The pathological phenotype of colon cancer with microsatellite instability

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ABSTRACT

INTRODUCTION: Colorectal cancer is a common malignant disease, caused by different aetiologies and molecular pathways. Heterogeneous results have been published regarding the association of microsatellite instability and clinicopathological features. The aim of this study was to compare clinicopathological features of microsatellite unstable tumours with stable ones.

METHODS: Data were collected retrospectively, but the pathological analyses were all made prospectively. The study included a total of 833 patients undergoing resection of their colon tumour at Nordsjællands Hospital – Hillerød, with mismatch repair analysis from 1 January 2007 to 30 November 2012. The study was performed in a setting with complete mesocolic excision surgery and post-operative expert pathological examination of the tumours. Mismatch repair analysis was done by immuno-histochemical staining for the mismatch repair proteins: pMLH1, pMSH2, pMSH6 and pPMS2 for the determination of microsatellite instability. Microsatellite instability was defined as deficient expression of one or more of these proteins.

RESULTS: Of the 833 patients, 177 had microsatellite instable tumours (21%). Using multivariable logistic regression analysis, we demonstrated that microsatellite unstable cancers were significantly associated with a lower degree of lymph node metastases (odds ratio (OR) = 0.92), distant metastases (OR = 0.33) and tumour budding (OR = 0.41).

CONCLUSIONS: We found that microsatellite unstable tumours show a pathological profile that appears less aggressive than the pathological profile of stable tumours.

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Colorectal cancer is a common malignant disease, which about one in 20 people will develop during their lifetime. It is the second leading cause of cancer-related death, and in the United States it is expected to have caused 50,000 deaths in 2014 [1]. With this huge burden in both morbidity and mortality from colorectal cancer, it is becoming increasingly evident that a personalised treatment approach is needed. Different aetiologies and molecular pathways of colorectal cancer are starting to become recognised [2], one such being microsatellite instability (MSI), which plays a pivotal role in Lynch Syndrome, but which also carries a prognostic value for patients with sporadic colon cancer [3].

MSI tumours are characterised by an impaired proofreading of the genome during replication because of a deficient mismatch repair (MMR) system. The MMR system consists of proteins that repair errors made in the DNA replication, and which, when deficient, will result in hypermutability of microsatellites (MS). MS are repetitive sequences of a few nucleotides and are frequent in the genome, in coding as well as non-coding sequences. When MS are shortened or extended compared with non-mutated sequences, the tumour is termed MSI as opposed to microsatellite stable tumours (MSS) [2]. Deficiency of the MMR system may result from a germline mutation, somatic mutations or methylation of the pMLH1 promotor [2]. It has been shown that MSI is associated with location in the proximal colon [4-6], and MSI is most frequent in females [5]. However, one study suggests no association with gender [4]. The pathological characteristics for MSI tumours are associated with a poor grade of differentiation [5], higher lymph node yield [4] and a lower rate of lymph node and distant metastasis [6, 7]. Several reviews have shown that patients with MSI tumours have a better prognosis for overall survival than those with MSS tumours [8, 9], but it remains unknown which mechanisms account for this positive prognostic value. Furthermore, MSI tumours respond differently to chemotherapy than MSS tumours do, and some studies have shown that MSI tumours are less sensitive to 5-FU-based adjuvant chemotherapy [8, 9].

Pathological examination was performed by an experienced colorectal cancer pathologist for all the tumours during the entire study period, and complete mesocolic excision (CME) surgery was performed in all elective patients from June 2008. To our knowledge, no previous study of a single large cohort has studied a wide pathological description of MSI tumours in patients undergoing CME surgery. The aim of this study was to describe the association between the clinicopathological phenotypes of patients with colon cancer with and without MSI.

ORIGINAL ARTICLE

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METHODS

Data for all patients undergoing a resection for colon cancer at Hillerød Hospital from 1 January 2007 to 30 November 2012 were retrieved from the local pathology registry. Data from the first tumour only were included for patients with metachronous tumours during the period. Patients with multiple tumours and those with tumours other than adenocarcinoma, mucinous adenocarcinoma, signet ring cell carcinoma, medullary and undifferentiated carcinoma were excluded. There were no further restrictions on patient inclusion.

From 1 June 2008, CME surgeries were performed in all elective procedures [10]. Prior to this date and in acute cases, surgery was performed with the objective of achieving a high lymph node yield. Demographic and operation data were obtained from our colon cancer database (2008-2012) or from the medical records. Pathology data were obtained from our pathology database. We noted if tumours were right-sided (caecum, ascending colon, right flexure or colon transversum) or left-sided (left flexure, descending colon or sigmoideum).

The data collection was approved by the Danish Data Protection Agency. As a retrospective study under Danish law, approval from the local ethics committee was not needed.

Pathologic assessment

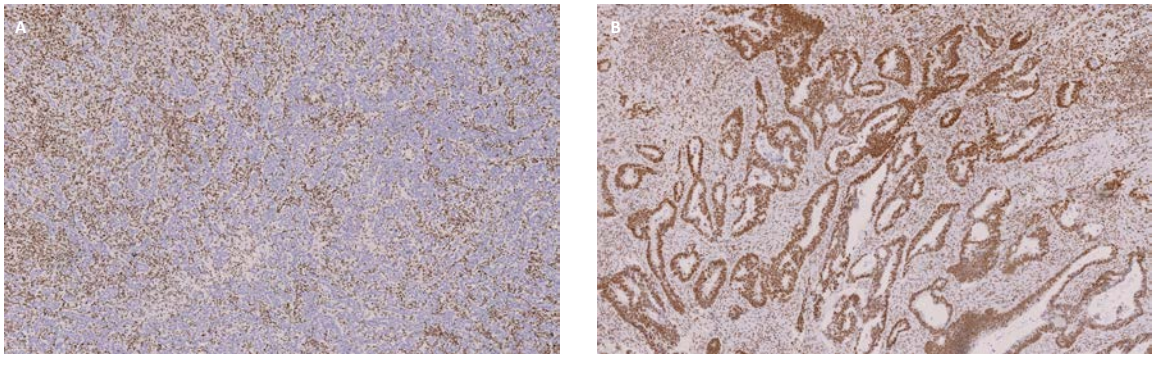
As described before [10], the tumour, lymph node and metastases (TNM) staging was defined in accordance with the American Joint Committee on Cancer, 5th edition [11]. The pathological examination was done in accordance with the same standards during the entire study period, except for the use of methylene blue for identification of lymph nodes, which was introduced in May 2010 [12]. This variable was included in the multivariable statistical analyses. All identified lymph nodes were completely embedded. The histological type of the tumour was recorded as adenocarcinoma not otherwise specified, mucinous adenocarcinoma, poorly differentiated adenocarcinoma, signet ring cell carcinoma, undifferentiated carcinoma or medullary carcinoma. We also determined tumour budding, extramural perineural involvement, extramural venous invasion and peritoneal involvement using haematoxylin and eosin staining. Tumour budding was present when ten or more foci of 1-4 dedifferentiated tumour cells were present at the invasive front at 200 × magnification, according to the national colorectal cancer pathology guidelines. No residual tumour (R0) was defined as more than 1 mm from the resection margin to the tumour or any tumour deposits. Microscopic residual tumour (R1) was defined as either direct tumour involvement from the tumour or tumour deposits within 1 mm of the resection margin found by microscopy. R1 was reported together with macroscopically verified residual tumour (R2).

The MMR analyses of the tumour were performed with immuno-histochemical (IHC) analysis of the proteins pMLH1, pMSH2, pMSH6 throughout the entire study period. The analysis of pPMS2 was added as from 2008. The sections used contained both tumour tissue and normal mucosa so the sections contained their own internal controls (**Figure 1**). MSS was defined as an expression of all the mismatch repair proteins, and MSI was defined as missing an expression of one or more of these proteins. In this paper, we do not discriminate between deficient MMR and MSI or proficient MMR and MSS.

Statistical analysis

A multivariable logistic regression model was used with purposeful predictor selection [13]. In short, univariable analysis of each predictor was done. Based on the Wald statistic and a p-value below 0.25, possible predictors were chosen. The multivariable model was made on the predictors identified in the univariable analysis. Interactions between "site of the tumour and the type of op-

FIGURE 1



Examples of immuno-histochemical staining for mismatch repair proteins. **A.** Staining for MLH1 showing nuclear staining in the intratumoral lymphocytes, but no staining of the tumour cells. Blue-coloured nuclei is negative staining. **B.** Normal expression of MLH1 in both the tumour cells and lymphocytes, brown-coloured nuclei is positive staining.

TABLE 1

Univariate logistic regression analysis of surgery-related variables.

	Total (n = 833)						
	MSI (n = 177)		MSS (n = 656)		Univariable logistic regression, p-value	Reduced multivariable logistic regression model	
	n (%)	mean (± SD), cm	n (%)	mean (± SD), cm		OR (95% CI)[a]	p-value
Elective or acute							
Elective	157 (22.6)		535 (77.4)		0.04	1	–
Acute	20 (16.1)		117 (83.9)		–	0.49 (0.25-0.95)	0.04
Unknown	0		3 (100)		–	–	–
Surgical approach							
Open	133 (27.0)		359 (73.0)		< 0.0001	1	–
Laparoscopic	37 (12.7)		255 (87.3)		< 0.0001	0.53 (0.31-0.88)	0.02
Converted	7 (14.3)		42 (85.7)		0.06	0.62 (0.22-1.60)	0.35
Intention							
Curative	151 (22.7)		515 (77.3)		0.08	–	–
Palliative	23 (16.0)		121 (84.0)		–	–	–
Unknown	3 (13.0)		20 (87.0)		–	–	–
Residual tumour							
R0	158 (22.4)		548 (77.6)		0.07	–	–
R1 or R2	19 (15.1)		107 (84.1)		–	–	–
Unknown	0		1 (100)		–	–	–
Length of specimen		30.87 (± 17.1)		25.6 (± 15.7)	< 0.001	–	–

CI = confidence interval; MSI = microsatellite instability; MSS = microsatellite stable tumours; OR = odds ratio; R0 = no residual tumour; R1 = microscopic residual tumour; R2 = macroscopic residual tumour; SD = standard deviation.
a) OR are from the multivariate regression model.

eration", "elective or acute indication of surgery and type of operation" or "methylene blue and number of lymph nodes" were investigated. The adequacy and fit of the model was checked [14]. All analyses were done using R 3.1.0. statistical programme (Windows).

Trial registration: not relevant.

RESULTS

In this study, we included 833 patients with colon cancer of whom 407 (48.8%) were female and 177 (21.3%) had MSI tumours. From 1 January 2007 to 30 November 2012, we obtained data on 910 patients from our local pathology registry; excluded were three patients with metachronous tumours, 13 patients with synchronous tumours, four patients with rare histological type and 57 patients with no MSI data.

The MSI and MSS groups were not significantly different from one another with respect to neoadjuvant chemotherapy, curative intention of surgery, and R0 or R1 and R2 stage (**Table 1**). In the multivariable analysis, we found no association between MSI status and the patient's age (p = 0.46), the mean age of patients with MSI tumours was 70.81 years (standard deviation (SD) = 12.2) and for those with MSS tumours 69.2 (SD = 10.8) years. Results from the univariate logistic regression model are shown in Table 1 and **Table 2**, and results from the multivariate logistic regression model are shown in **Figure 2**. A receiver operating characteristics curve analysis showed an area under the curve = 0.88, confirming a well-fitted model with an excellent discrimination [13].

MSI tumours were most likely to be found in the right side of the colon (OR = 10.0; 95% confidence interval (CI): 5.78-19.19) and were more common in females (OR = 2.27; CI: 1.47-3.54). In both the univariate and the multivariate analysis, the number of lymph nodes identified was found to be higher for MSI tumours (Table 2). The mean number of lymph node metastases was 1.9 in MSI tumours and 3.5 in MSS tumours, with a significantly lower lymph node ratio (number of lymph node metastases divided by number of lymph node harvested) of 0.06 for MSI tumours compared with 0.1 for MSS tumours, p < 0.001.

Only 29 (3.5%) patients had fewer than 12 lymph nodes examined. MSI tumours were associated with a lower risk of tumour budding, extramural perineural invasion, lymph node metastasis and distant metastasis in both the univariate and the multivariate analysis (Table 2 and Figure 2). TNM stage and rate of extramural venous invasion were significantly lower in MSI tumours in the univariate analysis (Table 2), but not in the logistic regression model.

No interactions were found between the tumour site and surgical approach, laparoscopic or open (p = 0.47); elective or acute indication and type of operation (p = 0.28); or methylene blue staining and number of lymph nodes examined (p = 0.63).

TABLE 2

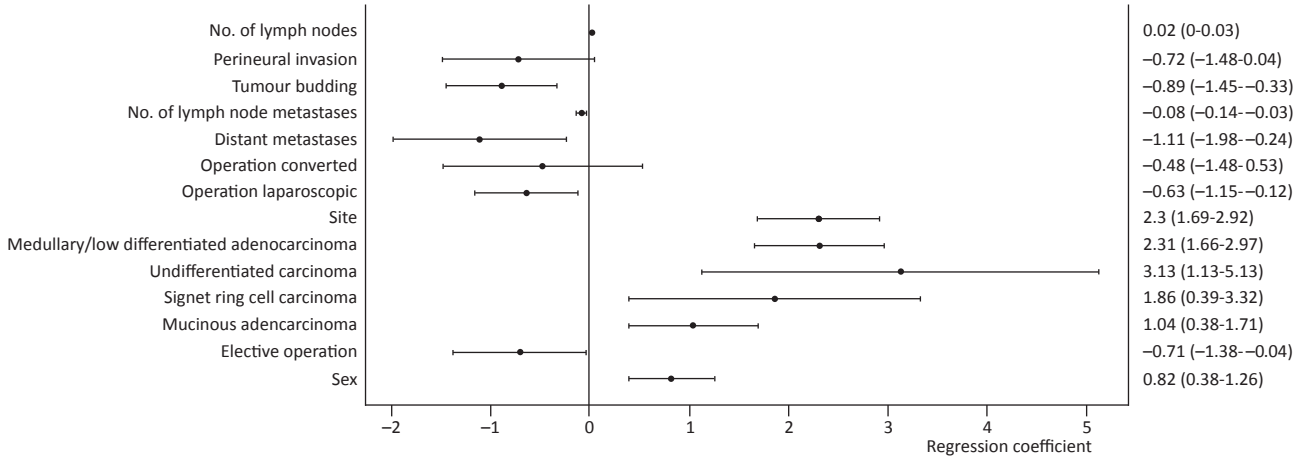
Univariate logistic regression analysis of tumour-related variables.

	Total, n (%) [range] (n = 833)		Univariate logistic regression, p-value	Reduced multivariable logistic regression model	
	MSI (n = 177)	MSS (n = 656)		OR (95% CI)[a]	p-value
Tumour location					
Right side	163 (35.4)	298 (64.6)	< 0.0001	10.0 (5.78-19.19)	< 0.0001
Left side	14 (3.8)	358 (96.2)	-	1	-
Tumour morphology					
Adenocarcinoma NOS	88 (14.3)	528 (85.7)	-	1	-
Mucinous carcinoma	25 (35.2)	46 (64.8)	< 0.0001	2.84 (1.46-5.51)	0.002
Signet ring cell carcinoma	6 (33.3)	12 (66.7)	0.03	6.41 (1.45-27.67)	0.01
Undifferentiated carcinoma	6 (66.7)	3 (33.3)	< 0.001	22.84 (3.56-222.99)	0.002
Medullary carcinoma or low differentiated carcinoma	51 (48.6)	54 (51.4)	< 0.0001	10.11 (5.34-19.84)	< 0.0001
Unknown histology	1 (7.1)	13 (92.9)			
Lymph nodes, mean	37.5 [6-107]	30.6 [6-127]	< 0.0001	-	-
Metastasis					
Lymph node (no: 427, unknown: 0)	62 (15.2)	344 (84.7)	0.005	0.92 (0.87-0.97)	0.002
Distant (no: 706, unknown: 8)	8 (6.7)	111 (93.3)	< 0.001	0.33 (0.13-0.75)	0.01
TNM stage					
I	16 (19.5)	66 (80.5)	-	-	-
II	97 (30.7)	219 (69.3)	0.05	-	-
III	55 (17.1)	267 (82.9)	0.61	-	-
IV	8 (7.6)	97 (92.4)	0.02	-	-
Unknown	1 (12.5)	7 (87.5)	-	-	-
Extramural invasion					
Perineural (no: 692)	13 (9.2)	128 (90.8)	< 0.001	0.49 (0.22-1.02)	0.07
Venous (no: 505)	46 (14.0)	282 (86.0)	< 0.0001	-	-
Tumour budding					
(no: 574, unknown: 28)	28 (12.1)	203 (87.9)	< 0.0001	0.41 (0.23-0.71)	0.002

CI = confidence interval; NOS = not otherwise specified; MSI = microsatellite instability; MSS = microsatellite stable tumours; OR = odds ratio; TNM = tumour, lymph node and metastasis staging.
a) OR are from the multivariable regression model.

FIGURE 2

Regression coefficients with 95% confidence interval (CI) of the multivariable logistic regression analysis. When the data are above 0, there is an association with microsatellite instability, while data below zero are associated with microsatellite stable tumours. When the CI contains zero, the association is insignificant. It is seen that number of lymph nodes, right side of the colon, all tumour morphologies but adenocarcinoma not otherwise specified, and female sex are associated with microsatellite instable tumours.



DISCUSSION

In this study, we found that MSI tumours were associated with a lower rate of tumour budding, extramural perineural invasion, lymph node metastasis and distant metastasis. The presence of tumour budding is an adverse prognostic factor, with a 2-3-fold higher relative risk of distant metastasis [15] and local recurrence [16]. We found a significantly lower rate of tumour budding in MSI tumours, which is consistent with previous findings [17]. To our knowledge, extramural perineural invasion has been compared to MSI status only once before and no significant difference was established [4]. The previous study reported extramural perineural invasion in only 82 patients and showed a non-significant difference between 9% of the MSI tumours and 23% of the MSS tumours. This could be the result of a type 2 error because of the low number of included patients failing to show the difference. In our study, we also found a non-significant, lower rate of extramural perineural invasion in MSI tumours with an OR of 0.49 (CI: 0.22-1.02). This difference can potentially be due to differences in the analyses of extramural perineural invasion.

Both lymph node and distant metastases are the most important factors for the course of colorectal cancer [18]. We found an association between a lower risk of these negative prognostic markers and MSI tumours. This is consistent with the results from several other studies [6, 7].

Surprisingly, we found that open operation was significantly associated with MSI tumours. After interaction analysis, this could not be explained by the site of the tumour or by the distinction between acute and elective surgery. We hypothesise that this interaction may be explained by MSI tumours having a larger tumour circumference than MSS tumours as has been shown in a study of 61 patients [6]. The standard procedure at Hillerød Hospital, Denmark, was open surgery in patients with a palpable tumour or if staged as T4 tumour at the preoperative computed tomography (CT) [10].

This study represents one of the largest cohorts with a detailed presentation of MSI status in patients with colon cancer and clinicopathological features, and based on a population of patients undergoing CME or surgery with the intention of achieving a high lymph node yield and a high-quality pathological examination of the tumours. In this study, we had a mean of more than 30 detected lymph nodes in both MSI and MSS tumours. Only 3.5% of the patients had fewer than 12 lymph nodes identified. A large number of detected lymph nodes is the basis for correct N-staging [18].

Our study results are limited by the fact that we performed IHC analysis only for determination of MMR status. According to Danish guidelines, all tumours should be examined by IHC for expression of MMR proteins. Furthermore, the analysis of pPMS2 was added as from 2008 leaving 198 cases without this analysis. This could potentially result in missing identification of MSI in these cases, but only two cases in total have pPMS2 as the only missing mismatch repair protein. Polymerase chain reaction (PCR) analysis is often used for determining MSS or MSI. With PCR, MSI can be further divided into either MSI high where 30% or more of the analysed genes are different from those of healthy tissue, or MSI low where fewer than 30% of the analysed genes are different from those of healthy tissue. A previous study has shown that IHC has a specificity of 100% and a sensitivity of 92.3% compared with PCR analysis [19]. The lower sensitivity is due to the fact that MMR proteins can be non-functional and still be detected by IHC, resulting in false-negative cases. One could argue that these IHC tests show deficient MMR proteins, and therefore the results should be reported as such. However, MSI is present when MMR is deficient and MMR deficiency causes MSI, leading to the same result [20].

MSI tumours show a pathological profile that is significantly different from that of MSS tumours. It remains unknown which mechanisms account for the favourable prognosis of MSI tumours reported in other studies, but the pathological profile described in this population shows that MSI tumours appear to be less aggressive than those due to a lower rate of tumour budding, extramural perineural invasion, lymph node metastasis and distant metastases.

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