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Mismatch repair deficiency as prognostic factor for stage III small bowel adenocarcinoma: A multicentric international study ☆,☆☆

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ABSTRACT

Background: Small bowel adenocarcinoma (SBA) is a rare cancer with an aggressive behavior. No study has specifically addressed the putative prognostic role of mismatch repair status in stage III SBAs.
Aims: We aimed to investigate whether mismatch repair deficiency is associated with cancer-specific survival in a Western cohort of patients with stage III SBAs.
Methods: In this retrospective multicentric international cohort study, we enrolled 70 patients who underwent surgically resection for stage III SBAs and we analyzed the frequency of mismatch repair deficiency, tested by immunohistochemistry for mismatch repair proteins and by polymerase chain reaction for microsatellite instability, and its association with cancer-specific survival and other clinic-pathologic factors.
Results: We found sixteen (23%) patients with mismatch repair deficient adenocarcinoma, without discordance between immunohistochemical and polymerase chain reaction for microsatellite instability analyses. Mismatch repair deficiency proved to be associated with a better outcome both at univariable analysis (hazard ratio: 0.28, 95% confidence interval: 0.08–0.91, p: 0.035) and in bivariable models adjusted for patient age or gender, tumor site, pT4 stage, tumor budding, and perineural invasion.
Conclusion: This study highlights the importance of testing mismatch repair status to improve prognostic stratification in stage III SBAs.

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1. Introduction

Small bowel adenocarcinomas (SBAs) are rare neoplasms (about 3% of total gastrointestinal cancers); however, they account for 30–40% of all small intestinal tumors and their age-standardized incidence has increased in the United States and Europe in the last decades [1–4]. Due to their non-specific symptoms at presentation, as well as to the well-known difficulties in their detection by imaging techniques, they are mainly diagnosed in stage III (24–29%) or IV (33–36%), especially in Western countries [3,5].

American Joint Committee on Cancer (AJCC) TNM stage and, in particular, lymph node involvement are well-established prognostic factors for SBA patients. Stage III (pT1–4, pN1–2, M0) SBA patients have been shown to have a worse outcome, having a 5-year cancer-specific survival (CSS) of 40%, compared to stage II (pT3–4, pN0, M0) SBA patients (5-year CSS: 55%), and to stage III colorectal carcinomas (CRCs) (5-year CSS: 63%) [6].

The National Comprehensive Cancer Network (NCCN) guidelines recommend adjuvant chemotherapy after surgery for stage III SBAs [7]. However, no randomized study has evaluated the benefit of this approach. The French Intergroup Thésaurus National de Cancérologie Digestive (TCND) provides no recommendations for post-surgery management of stage III SBA patients [8]. Therefore, the increasing incidence and aggressive behavior of such cancers require further efforts to improve the knowledge of this orphan disease with limited therapeutic options beyond surgery. An international randomized phase III trial (NCT02502370) investigating the benefit of adjuvant chemotherapy for stage I-III SBAs (BALLAD) is currently on-going.

Up to now, several stage-independent prognostic factors for SBAs have been described and they include patient age at diagnosis, ethnicity, etiology/predisposing condition, tumor location, histologic grade and subtypes, perineural invasion, tumor-infiltrating lymphocyte (TIL) density, tumor budding (Tb), resection margins status, lymph node ratio (LNR), log odds of positive lymph nodes (LODDS) and total number of histologically evaluated lymph nodes [5,9–16].

Mismatch repair deficiency (dMMR)/microsatellite instability-high (MSI-H) status has been shown to be: (i) associated with an earlier disease stage and lower recurrence rates in SBAs; (ii) a positive prognostic factor in stage II SBAs; (iii) a predictive factor for response to pembrolizumab in metastatic (stage IV) or unresectable SBAs [13,17–21]. A few studies on SBAs in general have also suggested a favorable impact of this molecular alteration on overall survival and/or relapse-free survival of stage III SBA patients [9,10,13,22]. However, these studies show several limitations, being either conducted on single- or two-institution cohorts or exclusively based on an Asian (Korean) population, whose genetic differences from Caucasian people have been extensively characterized, as well as they did not analyze the impact of MMR status on CSS of stage III SBA patients. In the present investigation, we aimed to specifically analyze the association between dMMR status and CSS and between dMMR and clinico-pathologic and other prognostic factors in a multicentric cohort of Western patients with surgically resected stage III SBAs, in order to improve their prognostic stratification and guide clinical management.

2. Materials and methods

2.1. Study population

This retrospective study included 70 patients who underwent surgical resection for primary, non-ampullary stage III SBAs, retrieved from: (i) a larger population of 162 SBA patients enrolled from several tertiary referral Italian Centers participating in the Small Bowel Cancer Italian Consortium, (ii) datasets of Department of Gastroenterology and Hepatology at the University Hospitals of Leuven (Belgium), and (iii) databases of Division of Anatomic Pathology, Department of Laboratory Medicine and Pathology, Mayo Clinic (Rochester, Minnesota, USA). Stage IV disease at the time of diagnosis was clinically and radiologically excluded in all cases. Diagnosis of celiac disease was based on serology and the presence of typical duodenal histopathological lesions [23]. Diagnosis of Crohn's disease was ascertained according to international criteria [24]. Lynch syndrome was defined by the presence of a constitutional pathogenic mutation affecting a MMR gene reported in patient medical charts [25]. This study was approved by the Ethics Committee of Pavia (protocol number: 20,140,003,980).

2.2. Histology, immunohistochemistry and molecular analyses

A centralized histologic review of all tumors was performed for all the parameters required by current College of American Pathologists (CAP) cancer reporting protocols and by the eighth edition of the AJCC TNM staging system [16,26]. On the basis of pT and pN stages of the AJCC TNM staging system, stage III SBAs were further subdivided into a low risk (pT1–3, pN1) and a high risk category (pT4 and/or pN2), as recently recommended for stage III CRCs [27].

Histologically, SBAs were subclassified as: (i) SBAs, not otherwise specified (SBAs-NOS) (neoplasms featuring conventional glandular structures, without a discrete poorly cohesive component), (ii) poorly cohesive carcinomas (PCCs) (tumors exhibiting a dyshesive cell invasion pattern, with individual cell or cord-like stromal infiltration, in >50% of the neoplastic growth, with or without a signet ring component), (iii) mixed-poorly-cohesive-glandular-SBAs (mixed-PCG-SBAs) (SBAs showing a combination of both glandular and poorly cohesive cell patterns, the latter constituting 10–50% of the neoplasm), or (iv) medullary-type carcinomas (tumors predominantly characterized by solid, "syncytial" sheets of tumor cells, showing pushing border infiltration, and with associated prominent peritumoral and/or intratumoral lymphoid infiltrates), as previously reported [11,12]. In a few survival analyses, SBAs-NOS and medullary-type SBAs were grouped together to form the cohesive histologic group, whereas PCCs and mixed-PCG-SBAs formed the non-cohesive histologic group.

Perineural invasion and lymphovascular invasion were searched using hematoxylin and eosin-stained tumor sections. Histologic grade was categorized as high (G3 or poorly differentiated tumors), when <50% of the tumor was composed of glands, or low (well-to-moderately differentiated tumors, G1-G2), when ≥50% of the neoplasm was composed of glands, according to the histologic grading system described in the CAP protocol for SBAs [16].

Tb was analyzed along the tumoral invasive front (peritumoral Tb) using the hotspot method (i.e., by counting the buds, defined as single neoplastic cells or small clusters of two to four tumor cells, on hematoxylin and eosin staining from the single field of view with the highest number of buds using × 200 total magnification), according to the International Tumor Budding Consensus Conference (ITBCC) criteria, as previously reported [28,29]. Cases were divided into a low (0–9 buds) and a high Tb class (10 or more buds), as recently proposed for SBAs [10].

Immunohistochemistry (IHC) for MMR proteins was centralized in the laboratory of Anatomic Pathology of the Department of Molecular Medicine, University of Pavia. Briefly, four μm-thick sections were stained on a Dako Omnis platform with the following antibodies: MLH1 (monoclonal, clone ES05, prediluted, Dako), MSH2 (monoclonal, clone FE11, prediluted, Dako), MSH6 (monoclonal, clone EP49, prediluted, Dako), and PMS2 (monoclonal, clone EP51, prediluted, Dako). Immunostaining of MMR proteins was considered as MMR-proficient (pMMR) if unequivocal nuclear expression of all

Table 1
Clinico-pathologic and prognostic features of the 70 stage III small bowel adenocarcinomas.

Variable		N of cases (%)	N of deaths (%)	Median survival (25th-75th), months	HR (95% CI), *P* value
dMMR	No	54 (77)	30 (56)	38.7 (13.8–65)	1
	Yes	16 (23)	3 (19)	Unreached	0.28 (0.08–0.91), 0.035
Age at SBA diagnosis	<64 years	37 (53)	16 (43)	53.1 (28.5-NA)	1
	>64 years	33 (47)	17 (51)	34.2 (13.8-NA)	1.33 (0.67–2.65), 0.411
	continuous				1.01 (0.98–1.04), 0.431
Sex	Female	25 (36)	8 (32)	Unreached	1
	Male	45 (64)	25 (56)	31.3 (13.6–65.0)	2.34 (1.05–5.20), 0.037
Predisposing condition^					Overall *P:* 0.003
	Crohn's disease	20 (29)	13 (65)	28.5 (4.8–55)	1
	Lynch syndrome	6 (9)	0	Unreached	NA
	Celiac disease	12 (16)	2 (17)	Unreached	0.21 (0.05–0.92), 0.039
	None (sporadic)	32 (46)	18 (56)	38.7 (15.6-NA)	0.57 (0.28–1.17), 0.124
Tumor site^					Overall *P:* 0.016
	Ileum	31 (44)	19 (61)	28.5 (6.5–55.9)	1
	Jejunum	25 (36)	8 (32)	Unreached	0.33 (0.14–0.77), 0.010
	Duodenum	14 (20)	6 (43)	63.6 (31.5-NA)	0.42 (0.17–1.06), 0.067
pT4 stage	No	37 (53)	12 (32)	65 (31.3-NA)	1
	Yes	33 (47)	21 (64)	28.5 (8.7–55.9)	2.51 (1.23–5.13), 0.011
pN stage*	pN1	39 (57)	15 (38)	53.1 (21.4-NA)	1
	pN2	29 (43)	17 (59)	32 (10.8-NA)	1.60 (0.80–3.21), 0.183
LNR**	≤0.4	45 (68)	20 (44)	45.6 (21.4-NA)	1
	>0.4	21 (32)	12 (57)	20.7 (10.8-NA)	1.23 (0.6–2.52), 0.579
	continuous				1.18 (0.74–1.87), 0.493
LODDS**	≤ −1.1	30 (45)	15 (50)	45.6 (28.5-NA)	1
	> −1.1	36 (55)	17 (47)	34.2 (15.6-NA)	1.02 (0.51–2.06), 0.948
	continuous				1.16 (0.91–1.46), 0.23
Total number of LN examined**	<8	22 (33)	10 (45)	45.6 (20.7-NA)	1
	≥8	44 (67)	22 (50)	42.4 (13–6-NA)	1.26 (0.59–2.66), 0.549
Risk category	Low	26 (37)	7 (27)	Unreached	1
	High	44 (63)	26 (59)	32 (13.8–65)	0.45 (0.19–1.03), 0.06
Histologic grade	Low (G1-G2)	35 (50)	16 (46)	45.6 (21.4-NA)	1
	High (G3)	35 (50)	17 (49)	42.4 (10.8-NA)	1.3 (0.65–2.56), 0.457
Histologic subtype^					Overall *p*: <0.001
	PCC	8 (12)	8 (100)	15.6 (4.8–42.4)	1
	SBA-NOS (glandular)	40 (57)	15 (38)	65 (28.5-NA)	0.27 (0.11–0.65), 0.004
	Medullary	5 (7)	0	Unreached	NA
	Mixed-PCG	17 (24)	10 (59)	15.6 (8–45.6)	0.82 (0.32–2.11), 0.682
Tumor budding	Low	31 (34)	11 (35)	65 (21–4-NA)	1
	High	39 (66)	22 (56)	31.3 (13.8–53.1)	2.56 (1.2–5.26), 0.014
Perineural invasion	No	45 (64)	18 (40)	63.6 (21.4-NA)	1
	Yes	25 (36)	15 (60)	20 (8.7–53.1)	2.44 (1.21–4.91), 0.013

Legend: CI: confidence interval; HR: hazard ratio; LN: lymph node; LNR: lymph node ratio; LOODS: log odds of positive lymph nodes; dMMR: mismatch repair deficiency; NA: not applicable; PCC: poorly cohesive carcinoma; PCG: poorly cohesive-glandular; SBA: small bowel adenocarcinoma; SBA-NOS: small bowel adenocarcinoma, not otherwise specified. *In two cases with matted lymph nodes, the pN stage was not ascertainable; **In four cases, total number of LNs examined, LNR and LOODS were not computable. ^For post-hoc comparisons, the significance was set at 0.017 (Bonferroni correction).

four MMR proteins was retained, or dMMR if complete loss of nuclear expression of one or more MMR proteins was observed, in the presence of an adequate internal positive control (intra-tumor inflammatory and stromal cells and non-neoplastic cells). In parallel, MSI molecular testing by polymerase chain reaction (MSI-PCR) was performed in the laboratory of molecular pathology of the University of Insubria, as previously described [30–32]. *MLH1* promoter methylation status was assessed in all SBAs showing a loss of immunohistochemical expression of MLH1 and/or PMS2, as previously reported [32].

2.3. Evaluation of lymph node status

In order to analyze the prognostic significance of various methods of assessment of the lymph node status, we evaluated the AJCC 8th edition pN stage, LNR and LODDS, as well as the number of examined lymph nodes. According to the AJCC staging system, pN1 was defined as up to two metastatic regional lymph nodes while pN2 as the presence of three or more metastatic regional lymph nodes [26]. LNR was calculated by dividing the number of positive lymph nodes by the total number of retrieved lymph nodes, and LODDS by using the formula $\ln[(pN + 0{\cdot}5)/(nN + 0{\cdot}5)]$, where pN and nN are the number of positive and negative lymph nodes, respectively. Both LNR and LODDS were analyzed as continuous and categorical variables, in the latter case using a cut-off of 0.4 and −1.1, respectively, as recently reported in a large recent retrospective study [15]. The total number of retrieved lymph nodes was regarded as low (when less than eight lymph nodes were identified) or high (≥8 lymph nodes excised).

2.4. Statistical analysis

Stata 17 (StataCorp, College station, TX, USA) was used for all analyses. A two-sided P value <0.05 was considered statistically significant. The Bonferroni correction was applied for post-hoc comparisons. The data were described with the median and 25–75th percentiles if continuous and with counts and percentages if categorical; they were compared between groups with the Mann Whitney U test or the Fisher test respectively. Median follow-up (25–75th percentile) was computed with the reverse Kaplan–Meier method. Follow-up was computed from diagnosis of cancer to death or last available follow-up for censored patients. Cumulative survival curves were plotted according to the Kaplan–Meier method and compared with the log-rank test. The strength

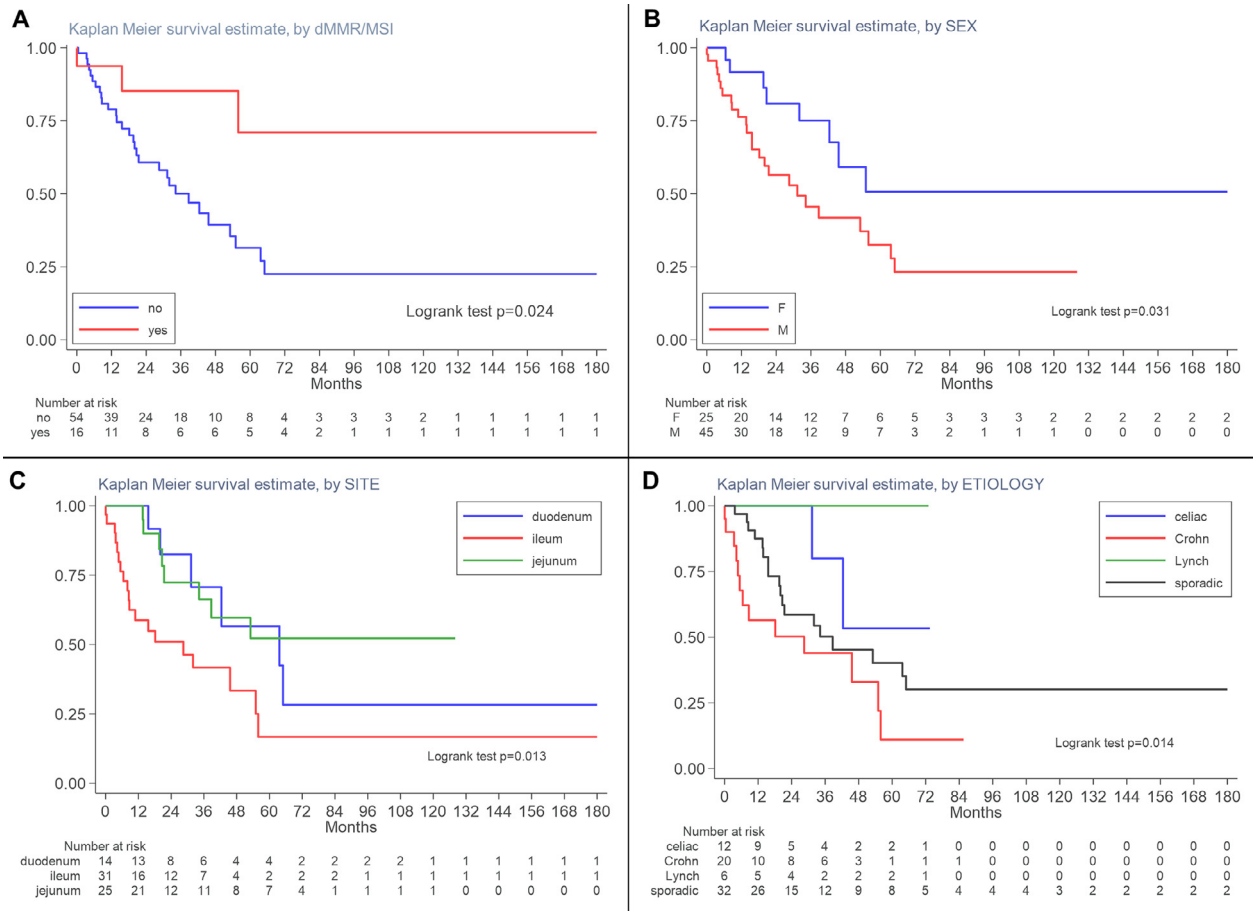

Fig. 1. Kaplan-Meier cancer-specific survival estimates by MMR status (**A**), patient sex (**B**), tumor site (**C**) and etiology (**D**).

of the association between series of candidate risk factors and cancer-specific mortality was assessed using Cox regression; hazard ratios (HR) and 95% confidence intervals (95% CI) were derived from the models. Owing to the small number of events, only bivariable models were fitted including dMMR and, respectively, patient's age and sex, tumor site, pT4, histologic groups, Tb, and perineural invasion.

3. Results

This retrospective study examined a series of 70 surgically resected, primary, non-ampullary stage III SBAs, part of which entered previous studies [11,12,19,29,32–35]. The median patient age at SBA diagnosis was 64 years (25th-75th: 54–73) and patients were predominantly males (64%). SBAs were mainly located in the ileum (44%), and less frequently in the jejunum (36%) or in the non-ampullary duodenum (20%). Twenty (29%) SBA cases were associated with Crohn's disease, 12 (16%) with celiac disease, six (9%) with confirmed Lynch syndrome, and 32 (46%) were considered sporadic (Table 1). The vast majority of patients (94%) underwent adjuvant chemotherapy, and an oxaliplatin-based doublet chemotherapy was the most common adjuvant regimen. No patient received neoadjuvant therapy or immunotherapy.

Thirty-three SBAs (47%) were staged as pT4 cancers. As for lymph node stage, 39 and 29 SBAs were classified as pN1 and pN2, respectively, while two stage III SBA specimens had only metastatic matted lymph nodes, thus preventing the exact number of metastatic lymph nodes to be reliably ascertained. Twenty-one (32%) cases had an LNR>0.4 while 36 (55%) cases had a LODDS>−1.1. In 67% of cases, the total number of harvested lymph nodes was >8. The majority of cases (44 SBAs, 63%) showed at least one high risk feature (pT4 or pN2) and were therefore included in the high risk category following the stage III colorectal cancer definitions.

Histologically, 50% of cases were classified as well-to-moderately differentiated (low grade) and 50% as poorly differentiated (high grade). As for histologic subtype, 40 cases (57%) were classified as SBAs-NOS (glandular), eight (12%) as PCCs, five (7%) as medullary SBAs and 17 (24%) as mixed-PCG-SBAs. An essentially cohesive histology was seen in 45 cases (64%), whereas a non-cohesive component was observed in 25 (36%) SBAs. High Tb and perineural invasion were identified in 39 (66%) and 25 (36%) cases, respectively. Lymphovascular invasion was identified in all cases, at least focally. dMMR was detected in sixteen (23%) SBAs by both immunohistochemistry and PCR-MSI assessment, whereas 54 cases (77%) were pMMR. No case showed any discordance between immunohistochemical and molecular analyses. Among the sixteen dMMR SBAs, three cases had a combined loss of expression of MSH2 and MSH6 and arose in Lynch syndrome patients. The thirteen remaining dMMR cases showed a combined loss of expression of MLH1 and PMS2; three of such cases lacked *MLH1* promoter hypermethylation and were associated with Lynch syndrome, whereas the remaining ten cases (four Crohn's disease-associated SBAs, four celiac disease-associated SBAs and two sporadic SBAs) harbored *MLH1* promoter hypermethylation.

Patients were followed up for a median of 45.5 months. As shown in Table 1 and Fig. 1, dMMR was identified as a significant predictor of favorable outcome at univariable analysis (HR: 0.28, 95% CI: 0.08–0–91, p: 0.035). Among the other examined clinicopathologic features, male sex, pT4 stage, high Tb, and perineu-

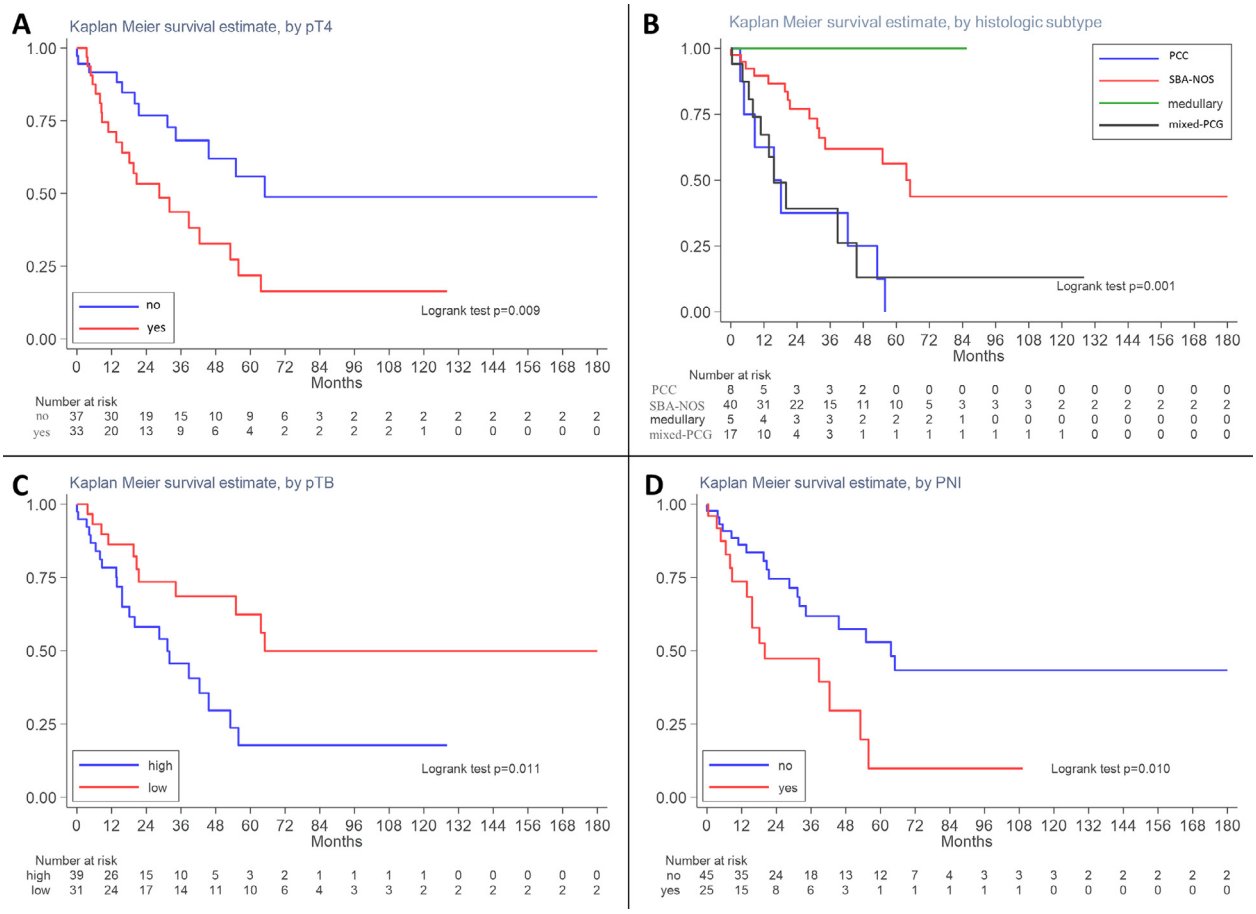

Fig. 2. Kaplan-Meier cancer-specific survival estimates by pT4 stage (**A**), histologic subtype (**B**), tumor budding (pTB) (**C**) and perineural invasion (PNI) (**D**).

ral invasion showed a significant association with a worse CSS. Tumor site was also associated with CSS, as jejunal cases had a better prognosis in comparison with ileal SBAs. Moreover, a more favorable outcome was observed in SBAs-NOS and medullary SBAs in comparison with PCCs and mixed-PCG-SBAs (Fig. 2). No tumor-related death was observed among medullary SBA cases, thus preventing their inclusion in Cox regression analysis. However, a log-rank test showed a significantly better CSS of medullary SBAs compared to PCC cases ($p = 0.009$). Although celiac disease- and Lynch syndrome-associated cases showed more favorable outcome compared with Crohn's disease-associated cancers, the differences did not reach statistical significance when Bonferroni correction was applied (Fig. 2). No significant difference in terms of CSS was found for patient age at diagnosis, risk category, and histologic grade. Furthermore, none of the parameters used for the evaluation of lymph node status seemed to have a statistically significant prognostic value. dMMR was confirmed as a significant predictor of favorable outcome in bivariable models including patient age, sex, tumor site, pT stage, Tb, and perineural invasion (Table 2). In a bivariable model adjusted for histotype, dMMR did not prove to be an independent prognostic factor, and a subgroup analysis showed that dMMR was associated with a better prognosis only among cohesive (medullary or glandular) SBAs (log rank test: $p = 0.046$), while among the non-cohesive cases the difference was not significant (log-rank test: $p = 0.499$).

Among stage III SBAs, MMR status was significantly associated with the predisposing condition ($p<0.001$) and histologic subtypes ($p = 0.008$) (Table 3). As expected, dMMR was more rarely seen in Crohn's disease-associated (6%) and sporadic (20%) SBAs in comparison with Lynch syndrome associated SBAs (100%, $p = 0.001$ versus Crohn's, $p<0.001$ versus sporadic) or celiac disease-associated cases (33%). Moreover, the frequency of dMMR in stage III SBAs with medullary histology (80%) was higher in comparison with PCCs (12.5%) or mixed-PCG-SBAs (6%), while glandular cases showed an intermediate dMMR percentage (25%). No significant association was found between dMMR and patient age at diagnosis, sex, tumor site, pT4 stage, pN stage, LNR, LODDS, number of lymph nodes retrieved, risk category, histologic grade, Tb and perineural invasion. In particular, the median of positive lymph nodes was 2 for both dMMR and pMMR cases.

When comparing low risk and high risk stage III SBAs, statistically significant differences were observed in terms of pT4 and pN2 stage, LNR, LODDS and perineural invasion (Table 3). In addition, when patients were subdivided into three groups based on MMR status and risk category (i.e., dMMR/MSI, pMMR/MSS low risk and pMMR/MSS high risk), a significant prognostic stratification was seen (Fig. 3), although post-hoc comparisons showed a significantly different CSS only between pMMR/MSS high risk and dMMR/MSI cases (HR: 4.26, 1.27–14.24; $p = 0.019$).

4. Discussion

In the present study, we analyzed the prevalence and the prognostic value of dMMR in a fairly large multicentric international cohort of stage III, primary, non-ampullary surgically resected SBAs. We found that SBAs harboring dMMR were associated with a more favorable CSS in comparison with pMMR cases. In addition, MMR status proved to be an independent prognostic factor in bivariable models adjusted for patient age at cancer diagnosis and sex, tumor site, pT4 stage, Tb, and perineural invasion.

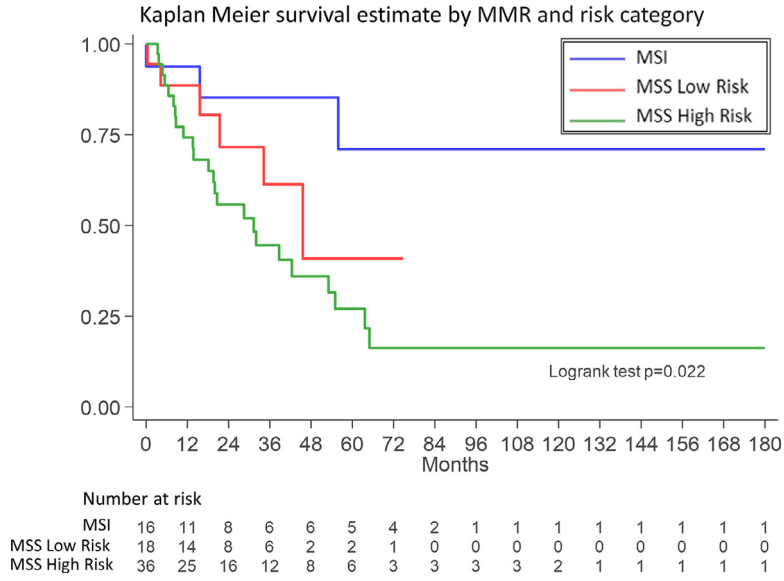

Fig. 3. Kaplan-Meier cancer-specific survival estimates by MMR status combined with risk category.

Table 2
Cancer-specific survival by bivariable Cox models of the 70 stage III small bowel adenocarcinomas.

Bivariable model		HR (95% CI)	*P* value
Model#1			Model *P*: 0.032
dMMR	No	1	
	Yes	0.28 (0.08–0.92)	0.036
Age at SBA diagnosis	≤ 64 years	1	
	>64 years	1.32 (0.66–2.63)	0.427
Model#2			Model *P*: 0.003
dMMR	No	1	
	Yes	0.26 (0.08–0.88)	0.030
Sex	Female	1	
	Male	2.43 (1.09–5.41)	0.030
Model#3			Model *P*: 0.002
dMMR	No	1	
	Yes	0.25 (0.08–0.84)	0.025
Tumor site^	Duodenum	1	
	Jejunum	0.85 (0.29–2.44)	0.759
	Ileum	2.64 (1.04–6.69)	0.041
Model#4			Model *P*: 0.002
dMMR	No	1	
	Yes	0.29 (0.09–0.97)	0.045
pT4	No	1	
	Yes	2.39 (1.17–4.89)	0.017
Model#5			Model *P*: <0.001
dMMR	No	1	
	Yes	0.34 (0.10–1.12)	0.076
Histologic group°	Cohesive	1	
	Non-cohesive	3.38 (1.67–6.85)	0.001
Model#6			Model *P*: 0.002
dMMR	No	1	
	Yes	0.29 (0.09–0.97)	0.044
Tumor budding	High	1	
	Low	0.41 (0.19–0.87)	0.020
Model#7			Model *P*: 0.003
dMMR	No	1	
	Yes	0.29 (0.09–0.94)	0.040
Perineural invasion	No	1	
	Yes	2.36 (1.17–4.75)	0.016

Legend: CI: confidence interval; HR: hazard ratio; dMMR: mismatch repair deficiency; NA: not applicable. ^For post hoc comparisons, the significance was set at 0.017 (Bonferroni correction), °Cohesive histology includes small bowel adenocarcinoma, not otherwise specified and medullary carcinomas, whereas non-cohesive histology includes poorly cohesive and mixed poorly cohesive-glandular carcinomas.

In our series, the frequency of dMMR stage III SBAs (23%) was similar to the one identified by Jun et al. (28%) but lower than the rates reported by Overman et al. (43%), Latham et al. (41%) and Aparicio et al. (36.5%) [9,17,36,37]. Such differences in dMMR frequencies may be partly related to different study populations in terms of ethnicity and etiologic factors, sample size and disparate methods performed to detect dMMR in the aforementioned studies (MMR-IHC with confirmatory MSI-PCR in dMMR cases only by Overman et al.; MSIsensor (next generation sequencing) and/or MMR-IHC by Latham et al., IHC-only approach by Aparicio et al.) [17,37]. In our study dMMR was defined by loss of immunohistochemical expression of at least one MMR protein, with an appropriate internal positive control, and/or MSI-H by PCR, as recommended by the recently published CAP guidelines for MMR and MSI testing for immune checkpoint therapy, and we found a perfect concordance between the two techniques (IHC and MSI-PCR) [30].

Interestingly, the dMMR rate of stage III SBAs we found was lower compared to our recently described series of stage II SBAs (42%), in keeping with findings by Aparicio et al., thus strengthening the already described association of dMMR with earlier stage disease in SBA patients [17,19,37]. However, this finding is still controversial as some Authors observed a similar or even higher percentages of dMMR cases in stage III compared to stage II SBAs and requires further investigation [9,36]. Of note, dMMR was about 2-fold more frequent in SBAs compared to stage III CRC [38]. As for etiology of dMMR cases, we found that 37.5% of dMMR stage III SBAs were associated with Lynch syndrome, a proportion very similar to that reported by Latham et al. in all stage SBAs, further supporting the clinical significance of MMR testing in stage III SBAs [17].

We identified a prognostic role of dMMR/MSI-high status in stage III SBAs, independently of patient age, sex, tumor site, pT4 stage, Tb, and perineural invasion. This finding is in keeping with a previous study by Colina et al., who observed a better overall and relapse-free survival of patients with stage III dMMR SBAs compared to stage III pMMR SBAs at univariate analysis [13]. Moreover Gonzalez et al. found that dMMR was a predictor of more favorable disease-free survival at stage III-inclusive multivariate analysis, although it was not significantly associated with CSS [22]. Further previous studies reported that dMMR/MSI-H was associated with a better overall survival or CSS regardless of SBA stage and other

Table 3
Clinico-pathologic features of the 70 stage III small bowel adenocarcinomas by mismatch repair status and risk category.

Variable		dMMR SBAs (n = 16, 23%)	pMMR SBAs (n = 54, 77%)	P value	Low risk SBAs (n = 26, 37%)	High risk SBAs (n = 44, 63%)	P value
Age at SBA diagnosis, years, median (25th-75th)		68 (55.5–78)	62.5 (52–71)	0.258	66.60 (58–77)	61 (51.50–70.75)	0.062
Sex, N (%)	Male	10 (62)	35 (65)	1.000	15 (58)	30 (68)	0.443
	Female	6 (38)	19 (35)		11 (42)	14 (32)	
Predisposing condition, N (%)	Crohn's disease	4 (25)	16 (30)	<0.001	8 (31)	12 (27)	0.680
	Celiac disease	4 (25)	8 (15)		6 (23)	6 (14)	
	Lynch syndrome	6 (37)	0 (0)		2 (8)	4 (9)	
	None (sporadic)	2 (13)	30 (55)		10 (38)	22 (50)	
Tumor site, N (%)	Duodenum	3 (19)	11 (20)	1.000	4 (15)	10 (23)	0.386
	Jejunum	6 (37)	19 (35)		12 (46)	13 (29)	
	Ileum	7 (44)	24 (45)		10 (39)	21 (48)	
pT4 stage, N (%)	Yes	7 (44)	26 (48)	0.784	0 (0)	33 (75)	<0.001
	No	9 (56)	28 (52)		26 (100)	11 (25)	
pN stage, N (%)*	N1	11 (73)	28 (53)	0.238	26 (100)	13 (31)	<0.001
	N2	4 (27)	25 (47)		0 (0)	29 (69)	
LNR, N (%)**	>0.4	4 (29)	17 (33)	1.000	2 (8)	19 (45)	0.002
	≤0.4	10 (71)	35 (67)		22 (92)	23 (55)	
	median (25th-75th)	0.23 (0.09–0.50)	0.26 (0.11–0.52)	0.736	0.16 (0.07–0.33)	0.36 (0.14–0.66)	0.005
LODDS, N (%)**	≤−1.1	7 (50)	23 (44)	0.768	14 (58)	16 (38)	0.131
	>−1.1	7 (50)	29 (56)		10 (42)	26 (62)	
	median (25th-75th)	−1.15 (−2.12–0)	−0.91 (−1.80–0.06)	0.606	−1.38 (−2.30- −0.59)	−0.48 (−1.65–0.59)	0.004
Total number of LN examined, N (%)**	≥8	6 (43)	38 (73)	0.054	12 (50)	32 (76)	0.056
	<8	8 (57)	14 (27)		12 (50)	10 (24)	
Risk category, N (%)	Low	8 (50)	18 (33)	0.251	–	–	–
	High	8 (50)	36 (67)		–	–	
Histologic grade, N (%)	Low (G1-G2)	7 (44)	28 (52)	0.777	15 (58)	20 (45)	0.458
	High (G3)	9 (56)	26 (48)		11 (42)	24 (55)	
Histologic subtype, N (%)	Medullary	4 (25)	1 (1)	0.008	3 (11)	2 (4)	0.357
	SBA-NOS (glandular)	10 (53)	30 (56)		16 (62)	24 (55)	
	Mixed-PCG	1 (6)	16 (30)		6 (23)	11 (25)	
	PCC	1 (6)	7 (13)		1 (4)	7 (16)	
Tumor budding, N (%)	Low	9 (56)	22 (41)	0.391	14 (54)	17 (39)	0.319
	High	7 (44)	32 (59)		12 (46)	27 (61)	
Perineural invasion, N (%)	Yes	5 (31)	20 (37)	0.772	5 (19)	20 (45)	0.039
	No	11 (69)	34 (63)		21 (81)	24 (55)	

Legend: LN: lymph nodes; LNR: lymph node ratio; LODDS: log odds of positive lymph nodes; PCG: poorly cohesive glandular; dMMR: mismatch repair deficient; pMMR: mismatch repair proficient; PCC: poorly cohesive carcinoma; SBA: small bowel adenocarcinoma; SBA-NOS: small bowel adenocarcinoma, not otherwise specified. *In two cases with matted LNs, the pN stage was not ascertainable; ** In four cases, the total number of LN examined, LNR and LODDS were not computable.

prognostic factors [10,39,40]. However, to the best of our knowledge, the present study is the first primarily and specifically designated to address whether dMMR showed prognostic value in terms of CSS in stage III SBAs.

Moreover, our data suggest that ileal tumor location, pT4 stage, high Tb, perineural invasion and a non-cohesive histology are adverse prognostic factors in stage III SBAs, in keeping with the Literature [5,9–13]. Worth of note, the prognostic relevance of dMMR in stage III SBAs seems to depend on tumor histologic cohesiveness, as it was not statistically significant in cases with non-cohesive histology (PCCs and mixed-PCG-SBAs). We also found that male sex is associated with a worse CSS, regardless of MMR status, at variance with findings by other authors [5,13]. As for the prognostic value of predisposing conditions, previous studies suggested a better prognosis in celiac disease-related SBAs when compared with sporadic or Crohn's disease-associated SBAs, regardless of tumor stage, and a trend towards a better survival of Lynch syndrome-related SBAs when compared to sporadic or Crohn's disease-related cases [5,32,41,42]. Although we observed a more favorable outcome of Lynch syndrome-associated and celiac disease-associated SBA patients (both enriched in dMMR cases) compared to those associated with Crohn's disease or without any predisposing conditions, the differences did not reach statistical significance, likely due to the limited number of cases in each category. In keeping with the previous Literature, pN2 stage, high LNR, high LODDS, and high risk category were all associated with a trend towards a worse patient outcome in our SBA cohort [13,15,36,43]; however, the survival differences did not reach statistical significance, likely due to the relatively small number of patients. In addition, high risk category SBAs were found to be associated with other adverse pathologic parameters, such as perineural invasion.

Even though histologic grade, based on tumor differentiation (percentage of the tumor forming glandular structures) has been previously associated with patient survival in SBAs, regardless of tumor stage, we did not find a significant association between tumor grade and CSS [5,13]. This finding may be partially explained by our relatively high number of cases featuring a medullary histotype (5 cases), which, despite its poorly differentiated morphology, has been associated with an improved prognosis in gastrointestinal carcinomas, especially in colorectal cancers [44]. In fact, we did not observe any tumor-related death in our cases of medullary-type stage III SBAs and our data suggest that medullary SBAs are characterized by a significantly better outcome when compared to PCC cases. Of note, we found a statistically significant association between medullary histotype and dMMR in stage III SBAs, as previously described in small intestinal carcinomas, as well as in colorectal and ampullary carcinomas [9,22,44,45].

Strengths of this study include the centralized proof-reading of MMR status and MSI by PCR with optimal concordance between techniques as well as the demonstration that dMMR/MSI status is associated with better CSS (and not only on overall survival as in other studies).

We do acknowledge that this study has several limitations, first of all its inherently retrospective nature. Nevertheless, the involvement of international centers with referral experience in the field and the centralized histologic review and MMR testing were guarantees of data quality. Moreover, given the rarity of the condition and events, no multivariable model, which does not suffer from overfitting, could be applied; nonetheless, we accounted for a series of potential confounders in the bivariable analyses we performed, that consistently confirmed the prognostic relevance of dMMR with HRs ranging from 0.25 to 0.34. It was not possible to investigate whether MMR status in SBA impacts on response to adjuvant treatment. It would be interesting to compare dMMR/MSI stage III SBAs with pMMR/MSS stage III SBAs with regards to response to adjuvant treatment; however, for now, NCCN guidelines [7], as well as a recent meta-analysis [46] state that stage III SBA patients should undergo adjuvant treatment and that this results in improved overall survival. As of yet, there is no distinction on management of stage III SBA patients based on MMR status (differently to stage II SBAs and CRCs for which MMR status impacts on adjuvant treatment), but this is definitely an important point which will require elucidation in the future. A further point, which requires reliable, standardized MMR status evaluation, is MMR/MSI testing at initial SBA diagnosis. Indeed, considering the breakthrough trials on the neoadjuvant use of immune checkpoint inhibitors in colon and rectal cancer, much the same could be postulated for SBA [46], even though the setting is very different as SBAs are often incidental findings with location dependent difficulty in the acquisition of tissue for diagnosis (on which MMR evaluation can be performed).

In conclusion, the present study highlights the prognostic importance of detecting MMR status in stage III SBAs, especially in those showing a conventional (glandular) or medullary histotype, as dMMR proved to be associated with a more favorable CSS, like in stage II SBAs [19]. In addition, MMR testing will identify a relevant proportion of Lynch syndrome-related cases (about one third of dMMR stage III SBAs), as well as enable selection of patients who could be potentially treated with immune checkpoint inhibitors.

Conflict of Interest

The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

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