



Original article

Colorectal cancer with microsatellite instability: Right-sided location and signet ring cell histology are associated with nodal metastases, and extranodal extension influences disease-free survival

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ABSTRACT

Colorectal cancer (CRC) with microsatellite instability (MSI) accounts for 15–18 % of all CRCs and represents the category with the best prognosis. This study aimed at determining any possible clinical/pathological features associated with a higher risk of nodal metastasization in MSI-CRC, and at defining any possible prognostic moderators in this setting. All surgically resected CRCs of the last 20 years (mono-institutional series) with a PCR-based diagnosis of MSI, with and without nodal metastasis, have been retrieved for histological review, which was performed following WHO guidelines. Furthermore, the most important prognostic moderators have been investigated with a survival analysis. The study of 33 cases of MSI-CRCs with nodal metastasis highlighted a high fidelity of histology maintenance between primary tumors and matched nodal metastases. At survival analysis, the strongest prognostic variable in MSI-CRCs with nodal metastasis was the extranodal extension (multivariate analysis, HR: 14.4, 95 %CI: 1.46–140.9, $p = 0.022$). Furthermore, through a comparison between nodal positive (33 cases) and nodal negative (71 cases) MSI-CRCs, right-sided location ($p < 0.0001$), pT4 stage ($p = 0.0004$) and signet-ring histology ($p = 0.0089$) emerged as parameters more commonly associated with nodal metastasization. These findings shed new light on the biology of MSI-CRC and can be of help for the prognostic stratification of MSI-CRC patients.

1. Introduction

Colorectal cancer (CRC) has been recently indicated to be the third most common cancer worldwide and the second leading cause of cancer-

related deaths, accounting for 9.2 % of all cancer's deaths [1,2]. CRC is a heterogeneous tumor category and includes tumors with different biological features and prognosis. Recent molecular classifications of CRC have subdivided it into different subgroups, each with clinical correlates

Abbreviations: CRC, colorectal cancer; MSI, microsatellite instability; PCR, polymerase chain reaction; HR, hazard ratio; IHC, immunohistochemistry; NOS, conventional adenocarcinoma, not otherwise specified; MUC, mucinous adenocarcinoma; MA, adenocarcinoma with mucinous aspects; MED, medullary carcinoma; SRC, signet ring cell adenocarcinoma; SRA, adenocarcinoma with signet ring cell aspects; TNM, tumor nodes metastasis staging system; AJCC, American Joint Committee on Cancer; ENE, extranodal extension of nodal metastasis.

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[3,4]. Notably, a specific subset of CRCs, representing about 15–18 % of all cases, shows the best prognosis [3–6]. This subgroup is represented by tumors with the functional impairment of genes involved in DNA Mismatch Repair System (MMR) [7]. As a consequence of a defective MMR (dMMR), such neoplasms typically accumulate mutations that are most frequently located in monomorphic microsatellites, and are thereby defined as having microsatellite instability (MSI) [7–9].

This genetic asset has important prognostic consequences, but presents also relevant predictive implications. On one hand, it is well established that MSI-CRCs cannot receive any benefit from adjuvant therapy with 5-fluorouracil [10,11]. On the other hand, MSI-CRCs have demonstrated a high-rate of response to anti-PD1 immunotherapy, with prolonged survival indexes also in the case of metastatic disease [12]. Notably, some particular features have been described as more common in MSI-CRCs. First, they are more frequently right sided and occur in women with higher frequency [13–15]. Histologically, MSI-CRCs show an increased number of intraepithelial lymphocytes and display more often mucinous, signet-ring and medullary features [16–20].

Although MSI-CRC appears as a distinctive category with unique biological features, also with important clinic-pathologic implications, little is known about MSI-CRCs with concurrent lymph node metastasis. This study aims at evaluating the histological features of primary tumors and matched nodal metastases of patients with MSI-CRCs, investigating also potential prognostic moderators within this tumor entity. Furthermore, through a comparison between nodal positive and nodal negative MSI-CRCs, we aim at defining any possible clinic-pathologic features associated with a higher risk of nodal metastasization in this tumor setting.

2. Materials and methods

This study has been approved by the local Ethical Committee (project name: MSI-CRC, number of approval: 2327/CESC, date of approval: 09/11/2019), and has been conducted in accordance with the Good Practice guidelines, the Declaration of Helsinki and current laws, ethics and regulations. For identifying all potential MSI-CRCs with concurrent nodal metastasis, all surgically resected cases with a PCR-based diagnosis of MSI-CRC in the last 20 years have been retrieved from the archive of ARC-Net biobank of Verona University Hospital. Only cases for which all tumor slides were available, were selected for this study. The selection of MSI-CRC cases was based on the presence of MSI-PCR indicative for MSI/dMMR.

MSI/dMMR assessment was established on MSI-based PCR through the analysis of mononucleotide repeats. After DNA extraction using a column-based purification kit (DNeasy Blood & Tissue kit; Qiagen, Valencia, CA) and DNA quantification by spectrophotometric and fluorometric analysis by Qubit DNA HS Assay kit (ThermoFisher) and NanoDrop ND-2000, the amplification of the mononucleotide microsatellites BAT25, BAT26, NR-21, NR-24 and NR-27 was performed using HNPCC KIT 1-FL kit (Experteam s.r.l.), followed by capillary-gel electrophoresis, using ABI Genetic Analyzer 3130XL platform (Applied Biosystems Inc., Foster City, CA). A case was considered microsatellite instable if two or more mononucleotide markers show repeats length alteration [7,21].

BRAF mutation status was assessed in the cohort of nodal positive MSI-CRC, using immunohistochemistry (IHC) as already described [22, 23]. Briefly, IHC was performed on a BenchMark XT automated slide stainer, (Ventana), using an already validated clone (VE1, Medical System, AZ, USA). IHC results were further validated by PCR in a cohort of 10 cases, randomly selected.

The histological analysis was performed following WHO 2019 guidelines [24], focusing in particular on CRCs variants typically associated with MSI: i) mucinous adenocarcinoma (MUC) was considered in cases of > 50 % of lesion composed of pools of extracellular mucin containing overt malignant epithelium; ii) adenocarcinoma with mucinous aspects (MA) was used if < 50 % of lesion showed mucinous

Table 1

Baseline Characteristics of the cohort of nodal-positive MSI-CRC.

Variables	Number of Nodal-positive Patients (%)	Number of Nodal-negative Patients (%)
Age		
≤ 65 years	11 (33.3 %)	21 (29.6 %)
> 65 years	22 (66.7 %)	50 (70.4 %)
Gender		
Male	13 (39.4 %)	40 (56.3 %)
Female	20 (60.6 %)	31 (43.7 %)
Anatomic Location		
Ascending/Transverse Colon	28 (84.9 %)	22 (30.9 %)
Descending/Sigmoid Colon	5 (15.1 %)	49 (69.1 %)
AJCC pT-stage		
T1–3	19 (57.6 %)	64 (90.1 %)
T4	14 (42.4 %)	7 (9.9 %)
AJCC N-stage		
N0	0 (0%)	71 (100 %)
N1	23 (69.7 %)	0 (0%)
N2	10 (30.3 %)	0 (0%)
<i>BRAF</i> status		
Wild-type	19 (57.6 %)	NA
Mutated	14 (42.4 %)	NA
Tumor Grade		
Low	14 (42.4 %)	43 (60.6 %)
High	19 (57.6 %)	28 (39.4 %)
Vascular Invasion		
No	6 (18.2 %)	50 (70.4 %)
Yes	27 (81.8 %)	21 (29.6 %)
Peri-neural Invasion		
No	14 (42.4 %)	62 (87.3 %)
Yes	19 (57.6 %)	9 (12.7 %)
Tumor Budding		
No	10 (30.3 %)	24 (33.8 %)
Yes	23 (69.7 %)	47 (66.2 %)
Mucinous		
Adenocarcinoma		
No	26 (78.8 %)	NA
Yes	7 (21.2 %)	NA
Histology		
NOS	12 (36.3 %)	39 (54.9 %)
MUC	7 (21.2 %)	8 (11.2 %)
MA	7 (21.2 %)	23 (32.4 %)
SR	4 (12.1 %)	0 (0%)
MED	3 (9.1 %)	1 (1.4 %)
Extra Nodal Extension		
No	22 (66.7 %)	NA
Yes	11 (33.3 %)	NA

Abbreviations: AJCC: American Joint Committee on Cancer; NOS: conventional adenocarcinoma, not otherwise specified; MUC: mucinous adenocarcinoma; MA: adenocarcinoma with mucinous aspects, SR: signet-ring cell carcinoma; MED: medullary carcinoma.

features; iii) medullary carcinoma (MED) was considered in cases of tumors characterized by sheets of malignant cells with vesicular nuclei, evident nucleoli, prominent inflammatory cell infiltration and syncytial growth pattern; iv) signet ring cell adenocarcinoma (SRC) was considered if > 50 % of tumor cells showed prominent intracytoplasmic mucin with a typical displacement and molding of the nucleus; v) adenocarcinoma with signet ring cell aspects (SRA) was used if < 50 % of cells showed signet-ring features.

Other histological variables, such as vascular invasion, perineural invasion and tumor budding were assessed by morphology and using existing guidelines [24,25]. In the statistical analysis, we reported such data as dichotomous variables (present vs. absent): for tumor budding we consider as “present” only cases with “high-grade” tumor budding. Pathological TNM was assessed adopting the 8th edition of the AJCC cancer staging system [26]. At last, extranodal extension of nodal metastasis (ENE) was assessed using a strict and specific definition, as already discussed and presented [27–31]. ENE is indeed the extension of tumor cells through the nodal capsule into the perinodal fatty tissue; it

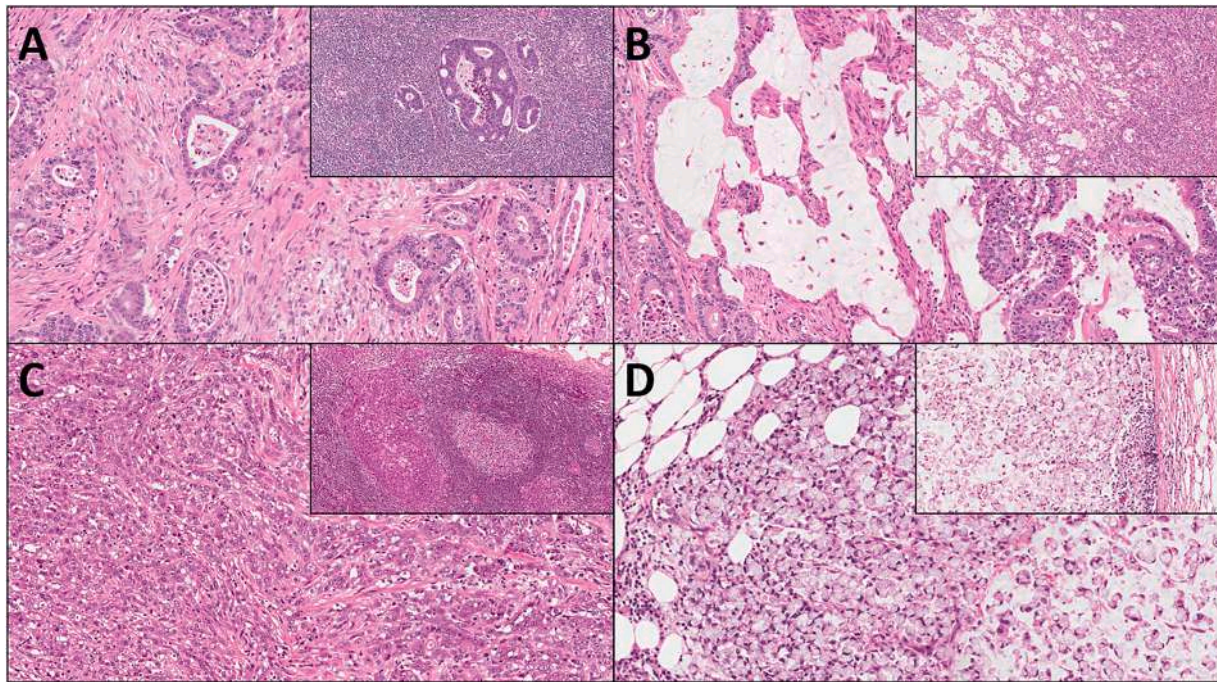


Fig. 1. Summarizing figure of the different types of histology of colorectal cancer, with associated nodal metastasis. The main image represents the primary tumor, while the small box shows the histology in the metastatic lymph node (original magnification 20x). A: primary conventional colorectal adenocarcinoma; small box: the same morphology is maintained in this metastatic lymph node; B: primary mucinous colorectal adenocarcinoma; small box: the same morphology is maintained in this metastatic lymph node; C: primary medullary colorectal adenocarcinoma; small box: the same morphology is maintained in this metastatic lymph node; D: primary signet-ring cell colorectal adenocarcinoma; small box: the same morphology is maintained in this metastatic lymph node.

has to be demonstrated histologically, documenting a structural rupture of the lymph node capsule by the metastasis. Neoplastic emboli even outside the lymph node, as well as metastasis in the marginal sinus or free tumor cells deposits in soft tissue have not be considered as true ENE, as already established [27].

For investigating the presence of any possible parameters associated to nodal metastasization, we collected from the same archive and for histological examination all available cases of MSI-CRC without nodal metastasis. To this aim, only MSI-CRCs without nodal metastasis and with complete data and tissue slides were retrieved.

For survival analysis, the clinical history of patients with MSI-CRC was recorded, obtaining data regarding disease-free survival, with presence and location of eventual relapses. Univariate and multivariate analyses have been used to investigate the prognostic significance of histological parameters, using STATA, version 12.0 (StataCorp LP, College Station, TX, USA).

3. Results

Among 1928 surgically resected CRCs, 147 (12.8 %) showed MSI and 33 (22.4 % of MSI-CRC) had at least one nodal metastasis.

Table 1 (left column) summarizes the most relevant clinical pathological features of the 33 MSI-CRC patients with nodal metastasis. It was composed of 13 male and 20 female patients, with an age at the time of the resection ranging from 39 to 91 years (mean: 67.5; median: 70). The vast majority of tumors (84.9 %) were right-sided, with only a minority of them involving the distal tracts. Regarding TNM classification, there was a similar distribution of cases between early and late T stages (T1–3 57.6 % and T4 42.4 % of cases); N1 cases, namely tumors with 1–3 metastatic lymph nodes, represented about 2/3 of N category, with the remaining 1/3 that was N2 (> 3 nodal metastases). Regarding *BRAF*, a control/validation of IHC results with PCR was performed on ten cases, randomly selected; it showed a perfect correspondence between IHC and *BRAF* mutational status, supporting the use of IHC as a surrogate of

BRAF mutational status also in this series. The majority of cases (19/33, 57.6 %) were thus considered as *BRAF* wild type, whereas 14/33 (42.4 %) showed a “mutated” pattern (paradigmatic images are reported in Supplementary Fig. 1). Moreover, the majority of cases was represented by high-grade CRC, and there was a sharp prevalence of histological features associated with an aggressive biological behavior, such as vascular invasion (81.8 % of cases), peri-neural invasion (57.6 %) and tumor budding (69.7 %). Extranodal extension of nodal metastasis was present in 1 out of 3 of cases.

Regarding the histological classification of primary tumors and matched nodal metastases, 12 primary MSI-CRC were conventional adenocarcinoma, not otherwise specified. All nodal metastases of these 12 tumors maintained the morphology of the primary, without any peculiar pattern of differentiation (Fig. 1A). Regarding mucinous differentiation, 7 cases were MUC and other 7 cases presented MA. Among the 7 MUC MSI-CRC, 5 presented mucinous histology also in their metastatic lymph nodes (Fig. 1B), whereas in 2 cases the nodal metastases presented conventional histology. In 2 of the MUC MSI-CRC there were 2 nodal metastases: in both cases one showed mucinous histology, and the other a conventional histology. Among the 7 MA MSI-CRC, 4 presented mucinous histology also in their metastatic lymph nodes, whereas in 3 cases the nodal metastases presented conventional histology. Three MSI-CRC displayed MED histology: 2/3 cases maintained MED histology also in the metastatic lymph node (Fig. 1C), whereas 1/3 case showed conventional histology in the nodal metastasis. Four MSI-CRC, at last, showed SRC morphology: three cases displayed the same morphology in the metastatic nodes (Fig. 1D), whereas 1 case present mucinous histology in the majority of nodal metastases (7/9 metastatic nodes) and conventional histology in the remaining (2/9 metastatic nodes). All these results indicate that there is a high fidelity of histology between primary tumors and nodal metastasis: 12/12 (100 %) conventional CRC, 5/7 (72 %) MUC, 4/7 (57 %) MA, 2/3 (66 %) MED and 3/4 (75 %) SRC, with only a minority of cases showing a discrepancy along this line.

Table 2
Univariate Survival Analysis.

Variables	3-year DFS (95 %CI)	p-value
Age		0.50
≤ 65 years	78.5 % (38.1–94.3)	
> 65 years	74.1 % (43.1–89.9)	
Gender		0.45
Male	78.5 % (36.1–94.5)	
Female	74.5 % (45.3–89.6)	
Anatomic Location		0.75
Ascending/Transverse Colon	75.0 % (49.0–89.1)	
Descending/Sigmoid Colon	80.0 % (20.4–96.9)	
AJCC T-stage		0.30
T1–3	84.0 % (48.7–95.9)	
T4	68.4 % (35.9–86.8)	
AJCC N-stage		<0.001
N1	92.8 % (59.1–98.9)	
N2	37.5 % (8.7–67.4)	
BRAF status		0.61
Wild-type	72.7 % (44.5–88.2)	
Mutated	76.9 % (44.2–91.9)	
Tumor Grade		0.88
Low	75.7 % (30.5–93.7)	
High	76.0 % (47.9–90.3)	
Vascular Invasion		0.46
No	100 %	
Yes	70.9 % (45.7–86.1)	
Peri-neural Invasion		0.037
No	100 %	
Yes	60.0 % (31.3–79.9)	
Tumor Budding		0.10
No	100 %	
Yes	64.6 % (35.6–83.1)	
Mucinous Adenocarcinoma		0.14
No	70.8 % (45.8–85.8)	
Yes	100 %	
Extra Nodal Extension		<0.001
No	94.7 % (68.1–99.2)	
Yes	33.3 % (7.8–62.3)	

Abbreviations: AJCC: American Joint Committee on Cancer. Note: total number of patients for survival analysis (disease-free survival): 28 (see also Fig. 2).

Table 3
Multivariate Survival Analysis.

Variables	Hazard ratio (95 % CI)	p-value
Tumor Budding (No vs Yes)	3.95 (0.1–187.3)	0.48
Perineural invasion (No vs Yes)	4.89 (0.14–163.4)	0.38
AJCC N-stage (N1 vs N2)	2.08 (0.34–12.5)	0.42
Extra Nodal Extension (No vs Yes)	14.4 (1.46–140.9)	0.022

Abbreviations: AJCC: American Joint Committee on Cancer.

For investigating the presence of any possible clinic-pathologic parameter associated to nodal metastases in MSI-CRC, we collected and analyzed the histological parameters of cases of MSI-CRC without nodal metastasis. In particular, 71 MSI-CRC without nodal metastasis were available to this aim; the main histological parameters of this cohort have been summarized in Table 1 (right column). We compared the prevalence of these parameters between the cohorts of MSI-CRC with nodal metastasis and MSI-CRC without nodal metastasis, founding some statistically significant differences (Fisher's exact test was used to this aim). Indeed, nodal positive MSI-CRC, compared with nodal negative MSI-CRC, were more often right-sided ($p < 0.0001$) and have a higher pT stage ($p = 0.0004$). Looking at histology, the morphological difference with a statistical significance regarded signet ring cell carcinoma, which were more often represented in nodal positive MSI-CRC ($p = 0.0089$).

Disease-free survival and related data were available for 28/33 nodal-positive MSI-CRC patients. Of them, 7 displayed a disease relapse, as follows: three patients in distant (abdominal, non-locoregional) lymph nodes, two patients in the liver and other two patients in the

peritoneum. For the survival analysis, all potential clinic-pathological variables have been studied with univariate analysis (Table 2). Then, a multivariable model was constructed using the variables with a p value of 0.10 or less in the univariate analysis (Table 3). The only variable that maintained its significance also in the multivariable model was ENE, demonstrating a strong prognostic value in this setting (HR: 14.4, 95 % CI: 1.46–140.9, $p = 0.022$). The prognostic role of ENE was also highlighted with a Kaplan-Meier curve (Fig. 2). Given this prognostic significance, we further examined if ENE might be associated to particular morphological aspects, founding that it was more commonly associated to perineural invasion ($p = 0.046$) and to mucinous histology ($p = 0.035$) (Table 4).

4. Discussion

In this study we first analyzed the histological aspects of a cohort of 33 MSI-CRCs with nodal metastasis. We found that there is a high fidelity of histology maintenance between primary tumors and matched nodal metastases. Furthermore, through a comparison between MSI-CRCs with vs. without nodal metastasis, we found that right-sided location ($p < 0.0001$), pT4 stage ($p = 0.0004$) and signet ring histology ($p = 0.0089$) were more common in the case of nodal metastasization. In survival analysis, ENE, which was strongly associated with perineural invasion ($p = 0.046$) and mucinous histology ($p = 0.035$), emerged as the strongest prognostic variable in MSI-CRCs with nodal metastasis (multivariate analysis, HR: 14.4, 95 %CI: 1.46–140.9, $p = 0.022$).

The histological correlation between primary tumors and matched metastasis that we found in our cohort was very high: overall, about 80 % of primary tumors reflected their histological aspects in matched nodal metastases. This indicates that the main histology of primary tumors is the most important determinant also of the histology of nodal metastases, suggesting that there is no a peculiar morphologic sub-clone with a higher propensity for metastasization. Regarding MSI-CRC histology, we found different histotypes that usually are very rare in conventional CRC, as for example MUC, MED and SR. This is in line with the already known morphological features typically present in the general category of MSI-CRC [15–18,24], and is confirmed also in cases of MSI-CRC with nodal metastases. The presence of peculiar histological variants that are enriched in MSI-tumors highlights the important role of pathologists in selecting cases for MSI assessment. This may be crucial not only in colorectal cancers but also in other tumor types, where specific morphological variants have been reported as more typical in the case of MSI, as for example MED and mucinous/colloid histology in pancreatic cancer [32,33]. Interestingly, SR morphology emerged as a morphological variant more commonly present in the case of nodal metastasis. This result confirms the biological aggressiveness of the signet ring cell variant in CRC [31], but at the same time represents a new finding in the specific subset of MSI-CRC. Notably, also pT4 stage and the right-sided location resulted as distinctive parameters of MSI-CRC with nodal metastasis. Thus, within the MSI-CRC group, our findings delineated a category of tumors with a higher risk of nodal metastasis: they are characterized by right-sided location, SR histology and pT4 stage.

An interesting finding regarded also the prognostic significance of ENE in MSI-CRC with nodal metastasis. Our results are in line with recent evidences that pointed out its strong clinical value in CRC [27, 34–36] as well as in other types of solid malignancies [28–30,37–42]. However, the prognostic importance of ENE in nodal positive MSI-CRC represents a new finding and calls for further considerations regarding this histologic features. First of all, knowing that ENE can be very focal in a metastatic lymph node, all the lymph nodes with their surrounding adipose tissue should be completely included, even if very large. According to current findings and due to its prognostic significance, ENE should be considered for prognostic stratification of nodal positive MSI-CRC patients, identifying a subgroup of subjects with a higher risk

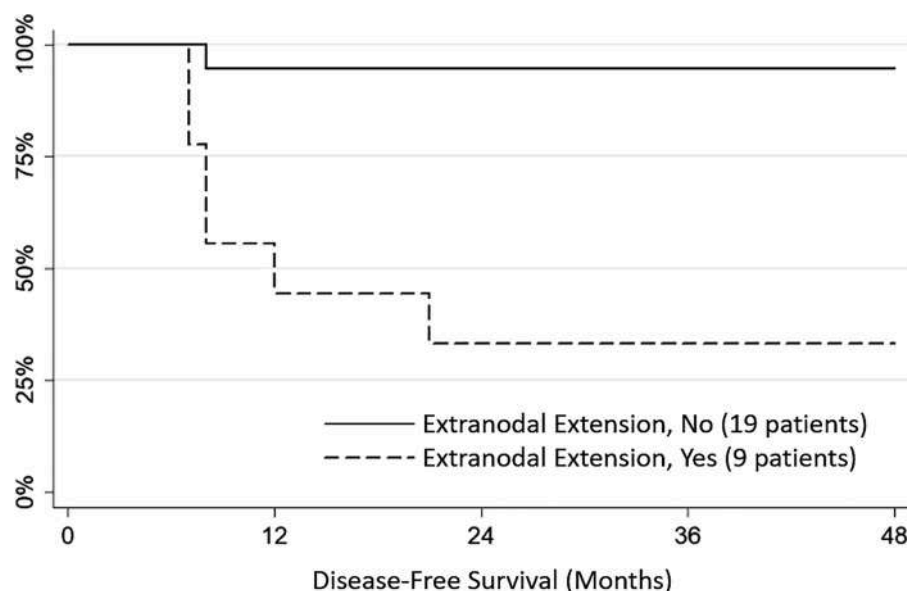


Fig. 2. Kaplan-Meier curve showing the adverse prognostic impact of extranodal extension within colorectal cancer with microsatellite instability and nodal metastasis.

Table 4

Comparison between baseline characteristics of extranodal extension vs. no extranodal extension disease.

Variables	No Extra Nodal Extension	Extra Nodal Extension	p-value
Number of Patients	22	11	–
Age			0.19
≤ 65 years	9 (40.9 %)	2 (18.2 %)	
> 65 years	13 (59.1 %)	9 (81.8 %)	
Gender			0.31
Male	12 (54.6 %)	8 (72.7 %)	
Female	10 (45.4 %)	3 (27.3 %)	
Anatomic Location			0.49
Ascending/Transverse Colon	18 (81.8 %)	10 (90.9 %)	
Descending/Sigmoid Colon	4 (18.2 %)	1 (9.1 %)	
AJCC T-stage			0.081
T1–3	15 (68.2 %)	4 (36.4 %)	
T4	7 (11.8 %)	7 (63.6 %)	
AJCC N-stage			0.003
N1	19 (86.4 %)	4 (36.4 %)	
N2	3 (13.6 %)	7 (63.6 %)	
BRAF status			0.32
Wild-type	14 (63.6 %)	5 (45.5 %)	
Mutated	8 (36.4 %)	6 (54.5 %)	
Tumor Grade			0.62
Low	10 (45.5 %)	4 (36.4 %)	
High	12 (54.5 %)	7 (63.6 %)	
Vascular Invasion			0.34
No	5 (22.7 %)	1 (9.1 %)	
Yes	17 (77.3 %)	10 (90.9 %)	
Peri-neural Invasion			0.046
No	12 (54.5 %)	2 (18.2 %)	
Yes	10 (45.5 %)	9 (81.8 %)	
Tumor Budding			0.061
No	9 (40.9 %)	1 (9.1 %)	
Yes	13 (59.1 %)	10 (90.9 %)	
Mucinous			0.035
Adenocarcinoma			
No	15 (68.2 %)	11 (100 %)	
Yes	7 (31.8 %)	0	

Abbreviations: AJCC: American Joint Committee on Cancer.

of relapse. A potential role of ENE in the management of patients with nodal positive MSI-CRC may be also investigated for therapeutic purposes. For example, in pancreatic cancer, Sergeant et al. demonstrated that ENE-positive patients had an improvement in the survival indexes in the case of adjuvant chemo-radiation, but not of chemotherapy alone [43]. At the same time, and in the setting of neo-adjuvant therapy, Depypere et al. reported ENE as the main moderator of survival in esophageal cancer after neo-adjuvant chemo-radiation [44]. Overall, these evidences highlighted that ENE might be of importance for designing specific therapeutic approaches in some gastrointestinal malignancies, and should be investigated along this line also in MSI-CRC. In our nodal-positive cohort, ENE was associated to mucinous histology and peri-neural invasion. These are new findings in MSI-CRC; metastatic lymph nodes of MSI-CRC and with this histologic features should be examined even more accurately, and entirely included during lymph node sampling. In the cohort of nodal positive cases, the fact that *BRAF* mutations were not clinically relevant is in line with data of current literature: in case of MSI, indeed, *BRAF* status appears not relevant in influencing the prognosis [4]. It is known that the use of IHC as a surrogate of *BRAF* mutational status may suffer from some discrepancies [45]; however, we validated this approach in our cohort showing a linear correspondence (100 % of concordance) between IHC and *BRAF* molecular (PCR) assessment in 10 cases, further supporting the reliability of the results. For clinical practice, cases with doubtful IHC pattern must be further investigated with molecular approaches.

Our study does have some limitations. First, the rarity of nodal metastasization in MSI-CRC has determined the relatively small sample size of nodal-positive MSI-CRC. Furthermore, the retrospective nature of the study may have affected the collection of cases. These limitations may have affected the power of statistical analyses. Notably, data on adjuvant treatment of node-positive cases are missing: although this specific sub-topic did not represent the main argument of this research, it should be taken into account by future investigations regarding this tumor setting. Lastly, specific data on other molecular alterations and of Lynch syndrome are lacking; however, these point may represent a relevant issue in the management of patients with colorectal cancer. Further studies should investigate in the future the possible role of other molecular alterations, including germline mutations, in influencing the process of nodal metastasization in a MSI/dMMR context.

Concluding, with this study we have demonstrated that the histology between primary MSI-CRC and matched nodal metastases is generally

maintained. Furthermore, in MSI-CRC, right-sided location, pT4 stage and signet ring histology are parameters strictly associated with nodal metastasis. At last, ENE emerged as the strongest prognostic variable in MSI-CRCs with nodal metastasis. These findings shed new light on the biology of MSI-CRC, also with potential clinical implications.

Authors contribution

CL: conceptualization; MLP, SA, CS, GT, AS, CP, CL: data curation; MLP, SA, CS, GT, FB, MF, AS, CP, CL; formal analysis; CL, FB: statistical analysis; all authors: data interpretation and overall discussion of the findings; AS, CP, CL: supervision of the project; MLP, SA, CS, GT, CP, AS, CL: writing - original draft; all authors: writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.prp.2021.153519>.

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