Impact of microsatellite status in early-onset colonic cancer

REACCT Collaborative

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Abstract

Background: The molecular profile of early-onset colonic cancer is undefined. This study evaluated clinicopathological features and oncological outcomes of young patients with colonic cancer according to microsatellite status.

Methods: Anonymized data from an international collaboration were analysed. Criteria for inclusion were patients younger than 50 years diagnosed with stage I–III colonic cancer that was surgically resected. Clinicopathological features, microsatellite status, and disease-specific outcomes were evaluated.

Results: A total of 650 patients fulfilled the criteria for inclusion. Microsatellite instability (MSI) was identified in 170 (26.2 per cent), whereas 480 had microsatellite-stable (MSS) tumours (relative risk of MSI 2.5 compared with older patients). MSI was associated with a family history of colorectal cancer and lesions in the proximal colon. The proportions with pathological node-positive disease (45.9 versus 45.6 per cent; P=1.000) and tumour budding (20.3 versus 20.5 per cent; P=1.000) were similar in the two groups. Patients with MSI tumours were more likely to have BRAF (22.5 versus 6.9 per cent; P,0.001) and KRAS (40.0 versus 24.2 per cent; P=0.006) mutations, and a hereditary cancer syndrome (30.0 versus 5.0 per cent; P,0.001; relative risk 6). Five-year disease-free survival rates in the MSI group were 95.0, 92.0, and 80.0 per cent for patients with stage I, II, and III tumours, compared with 88.0, 88.0, and 65.0 per cent in the MSS group (P=0.753, P=0.487, and P=0.105 respectively).

Conclusion: Patients with early-onset colonic cancer have a high risk of MSI and defined genetic conditions. Those with MSI tumours have more adverse pathology (budding, KRAS/BRAF mutations, and nodal metastases) than older patients with MSI cancers.

Introduction

The incidence of colorectal cancer among adults aged less than 50 years is rising globally[1,2]. It represents the second most common cancer and the third leading cause of cancer-related death in this age group[3]. Based on current trends, it is estimated that by 2030 the incidence rates of colonic and rectal cancer will have increased by 90 and 124 per cent respectively among adults aged 20–34 years, and by 27 and 46 per cent respectively for those

aged 35–49 years[2]. As the volume of data on early-onset colorectal cancer increases, distinct clinical and pathological patterns have emerged. Young patients typically present with an advanced disease stage, and more frequently exhibit adverse histopathological features, such as poor differentiation, perineural invasion, venous invasion, and mucinous and/or signet cell morphology[4–7]. Prognostication and therapeutic decision-making in colorectal cancer is based largely on histopathological analysis of the resected specimen and the TNM staging system. Clinical outcome, however, varies among patients with the same disease stage, probably in relation to tumoral molecular heterogeneity. The majority of colorectal cancers develop via chromosomal instability (and are also known as microsatellite-stable, MSS), whereas 15 per cent are characterized by microsatellite instability (MSI)[8,9]. MSI is due to defective DNA mismatch repair (MMR) which leads to the accumulation of errors in DNA replication. This defect may be the result of sporadic epigenetic silencing of the MLH1 gene and the CpG island methylator phenotype, or constitutive mutations in one of the MMR genes (MLH1, MLH6, MSH2, PMS2), that is Lynch syndrome[10]. Reflex testing for MSI (either by PCR or immunohistochemistry) is recommended in all patients with colorectal cancer, regardless of age or family history. Tumours with MSI have a unique clinical and immunological phenotype. They are typically located in the proximal colon, are less likely to metastasize to lymph nodes and distant organs, and demonstrate a strong intratumoral lymphocytic reaction[11–13]. They are associated with better stage-adjusted survival than MSS tumours, and are relatively resistant to 5-fluorouracil-based chemotherapy[14–17]. Historically, studies evaluating MSI have included all-age colorectal cancer and little is known about MSI in young patients. It is unclear whether the same clinicopathological patterns and survival trends exist as those observed in late-onset disease. Individual institutional data in isolation are too small for meaningful analyses. The REACCT Collaborative was established to aggregate large-volume real-world data from specialist centres across the world. This study compared the clinicopathological features and oncological outcomes of MSI and MSS colonic cancers in young patients.

Methods

Study participants

A retrospective international multicentre observational study was performed to assess the clinicopathological features, molecular characteristics, and disease-specific outcomes of patients diagnosed with early-onset colonic cancer. Inclusion criteria were adults aged between 18 and 49 years with a histologically confirmed diagnosis of stage I–III colonic cancer, who underwent surgery with curative intent, and with known MSI status.

Data collection

All participating institutions are tertiary referral units with specialist expertise in colorectal cancer. A principal investigator from each participating centre collected data from the institutional database or by independent review, and submitted the data centrally for analysis. Ethical approval was sought at an individual institutional level. Data collected included: baseline patient demographics, clinical information, stage, surgical, and treatment data, histopathological

and molecular features, and cancer-specific as well as overall survival information. Clinical staging was according to the eighth edition of the AJCC TNM staging system. Microscopically clear resection (R0) was defined by a tumour-free resection margin of at least 1mm. MSI was determined by PCR or immunohistochemistry (IHC). Loss of MMR proteins MLH1, PMS2, MSH2 or MSH6 on IHC was classified as MSI. A hereditary cancer syndrome was defined as diagnosis of a constitutive pathogenic variant on germline testing.

Statistical analysis

Continuous variables are presented as median (range), and were compared by Student's t test or Mann–Whitney U test, depending on distribution. Categorical variables are reported as numbers with percentages, and were analysed using $\chi 2$ test or Fisher's exact test, as appropriate. Survival statistics were calculated using the Kaplan–Meier method, and the log rank test was used to assess differences in survival between groups. Independent variables were entered into a multivariable binary logistic regression model. Variables found to be significant in univariable analysis, or with $P < 0.100$, were entered into the multivariable model. A significance level of 0.05 was used for all analyses; reported P values are two-tailed. Data were analysed using SPSS® version 24.0 (IBM, Armonk, New York, USA).

Results

Baseline demographics

A total of 650 patients aged less than 50 years and diagnosed with stage I–III colonic cancer were included. Median age was 43 (range 18–49) years and there were 332 men (51.1 per cent). Defined MSI was identified in 170 patients (26.2 per cent). The remaining 480 had MSS tumours. MSI was associated with younger age at diagnosis, a first-degree relative with colorectal cancer, and right-sided lesions (caecum and ascending colon), but not with sex or BMI. Demographics and clinical characteristics of the study population are summarized in Table 1.

Pathological features

MSI tumours were more likely to be poorly differentiated or undifferentiated (28.1 versus 21.2 per cent; P=0.026), and to display signet ring morphology (10.9 *versus* 4.4 per cent; *P*=0.013). They were less likely to exhibit lymphovascular (38.0 versus 47.5 per cent; *P*=0.027) or extramural venous (31.7 *versus* 44.9 per cent; *P*=0.008) invasion, whereas the rate of tumour budding was similar (20.3 *versus* 20.5 per cent; *P*=1.000). The proportion of patients with pathological stage I–III disease did not differ significantly.

Molecular characteristics

BRAF (22.5 *versus* 6.9 per cent; $P < 0.001$) and KRAS (40.0 versus 24.2 per cent; $P=0.006$) mutations were more common in the MSI group. MSI tumours were more likely to occur in the context of genetic predisposition. A hereditary cancer syndrome was diagnosed in 51 patients (30.0 per cent) with MSI tumours *versus* 24 (5.0 per cent) with MSS tumours (hazard ratio (HR) 8.14, 95 per cent c.i. 5.12 to 12.95; $P < 0.001$). Genetic testing had not been performed in 38 per cent at the time of data collection.

Survival

Overall median follow-up was 48 (range 1–221) months. Five-year overall survival rates in the MSI group were 100, 97, and 86 for patients with stage I, II, and III tumours respectively. Corresponding values in the MSS group were 98, 95, and 77 per cent. Five-year disease-free survival (DFS) rates in the MSI group were 95, 92, and 80 per cent for stage I, II, and III disease respectively, compared with 88, 88, and 65 per cent in the MSS group (P=0.753, P=0.487, and P=0.105 respectively).

Disease recurrence

Seventeen patients (10.0 per cent) in the MSI group developed disease recurrence compared with 71 (14.8 per cent) in the MSS group (P=0.053). Locoregional recurrence developed in 5 patients (2.9 per cent) in the MSI group and 18 (3.8 per cent) in the MSS group (P=0.638), and distant disease in 16 (9.4 per cent) and 76 (15.8 per cent) respectively (P=0.083). The median time to recurrence was 12 (range 1–84) months after surgery among patients with MSI tumours, and 13 (1–63) months in those with MSS tumours (P=0.480).

Factors predictive of disease-specific outcomes

In univariable analysis, in the MSI group, lymphovascular and extramural venous invasion were associated with worse DFS (Table 2). Only extramural venous invasion was significant in multivariable analysis (HR 7.81, 95 per cent c.i. 1.89 to 32.22; P=0.004). In the MSS group, R0 resection was significantly associated with better DFS in univariable analysis, whereas signet ring morphology, and lymphovascular, extramural, and perineural invasion were associated with worse DFS. In multivariable analysis, only lymphovascular invasion was significantly associated with worse DFS (HR 2.294, 1.36, 3.94; P=0.003).

Comparison of sporadic MSI tumours and MSI tumours arising in the context of a hereditary cancer syndrome

A subgroup analysis of MSI tumours was undertaken comparing patients with sporadic tumours with those with tumours and a confirmed genetic predisposition. Of 170 patients with MSI tumours, genetic testing had been carried out in 95 at the time of data collection. A defined

hereditary cancer syndrome was diagnosed in 51, whereas 44 had confirmed sporadic tumours. No significant differences in baseline demographics, clinical characteristics or pathological features were observed. As expected, patients with tumours arising in the context of a hereditary cancer syndrome were more likely to have a first-degree relative with colorectal cancer (35.5 versus 7.5 per cent; P=0.001). Disease-specific survival did not differ significantly between the two groups. The 5-year DFS rate was 90 per cent for patients with a genetic predisposition compared with 86 per cent for those with sporadic disease (P=0.792).

Table 1 Comparison of demographics and clinicopathological data between microsatellite instability and microsatellite-stable groups

	Overall (*n* = 650)	Microsatellite instability (*n* = 170)	Microsatellite-stable (*n* = 480)	P†
Ages (years)*	43(18–49)	40 (18–49)	44 (19–49)	<0.001‡
Men	332 (51.1)	111 (54.1)	317 (50.1)	0.176
BMI (kg/m^2)*	24.3 (13.3–58.6)	24.6 (13.3–47.0)	24.3 (16.0–58.6)	0.079‡
Inflammatory bowel disease	27 (4.2)	5 (2.9)	22 (4.6)	1.000
First-degree relative with colorectal cancer	127 (19.6)	52 (30.7)	75 (15.6)	0.001
Tumour site				
Rectosigmoid junction	98 (15.1)	10 (5.9)	88 (18.3)	<0.001
Sigmoid colon	200 (30.8)	41 (23.9)	159 (33.1)	0.015
Descending colon	50 (7.7)	12 (7.3)	38 (7.9)	0.881
Splenic flexure	64 (9.8)	21 (12.7)	43 (9.0)	0.144
Transverse colon	50 (7.7)	13 (7.8)	37 (7.9)	1.000
Hepatic flexure	16 (2.5)	7 (4.4)	9 (1.9)	0.080
Ascending colon	90 (13.8)	32 (19.0)	58 (12.1)	0.019
Caecum	82 (12.6)	34 (20.0)	48 (10.0)	0.001
Synchronous tumour pTNM stage	6 (0.9)	2 (1.2)	4 (0.8)	0.456
I	118 (18.2)	27 (15.8)	91 (19.0)	0.418
II	235 (36.2)	65 (38.2)	170 (35.4)	0.517
III	297 (45.7)	78 (45.9)	219 (45.6)	1.000
Adjuvant chemotherapy	408 (62.8)	102 (60.0)	306 (63.8)	0.169

Values in parentheses are percentages unless indicated otherwise; *values are median (range). †χ2 test or Fisher's exact test, except ‡Mann–Whitney U test.

Table 2 Univariable logistic regression analysis of factors predicting disease-free survival

	Microsatellite instability		Microsatellite-stable	
	Hazard ratio	P	Hazard ratio	P
Age	0.997 (0.05, 1.04)	0.888	0.995 (0.97, 1.02)	0.747
Poor differentiation	1.185 (0.79, 1.79)	0.418	1.194 (0.98, 1.46)	0.082
Tumour budding	1.022 (0.19, 5.47)	0.980	1.133 (0.57, 2.19)	0.711
Signet ring morphology	2.933 (0.68, 12.65)	0.149	2.780 (1.17, 6.61)	0.021
Mucin ≥50 (per cent)	2.209 (0.74, 6.56)	0.153	1.718 (0.98, 3.02)	0.060
Lymphovascular invasion	3.357 (1.22, 9.25)	0.019	2.478 (1.67, 3.72)	<0.001
Extramural vascular invasion	4.494 (1.76, 12.55)	0.002	2.088 (1.38, 3.17)	0.001
Perineural invasion	2.682 (0.95, 7.54)	0.061	2.302 (1.52, 3.50)	<0.001
R0 resection	0.143 (0.01, 2.77)	0.198	0.510 (0.29, 0.89)	0.018
Node-positive (pN)	1.333 (0.50, 3.55)	0.565	1.171 (0.79, 1.74)	0.437

Values in parentheses are 95 per cent confidence intervals.

Discussion

The incidence of early-onset colorectal cancer is rising globally. Understanding the biological and pathological mechanisms is important for optimization of outcomes. This study compared the clinicopathological features and oncological outcomes of young patients with MSI and MSS colonic cancer. MSI was identified in one in four patients, and was associated with a family history of colorectal cancer, and lesions located in the proximal colon. Unlike in older age groups, there was no female preponderance, and the proportion of patients with pathological node-positive cancer was similar in the MSI and MSS groups. Patients with MSI had better disease-specific outcomes in all stages, although the differences observed were not statistically significant.

Multiple population-based studies, and systematic reviews[18–21] have shown that patients with MSI colorectal cancers have better stage-adjusted survival than those with MSS disease. This survival advantage is despite an increased likelihood of high T status, poor differentiation or lack of differentiation, and mucinous histology among MSI tumours, all of which are suggestive of unfavourable tumour biology[22]. Plausible reasons for the apparent favourable prognosis of MSI cancers include less nodal positivity[12]. In the present study, however, young patients with MSI tumours had the same rate of stage III disease as those with MSS disease.

Poor prognostic pathological features in all-age MSI colorectal cancer have historically not been associated with poor outcome. In the present study, different unfavourable histopathological features were identified in MSI and MSS cancers. Poor differentiation and signet ring morphology were associated with MSI, whereas lymphovascular and extramural venous invasion were more common in MSS tumours. The rates of tumour budding, a biomarker of metastatic potential and negative prognostic indicator[23,24], were similar. Several studies of all-age colorectal cancer have shown that tumour budding is less common in tumours with MSI[25,26]. BRAF and KRAS mutations were more frequent in MSI cancers, the clinical implication of which includes the potential for targeted molecular therapy.

Previous studies evaluating MSI as a prognostic marker have analysed patients of all ages, with relatively few cases of early-onset disease. The MSI group in the present study demonstrated better survival at all disease stages, although this did not reach statistical significance. Despite a relatively large cohort of patients, it is possible that the findings are due to a type II statistical error and lack of statistical power. The differences in survival rates however, are likely to be of clinical significance, in particular for patients with stage III disease, in whom the 5-year DFS rate was 80 per cent in the MSI group and 65 per cent in the MSS group. Larger numbers would be required to identify statistical significance.

Although the majority of young patients have sporadic disease, they are more likely to harbour genetic mutations and have a defined hereditary cancer syndrome than their older counterparts[27–29]. The prevalence estimates of hereditary cancer syndromes in patients with early-onset colorectal cancer range between 5 and 35 per cent, compared with 2–5 per cent of colorectal cancers overall[30–32]. MSI is a common feature of genetic predisposition, serving as a screening tool to identify patients who should undergo genetic testing for Lynch syndrome. Lynch syndrome, the most common hereditary cancer syndrome, is associated with a lifetime

risk of colorectal cancer of between 50 and 70 per cent, and accounts for one-third of colorectal cancer cases in people aged less than 35 years[32,33]. In the present series, pathogenic constitutive variants were six times more common in the MSI group (1 in 3 patients) than the MSS group (1 in 20 patients). The clinical implications of identification of genetic predisposition include increased cancer surveillance, the potential for prophylactic risk-reducing surgery, and testing of at-risk relatives.

This study has limitations, including its retrospective nature, lack of a complete data set for the entire study group, and heterogeneity in treatment across the collaborative group. Larger numbers would be required to detect statistical significance in survival between patients with MSI and MSS tumours. Nonetheless, this study presents large-volume real-world data. Routine assessment of MSI for all colorectal cancers (regardless of family history) has been introduced only recently in many institutions, and data on young patients with MSI colonic cancer are lacking. Importantly, early-onset MSI tumours appear to show several differences compared with later-onset disease. Unlike in older age groups, MSI cancers in young patients are not associated with a female preponderance, and exhibit rates of node positivity and tumour budding similar to those of MSS tumours. They are more likely to have BRAF and KRAS mutations representing potential therapeutic targets. Despite the presence of these negative histopathological and molecular features, disease-specific survival is better than that for patients with MSS cancers. Increased understanding of the biological spectrum of MSI will guide oncotherapeutic decision-making and optimize survivorship.

Collaborators

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