	1 (6.25%)	1 (1.4%)	NS
IIIA	–	1 (6.25%)	2 (2.9%)	NS
IIIB	7 (38.9%)	3 (18.75%)	20 (29%)	NS
IIIC	1 (5.6%)	3 (18.75%)	7 (10.1%)	0.9
Number of excised nodes	13 (12;12.75)	13 (12;17)	12 (10;14)	0.01
LNR1	5 (62.5%)	1 (14.3%)	9 (31.03%)	NS
LNR2	2 (25%)	1 (14.3%)	8 (27.6%)	NS
LNR3	–	4 (57.1%)	5 (17.23%)	0.6
LNR4	1 (12.5%)	1 (14,3%)	4 (13.8%)	NS
LNR5	-	-	3 (10.34%)	NS
Macroscopic appearance				
- vegetant	13 (72.2%)	6 (37.5%)	15 (21.8%)	NS
-ulcero-vegetant	4 (22.2%)	5 (31.25%)	29 (42%)	0.05
-ulcero-infiltrative	1 (5.6%)	5 (31.25%)	25 (36.2%)	NS
TUMOUR SIZE				
< 4 cm	5 (27.8%)	1 (6.2%)	26 (37.7%)	NS
> 4 cm	13 (72.2%)	15 (93.8%)	42 (62.3%)	NS
median	5 (3,75;7)	7 (5;8)	5 (3,5;6)	0.003
TUMOUR GRADING				
G1	4 (22.2%)	9 (56.2%)	19 (27.5%)	NS
G2	12 (66.7%)	5 (31.5%)	44 (63.7%)	0.7
G3	2 (11.1%)	2 (12.3%)	5 (7.2%)	NS
G4			1 (1.6%)	NS
KLINTRUP SCORE				
Low grade (0;1)	5 (27.8%)	9 (56.2%)	37 (53.6%)	NS
High grade (3;4)	13 (72.2%)	7 (43.8%)	32 (46.4%)	0.3
PETERSEN SCORE				
Low risk (0-1)	17 (94.45%)	12 (75%)	59 (85.5%)	NS
High risk (2-5)	1 (5.55%)	4 (25%)	10 (14.5%)	1
Venous invasion	2 (11.1%)	4 (24%)	9 (13%)	0.5
PERINEURAL INVASION	–	1 (6.25%)	5 (7.2%)	0.6
NECROSIS				
0	2 (11.1%)	3 (18.8%)	13 (19.1%)	NS
1	8 (44.4%)	6(37.5%)	26 (38.2%)	NS
2	5 (27.8%)	4 (25%)	22 (32.4%)	NS
3	3 (16.7%)	3 (18.8%)	7 (10.3%)	0.6
MUCINOUS COMPONENT				
0			48 (70.6%)	NS
1	12 (66.7%)	9 (56.2%)	7 (10.3%)	NS
2	2 (11.1%)	1 (6.2%)	5 (7.4%)	NS
3	3 (16.7%)	2 (12.5%)	8 (11.8%)	>0.05
	1 (11.1%)	4 (25%)		

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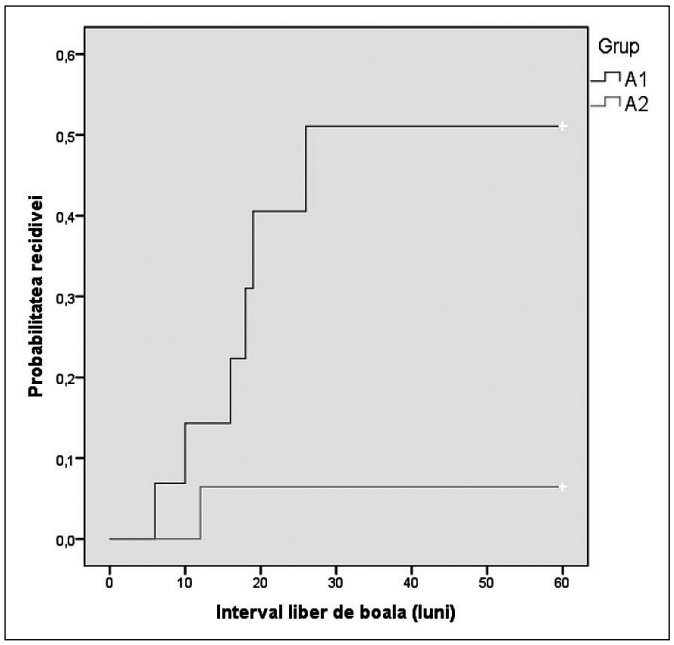

Fig. 1: The Kaplan-Meier curve of recurrence for MSI (A1) and MSI (MLH1/ BRAF+) (A2) groups.

In terms of size, median tumour size was 7 cm [5,8] in the MSI group, 5 (3.75; 7) cm in the MSI group (MLH1/BRAF+), and 5 cm[3,5,6] in the group MSS group. There was a statistically significant difference between the MSI group and the MSS group (p=0.003).

Our findings show that there are no statistically significant differences between patients with MSI (MLH1/BRAF+), MSI and MSS tumours in terms of TNM staging, T or N stage. However, there are differences in terms of LNR values, which were higher in the MSS group.

The 5-year survival of patients with MSI tumours (MLH1/BRAF+) was 83.3%, where 15 out of 18 patients survived, and recurrence rate was 5.6%, 1 patient in 18 presenting distant recurrence. The patients in the MSI group had a survival rate of 56.2%, 9 out of 16 patients survived 5 years after surgery, and recurrence rate was 37.5%, 6 out of 16 patients had recurrence, of which 3 had distant recurrence and 3 local recurrence. When comparing survival and recurrence rates in these two groups of patients, there was a significant statistical difference in terms of recurrence (p=0.035, Fisher test). The patients in the MSI (A1) group were more likely to have recurrent disease in 5 years than those in the MSI (MLH1/BRAF+) group (A2) (6 (37.5%) vs. 1 (5.6%) (p=0.03) (Fig. 1).

In the group of patients with microsatellite stable colorectal tumors, the 5-year survival rate was 63.8% (44/69) and recurrence rate was 40.6% (28/69).

There were no statistically significant differences in survival (p=0.58) or recurrence (p=1) when comparing survival and recurrence rates between patients in the MSI group and those with microsatellite stable tumours. On the other hand, there was a statistically significant difference in terms of recurrence (Fisher exact test, p=0.004) between the MSI (MLH1/BRAF+) group and the MSS group, but no statistically significant difference in terms of survival (p=0.1). Recurrence after 5 years was more likely to occur in patients in the MSS group than in those in the MSI (MLH1/BRAF+) group (28 (40.6) vs. 1 (5.6)) (p=0.01).

A thorough analysis of the group of patients with MSI tumours showed that in 9 out of 16 cases the PMS2 gene stood out as the only one affected. In these cases, survival rate was 44.4%, with 4 out of 9 patient dying, and recurrence rate was 55.5%, where 33.3% distant recurrence and 22.2% local recurrence, with no statistically significant differences compared with the other tumours, both in terms of 5-year survival (p=0.35), and recurrence (p=0.14). In two cases, the affected PMS2 gene was accompanied by MLH1 gene defect and in these situations survival was 50%.

Discussions

The data in our study show that following immunohistochemistry aimed at identifying the absence of MMR gene expression (MLH1, MSH2, MSH6, PMS2), a number of 34 patients revealed absent expression, indicating microsatellite instability.

MLH1 gene expression was absent in 23 patients, of which 18 had BRAF gene mutations, where loss of MLH1 gene expression occurred through promoter hypermethylation resulting in spontaneous MMR system impairment. Data from the literature show that 15-20% of colorectal cancers result from this mechanism [1].

In the present study, the involvement of MLH1 and MSH2 genes was low compared to data in the literature, where values go up to 40% [3]. We identified 9 patients with the absence of PMS2 gene expression, a gene whose involvement was significant in our study, whereas data in the literature show that the involvement of this gene occurs in 5-10% of cases [3]. In Romania, there are no genetic studies on extensive groups of patients with colorectal cancer regarding the clinical expression of MMR gene mutations. Studies in the literature refer to population groups such as the US [3], Japan 7, Norway [15] and Iran [16]. According to our study, the number of patients with mutations in MLH1 and MLH2 genes was low, and patients with mutations in PMS2 gene were in greater numbers, which may be a characteristic of the population studied.

According to data in our study, in groups of patients with MSI tumours (MLH1/BRAF+) the predominant location was in the colon (88.9%), namely on the right side of the colon, which explains the predominant symptom - pain. In patients with MSI tumours (MLH1/BRAF+) there

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was no case of lower rectal location and differences were statistically significant.
MSI tumours had larger long-axis colonic dimensions than other cases, and MSI tumours (MLH1/BRAF+) were mainly vegetant, probably due to their prevalent location on the right side of the colon.
Our findings show that there are no statistically significant differences between patients with MSI (MLH1/BRAF+), MSI and MSS tumours in terms of TNM staging, T or N stage. However, there are differences between LNR values, which were higher in MSS tumours.
Although there are studies in the literature showing the importance of the inflammatory response in colorectal cancer in general and in colorectal tumors with microsatellite instability in particular [1,4,17,18], our findings do not confirm the data in the literature regarding inflammation in tumours displaying microsatellite instability. Cellular components that make up the general inflammatory system (leukocytes, neutrophils, lymphocytes, neutrophil-to-lymphocyte ratio, platelets) did not differ significantly, and Klintrup score assessing local inflammatory response also showed no statistically significant differences between the groups of patients with MSI (MLH1/BRAF+), MSI and MSS tumours.
The results of the present study show high 5-year survival rates and low recurrence rates in MSI (MLH1/BRAF+) tumours, while in MSI tumours survival rates were lower and recurrence rates were statistically significantly higher. The patients in the MSI group were more likely to develop recurrence after 5 years than those in the MSI (MLH1/BRAF+) group. The explanation could be that MSI tumours might be more aggressive or that these types of tumours do not respond to adjuvant therapy. There are no studies in the literature separately determining the phenotypic characteristics and the clinical and therapeutic implications of MSI (MLH1/BRAF+) and MSI tumours, the only differences being recorded between tumours displaying general microsatellite instability and microsatellite stable tumours.
Literature data on the response of colorectal tumours with MSI to therapy with 5FU are still contradictory (19,20,21). In our study, only stage III patients received chemotherapy. On the one hand, we observed that MSI tumours were discovered in advanced stages, and on the other hand we found that MSI (MLH1/BRAF+) tumours responded better to chemotherapy than MSI tumours.
Patients with MSI (MLH1/BRAF+) tumours had better 5-year survival rates and recurrence rates than patients with microsatellite stable tumours, which correlates with literature data showing that MSI tumours have a better prognosis compared to MSS tumours [3,22].
In our study, patients who had mutations in the PMS2 gene had lower survival rates and higher recurrence rates, especially for distant recurrence, compared to other patients.
In patients where the genetic defect was in the MLH1 gene, without any association with PMS2, the 5-years survival rate was higher. Literature data on MSI tumours in general, show better prognosis in tumours where MLH1 gene expression is absent, but also including colorectal tumours where MLH1 gene defect occurs via hypermethylation [23].
A limitation of our study is the small number of patients involved, due to the short time period and extensive exclusion criteria. Also, we could not have the opportunity to conduct further genetic testing. However, to the best of our knowledge, this is the first evaluation of the prognostic role of different phenotypic and clinic pathological characteristics between microsatellite unstable colorectal cancer occurring as a result of different mechanisms (hypermethylation vs genetic mutations) and microsatellite stable tumours.

Conclusions

Our results show that there are differences in certain phenotypic and clinic pathological criteria that have a prognostic role, and between survival, and recurrence in patients with tumours with microsatellite instability triggered by different mechanisms and microsatellite stable tumours. Patients with microsatellite instability should be further investigated by means of genetic testing. The identification of patients with colorectal tumours with microsatellite instability is an important molecular marker that can help identify new subgroups of patients with cancers that have different clinical, pathological and molecular characteristics, requiring the use of individualized therapies, representing one more step towards personalized therapy for colorectal cancer.

Riassunto

In letteratura i dati riferiti al ruolo prognostico del sistema DNA di riparazione nel cancro del colon-retto sono tutt'ora controversi.
Lo scopo dello studio era quello di identificare il ruolo prognostico dell'adenopatia micro satellitare, con riferimento alle caratteristiche fenotipiche, alle caratteristiche cliniche e patologiche ed alla loro correlazione con il tasso di sopravvivenza e di intervallo libero da malattia su pazienti affetti da cancro colorettale.
Si è trattato di uno studio retrospettivo comprendente 103 pazienti sottoposti ad intervento chirurgico curativo per cancro colo-rettale.
I test di immunoistochimica hanno rilevato la presenza dei geni MLH1, MLH2, MLH6, PMS2, nonché una mutazione al livello del gene BRAF.
In base a queste determinazioni, sono stati individuati tre gruppi di pazienti: Gruppo A: pazienti sofferenti di cancro colorettale con instabilità micro satellitare, pro-

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dotto dall'ipermetilazione (MLH1/BRAF+); Gruppo B: pazienti sofferenti di tumori con instabilità micro satellitare. In base ai risultati ottenuti, abbiamo elaborato una banca dati che contiene informazioni cliniche, di laboratorio e di anatomia patologica. I pazienti sono stati sorvegliati per cinque anni postoperatorio.
Lo studio ha rilevato il fatto che i tumori con instabilità micro satellitare (MLH1/BRAF+) si manifestano più frequentemente al sesso femminile (p=0,05) ed a livello del colon destro.
Il tasso di sopravvivenza a 5 anni è più elevato nei pazienti con tumori MSI (MLH1 / BRAF +) rispetto a quelli con tumori microsatelliti stabili, con differenze non statisticamente significative.
La frequenza di recidiva è stata superiore nei pazienti con tumori con instabilità micro satellitare - MSI - rispetto a quelli con tumori (MLH1 / BRAF +) (p = 0,03) o con tumori con stabilità micro satellitare MSS (p = 0,004).
In conclusione l'identificazione dei tumori del colon-retto micro satellite instabile è un importante marcatore molecolare per classificare i pazienti in diversi sottogruppi fenotipici. A loro turno, l'importanza clinica di questi è stata anche dal punto di vista della possibilità di valutare la sopravvivenza e il rischio di recidiva.

References

1. Kloor M, Staffa L, Ahadova A, et al.: *Clinical significance of microsatellite instability in colorectal cancer*. Langenbecks Arch Surg. 2014; 399(1):23-31. doi: 10.1007/s00423-013-1112-3.

2. Gologan A, Sepulveda AR: *Microsatellite instability and DNA mismatch repair deficiency testing in hereditary and sporadic gastrointestinal cancers*. Clin Lab Med. 2005; 25(1):179-96. doi: 10.1016/j.cll.2004.12.001.

3. Boland CR, Goel A: *Microsatellite instability in colorectal cancer*. Gastroenterology. 2010; 138(6):2073-2087. doi: 10.1053/j.gastro.2009.12.064

4. Copija A, Waniczek D, Witko A, Walkiewicz K, Nowakowska-Zajdel E: *Clinical significance and prognostic relevance of microsatellite instability in sporadic colorectal cancer patients*. Int J Mol Sci. 2017; 6;1 8(1). doi: 10.3390/ijms18010107.

5. Stephen BE, Byrd DR, Compton CC, et al.: *Part III Digestive system: Colon and Rectum.* In Anonymous AJCC Cancer Staging Manual, 2010, 7th edition. New York, Springer, 2010; 173-206

6. Leitch EF, Chakrabarti M, Crozier JE, et al.: *Comparison of the prognostic value of selected markers of the systemic inflammatory response in patients with colorectal cancer.* Br J Cancer, 2007; 97(9):1266-70. doi: 10.1038/sj.bjc.6604027.

7. Sasaki K, Kawai K, Tsuno NH, et al.: *Impact of preoperative thrombocytosis on the survival of patients with primary colorectal cancer*. World J Surg, 2012; 36(1):192-200. doi: 10.1007/s00268-011-1329-7.

8. Petersen VC, Baxter KJ, Love SB, et al.: *Identification of objective pathological prognostic determinants and models of prognosis in Dukes' B colon cancer*. Gut, 2002; 51(1):65-9.

9. Klintrup K, Mäkinen JM, Kauppila S, et al.: *Inflammation and prognosis in colorectal cancer*. Eur J Cancer. 2005; 41(17):2645-54. doi: 10.1016/j.ejca.2005.07.017.

10 Pollheimer MJ, Kornprat P, Lindtner RA, et al.: *Tumour necrosis is a new promising prognostic factor in colorectal cancer.* Hum Pathol. 2010; 41(12):1749-57. doi: 10.1016/j.humpath.2010.04.018.

11. Richards CH, Roxburgh CS, Anderson JH, et al.: *Prognostic value of tumour necrosis and host inflammatory responses in colorectal cancer.* Br J Surg, 2012; 99(2):287-94. doi: 10.1002/bjs.7755.

12. Kelly N, Samaha G, Duff G, et al.: *An appraisal of lymph node ratio in colon and rectal cancer: Not one size fits all*. Int J Colorectal Dis 2013; 28: 1377-1384. doi: 10.1007/s00384-013-1707-8.

13. Chen SL, Steele SR, Eberhardt J, et al.: *Lymph node ratio as a quality and prognostic indicator in stage III colon cancer*. Ann Surg 2011; 253: 82-87. doi: 10.1097/SLA.0b013e3181ffa780.

14. Jensen SA, Vainer B, Kruhøffer M, Sørensen JB: *Microsatellite instability in colorectal cancer and association with thymidylate synthase and dihydropyrimidine dehydrogenase expression*. BMC Cancer. 2009; 9:25. doi: 10.1186/1471-2407-9-25.

15. Søreide K: *High-fidelity of five quasimonomorphic mononucleotide repeats to high-frequency microsatellite instability distribution in early-stage adenocarcinoma of the colon*. Anticancer Res, 2011;31(3):967-71

16. Moghbeli M, Moaven O, Dadkhah E, et al.: *High frequency of microsatellite instability in sporadic colorectal cancer patients in Iran.* Genet Mol Res. 2011; 10(4):3520-9. doi: 10.4238/2011.

17. Carethers JM: *Hereditary, sporadic and metastatic colorectal cancer are commonly driven by specific spectrums of defective DNA mismatch repair components*. Trans Am Clin Climatol Assoc. 2016; 127:81-97.

18. Cohen R, Svrcek M, Dreyer C, Cervera P, Duval A, Pocard M, Fléjou JF, de Gramont A, André T: *New Therapeutic Opportunities Based on DNA Mismatch Repair and BRAF Status in Metastatic Colorectal Cancer*. Curr Oncol Rep. 2016;18(3):18. doi: 10.1007/s11912-016-0504-2.

19. Hemminki A, Mecklin JP, Järvinen H, Aaltonen LA, Joensuu H: *Microsatellite instability is a favorable prognostic indicator in patients with colorectal cancer receiving chemotherapy*. Gastroenterology 2000; 119(4):921-28.

20. Thomas ML, Hewett PJ, Ruszkiewicz AR, Moore JW: *Clinicopathological predictors of benefit from adjuvant chemotherapy for stage C colorectal cancer: Microsatellite unstable cases benefit*. Asia Pac J Clin Oncol, 2015; 11(4):343-51.doi: 10.1111/ajco.12411.

21. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, et al.: *Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer*. J Clin Oncol. 2010; 28(20):3219-26. doi: 10.1200/JCO.2009.27.1825.

22. Guastadisegni C, Colafranceschi M, Ottini L, Dogliotti E: *Microsatellite instability as a marker of prognosis and response to therapy: a meta-analysis of colorectal cancer survival data*. Eur J Cancer. 2010; 46(15):2788-98. doi: 10.1016/j.ejca.2010.05.009.

23. Sinicrope FA, Sargent DJ: *Molecular pathways: Microsatellite instability in colorectal cancer: prognostic, predictive, and therapeutic implications*. Clin Cancer Res. 2012; 18(6):1506-12. doi: 10.1158/1078-0432.CCR-11-1469.

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