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Is MSI-H of value in predicting the development of metachronous colorectal cancer?

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ABSTRACT

Nearly 10% of patients with colorectal cancer (CRC) develop a metachronous cancer after curative resection of their primary malignancy, however identifying these patients is problematic. Although microsatellite instability (MSI) is associated with the development of multiple CRC, this is predominantly seen in those with hereditary non-polyposis colon cancer (HNPCC). This study has examined the value of MSI analysis in identifying patients at risk of developing metachronous cancer from the general population. MSI analysis was performed at the *Bat25*, *Bat26*, *Bat40*, *D2S123*, *D5S346* and *D17S250* loci using polymerase chain reaction and single-stranded conformational polymorphism on DNA extracted from 62 specimens taken from 49 patients with metachronous CRC, and from 71 primary single CRCs. MSI status was classified into MSI-H, MSI-L and MSS. MSI-H was more prevalent in metachronous cancers, 34/62 compared to 8/71 single cancers ($P < 0.0001$). The incidence of MSI-H from proximal colon cancers in index metachronous group, 4/22 was similar to single cancer group, 7/71 ($P = 0.28$), however MSI-H was more commonly identified in index metachronous cancers located distal to the splenic flexure 9/22 than single cancers 1/71 ($P < 0.0001$). Patients presenting with MSI-H colorectal cancers distal to the splenic flexure are more likely to develop a metachronous cancer and will benefit from surveillance.

1. Introduction

Metachronous colorectal cancer (CRC) occurs in nearly 10% of patients[1] and although some have an underlying genetic predisposition such as familial adenomatous polyposis coli (FAP) or hereditary non-polyposis colon cancer (HNPCC) these conditions account for only 2–3% of colorectal cancer in the general population.[2–4] Accurately identifying those at risk of developing a metachronous cancer has important implications in their management but remains problematic.

Studies have reported greater incidence of high-level microsatellite instability (MSI-H) in either synchronous or metachronous CRC than in single CRC.[5,6] Although many of these studies made no allowance for HNPCC, in which >90% of cancers demonstrate MSI-H, others that excluded HNPCC based on family history, have confirmed an association between MSI-H and multiple cancers.[7,8] Since 15% of CRC from patients who develop a single cancer demonstrate MSI-H,[9] this finding alone is an unreliable indicator for the risk of metachronous cancer however, the site of the presenting MSI-H cancer may be predictive. The majority of single CRC with MSI-H occur in the right side of the colon, proximal to the splenic flexure[10–12] with studies suggesting an increased risk of metachronous cancer in patients with a primary MSI-H cancer distal to the splenic flexure.[8,13] This study investigated the value of MSI-H in

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predicting the risk of metachronous CRC in the general population.

2. Patients and methods

2.1. Patients

Patients who developed metachronous CRC in South East England between 1972 and 1997, were identified from the Thames Cancer Registry. A metachronous cancer was defined as a second CRC resected at least 36 months after the presenting (index) cancer. Regional Ethics Committee approval was granted to obtain paraffin embedded cancer tissue and normal mucosa however, obtaining family history data was expressly forbidden. Sixty-two paraffin embedded archival samples of cancers and corresponding normal mucosa were obtained from 49 patients. In 22 cases only the index cancer, and in 40 cases only the second cancer, was available for analysis. In 13 patients, both first and second cancers were analysed. As a control group, paraffin embedded samples were also obtained from 71 patients who underwent colonic resection for a single cancer. These patients had been followed-up for a minimum of 7 years. This time interval was chosen since it was the median interval between resection of the index cancer and a second cancer seen in the metachronous group (range 3–23 years) with 65% of patients developing a second cancer within this period. Patients who died during follow-up were included to ensure that tumours with a good prognosis were not over represented within the group. Patient's age, time of colonic resection and the cancer site was recorded. Cancers were classified as right sided when proximal and left sided when distal to the splenic flexure and rectal if below the sacral promontory.

2.2. Analysis of MSI

DNA was extracted from the paraffin embedded tissue using standard techniques[6] and amplification of the microsatellite DNA performed by polymerase chain reaction (PCR) at the mononucleotide loci *Bat 26*, *Bat 25* and *Bat 40* and the dinucleotide loci *D2S123*, *D5S346* and *D17S250* in accordance with the National Cancer Institute's guidelines.[9] Each PCR reaction mixture consisted of: 2′-deoxynucleoside 5′-triphosphates 0.2 mmol/l (Amersham Pharmacia, Biotech, St. Albans, UK); 1 × PCR buffer containing Tris–HCl 50 mmol/l (pH 9); KCl 50 mmol/l; $MgCl_2$ 7 mmol/l; $(NH_4)_2SO_4$ 16 mmol/l (HT Biotechnologies, Cambridge, UK): 0.5 U SuperTaq polymerase (HT Biotechnologies); 25 pmoles of each primer and 200 ng of genomic DNA and sterile water to make a uniform volume of 50 μl. The reaction was performed using an Omnigene thermal cycler (Hybaid, Teddington, UK). PCR products were analysed by single-stranded conformational polymorphism (SSCP) using ExcelGel® DNA Analysis Kit (Pharmacia Biotech) and a Plus One™ DNA silver staining kit (Pharmacia Biotech). MSI was identified when a characteristic ladder pattern was identified in tumour DNA that was not present in corresponding normal mucosa. Cancers which demonstrated MSI in >30% of the loci investigated were designated as showing high-level instability (MSI-H), those with MSI in <30% of the loci were considered to show low-level instability (MSI-L) and those failing to demonstrate instability at any locus were classified as stable (MSS), in accordance with guidelines laid down by the National Cancer Institute workshop on MSI.[9]

2.3. Statistical analysis

Data were analysed using Mann–Whitney U test and Fisher's exact test. All statistical analysis was performed using GraphPad InStat™ version 3.00 for Windows 95, GraphPad Software, San Diego, CA, USA.

3. Results

The median age at diagnosis of the single cancers 74 (range 38–100 years), was higher than of the index cancers 66.5 (range 40–80 years), $P = 0.016$, but comparable to that of the second cancers 73 (range 60–91 years), $P = 0.68$ (Table 1). The site distribution of cancers was comparable with 36/60 (60%) metachronous and 42/71 (59%) single cancers located distal to the splenic flexure, $P = 0.9$; the site of two second cancers was unknown. MSI-H was more prevalent in metachronous cancers 34/62 (55%) than single cancers, 8/71 (11%), $P < 0.0001$. The incidence of MSI-H was similar in the index 13/22 (59%) and second, 21/40 (52%) cancers from the metachronous group, $P = 0.8$. While MSI-H metachronous cancers were evenly distributed throughout the colon, with 14/34 (41%) proximal, 15/34 (44%) distal to the splenic flexure and 5/34 (15%) rectal, MSI-H single cancers were mostly located in the right colon with, 7/8 (87.5%) proximal to the splenic flexure and 1/7 (12.5%) rectal (Fig. 1). Although there was no difference in the incidence of MSI-H in index or single cancers presenting proximal to the splenic flexure (4/22 vs. 7/71, $P = 0.28$), MSI-H was more commonly identified in index cancers than single cancers arising distal to the splenic flexure (9/22 vs. 1/71, $P < 0.0001$). This difference remained if cancers of the distal colon (6/11 vs. 0/20, $P = 0.0006$) or rectum (3/5 vs. 1/22, $P = 0.012$) were considered separately. Of 13 paired metachronous CRC, MSI-H was demonstrated in both cancers from four patients and in three of these cases, the index cancers presented distal to the splenic flexure. In four patients, only one of the paired cancers demonstrated MSI-H, and this was an index cancer presenting

Table 1 – Age, site distribution and MSI status of cancers

	Index ($n = 22$)	Metachronous ($n = 40$)	Single ($n = 71$)
Median age (yrs)	66.5 (38–100)	73 (60–91)	74 (38–100)
Site distribution[a]			
Proximal	6 (27%)	18 (47%)	29 (41%)
Distal	11 (50%)	15 (40%)	20 (28%)
Rectal	5 (23%)	5 (13%)	22 (31%)
MSI-H distribution			
Proximal	4/6 (66%)	10/18 (55%)	7/29 (24%)
Distal	6/11 (54%)	11/15 (73%)	0/20 (0%)
Rectal	3/5 (60%)	2/5 (40%)	1/22 (4.5%)

a Sites of two metachronous cancers were unknown.

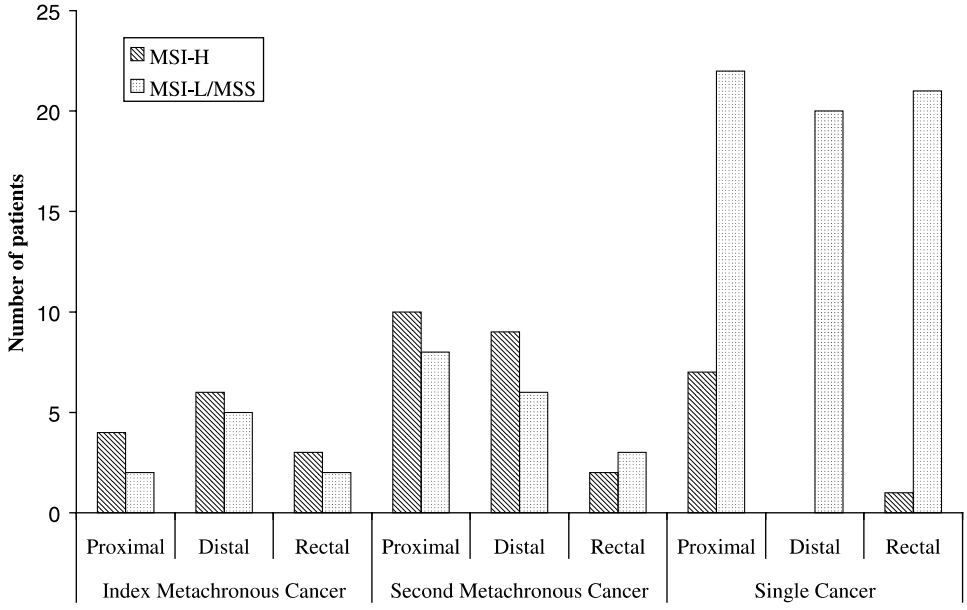

Fig. 1 – MSI status and site of metachronous and single colorectal cancers.

distal to the splenic flexure. In the remaining five patients neither cancer showed MSI-H.

4. Discussion

MSI has been reported in 70–89% of cancers from patients who develop multiple CRCs compared to 7–11% with single cancers.[5,6] However, these studies did not account for the possibility of an underlying genetic predisposition, namely HNPCC in which 90% of cancers demonstrate MSI-H, of whom around 45% develop a metachronous CRC following curative segmental resection.[14,15] Subsequent work, that excluded those with family history consistent with HNPCC reported MSI-H in 26–33% of multiple cancers compared to 0–8% with single cancers,[7,16,17] although this has been disputed.[18] Our findings confirm that MSI-H is more commonly identified in those with metachronous cancers than single cancers. Although the finding of MSI-H alone is not specific enough to predict the development of metachronous cancer, the site distribution of the MSI-H malignancy may be of use. It is known that 90% of MSI-H CRCs arising in those without HNPCC occur proximal to the splenic flexure.[11] In this series, comparisons between the incidence of MSI-H in single and index cancers occurring in the proximal colon suggest no difference, although MSI-H is more commonly seen in metachronous cancers located distal to the splenic flexure, a finding that is rare in patients with single cancers.

These results are in accordance with those of Masubuchi and colleagues who detected MSI-H in 33% of left sided cancers in patients who developed metachronous cancers, and none in patients who had a single cancer, having excluded HNPCC on the basis of family history.[13] Due to the lack of family history, our study can not exclude the possibility that those with MSI-H cancers distal to the splenic flexure are unrecognized cases of HNPCC. Although recent work has suggested that patients who develop multiple colorectal cancers, despite demonstrating an increased incidence of MSI-H, are unlikely to develop via the HNPCC pathway.[19,20] It is of note that three of four patients with paired MSI-H cancers presented with index cancers distal to the splenic flexure, these four patients have a genotype that would be consistent with HNPCC. In addition the incidence of MSI-H in rectal cancer is low but strongly associated with inherited *MMR* gene mutations. Nilbert et al. identified MSI-H in 3/165 (2%) consecutive rectal cancers, identifying germline mutations in *hMLH1* or *hMSH2* compatible with HNPCC in two of the cases.[21] The high incidence of MSI-H rectal cancers in the index and metachronous group in this study is suspicious of HNPCC.

Although the underlying genetic cause for the increased incidence of MSI-H cancers from patients who develop multiple colorectal cancers remains unclear, identifying an MSI-H cancer distal to the splenic flexure suggests that these patients are at risk of developing metachronous cancer. In such cases, a thorough family history should be obtained and the possibility of HNPCC should be excluded. In the event that HNPCC is not identified these patients may still benefit from close post-operative surveillance.

Conflict of interest statement

None declared.

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