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Mismatch repair status in patients with primary operable colorectal cancer: associations with the local and systemic tumour environment

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Background: Mismatch repair-deficient (dMMR) colorectal cancer (CRC) is associated with a conspicuous local immune infiltrate; however, its relationship with systemic inflammatory responses remains to be determined. The present study aims to examine the relationships and prognostic value of assessment of the local and systemic environment in the context of MMR status in patients with CRC.

Methods: The relationship between MMR status, determined using immunohistochemistry, and the local inflammatory cell infiltrate, differential white cell count, neutrophil : platelet score (NPS), neutrophil : lymphocyte ratio and modified Glasgow Prognostic Score (mGPS), and cancer-specific survival was examined in 228 patients undergoing resection of stage I–III CRC.

Results: Thirty-five patients (15%) had dMMR CRC. Mismatch repair deficiency was associated with a higher density of $CD3^+$, $CD8^+$ and $CD45R0^+$ T lymphocytes within the cancer cell nests and an elevated mGPS (mGPS2: 23% *vs* 9%, $P=0.007$) and NPS (NPS2: 19% *vs* 3%, $P=0.001$). $CD3^+$ density ($P<0.001$), mGPS ($P=0.01$) and NPS ($P=0.042$) were associated with survival independent of MMR status ($P=0.367$) and stratified 5-year survival of patients with MMR-competent CRC from 94% to 67%, 83% to 46% and 78% to 60% respectively.

Conclusions: Mismatch repair deficiency was associated with local and systemic environments, and in comparison with their assessment, dMMR had relatively poor prognostic value in patients with primary operable CRC. In addition to MMR status, local and systemic inflammatory responses should be assessed in these patients.

Colorectal cancer (CRC) is the second most common cause of cancer-related death in the United Kingdom (Ferlay *et al*, 2013). Although prognosis and the need for post-operative treatment are presently determined by pathological staging, obvious heterogeneity in outcome exists among patients with similar disease stage (Horgan and McMillan, 2010). Indeed, other tumour-associated characteristics, intrinsic to the tumour cell and pertaining to both the tumour microenvironment and the patient, may similarly influence oncological outcome and be used to determine the need for further treatment (McAllister and Weinberg, 2014).

One such tumour characteristic is loss of mismatch repair (MMR) protein activity. Approximately 15–18% of tumours arise

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through genomic instability as a result of loss of MMR competency, whereas 2% of MMR-deficient (dMMR) tumours occur through inherited germline mutations, the remaining 13–15% account for sporadic cases of CRC, often as a result of hypermethylation-induced silencing of the *hMLH1* promoter region (Boland and Goel, 2010). Tumours arising through dMMR activity accumulate mutations at an exponential rate, in particular within repeating microsatellite regions, and are characterised by the presence of MSI as well as distinct phenotypic characteristics, such as proximal tumour location and poor or mucinous differentiation (Ward *et al*, 2001; Jass *et al*, 2002; Greenson *et al*, 2009). Furthermore, dMMR status is associated with improved survival, in particular in patients with Stage II/III CRC (Popat *et al*, 2005; Guastadisegni *et al*, 2010; Saridaki *et al*, 2014).

In addition to such phenotypic characteristics, dMMR CRC is associated with characteristic features within the tumour microenvironment; in particular, the presence of a high density of tumour-infiltrating lymphocytes, a stage-independent predictor of increased survival in patients with CRC (Mei *et al*, 2014), has been consistently reported in this patient group (Smyrk *et al*, 2001; Ward *et al*, 2001; Greenson *et al*, 2009; De Smedt *et al*, 2015). Furthermore, the presence of a low proportion of tumour-associated stroma has similarly been associated with both favourable prognosis and dMMR status (Huijbers *et al*, 2013). Indeed, it has previously been suggested that the improved prognosis attributed to dMMR status may not be independent of such favourable characteristics within the tumour microenvironment (Ogino *et al*, 2009; Deschoolmeester *et al*, 2011; Huijbers *et al*, 2013).

Despite extensive characterisation of the tumour microenvironment, it is of interest that the relationship between MMR status and the systemic environment remains to be fully defined. Dysregulated systemic inflammatory responses promote cancer progression (McAllister and Weinberg, 2014) and the presence of a systemic inflammatory response, as measured by routinely available biomarkers, such as circulating acute-phase proteins and components of the differential white cell count, is associated with reduced survival independent of pathological staging (Roxburgh and McMillan, 2010). Given the favourable prognosis associated with dMMR status, it would be expected that patients with tumours arising through this pathway would be less likely to exhibit evidence of a cancer-associated systemic inflammatory response at diagnosis. Therefore, the aim of the present study was to characterise the relationships between MMR status, host local and systemic inflammatory responses and survival of patients undergoing elective, potentially curative resection of CRC.

MATERIALS AND METHODS

Clinicopathological characteristics. Patients were identified from a prospectively collected and maintained database of elective and emergency CRC resections in a single surgical unit at Glasgow Royal Infirmary. Patients who, on the basis of preoperative thoracoabdominal computed tomography and laparotomy findings, were considered to have undergone elective, potentially curative resection of Stage I–III CRC between January 1997 and May 2007, and whose tumour resection was included in a previously constructed CRC tissue microarray (TMA) were included. Exclusion criteria were as follows: (1) emergency resection, (2) inflammatory bowel disease-related CRC or known hereditary CRC syndrome, (3) pre-operative chemoradiotherapy, (4) surgery with palliative intent and (5) death within 30 days of surgery.

Tumours were routinely staged by gastrointestinal pathologists using the fifth edition of the tumour, node and metastases classification as is the current practice in the United Kingdom (Loughrey *et al*, 2014). Tumour differentiation, graded as well/moderate or poor in accordance with Royal College of Pathologists guidelines (Loughrey *et al*, 2014), and additional data were taken from pathological reports issued following resection. At multidisciplinary meetings following surgery, patients with stage III and high-risk stage II disease were considered for 5-fluorouracil-based adjuvant chemotherapy according to treatment guidelines at the time. Patients were routinely followed up for 5 years following surgery. Date and cause of death were cross-checked with the cancer registration system and the Registrar General (Scotland), and death records were complete until 31 March 2014 that served as the censor date. Cancer-specific survival was measured from date of index surgery until the date of death from recurrent or metastatic disease. Patients were censored at date of non-CRC death or date of last follow-up.

Assessment of the tumour microenvironment. Using routine haematoxylin and eosin-stained sections of the deepest point of invasion, the generalised inflammatory cell infiltrate at the invasive margin was assessed using Klintrup–Mäkinen (KM) grade and the extent of tumour stroma was assessed using tumour stroma percentage (TSP), both as previously described (Klintrup *et al*, 2005; Mesker *et al*, 2007). Briefly, using KM grade, the inflammatory cell density at the invasive margin was graded as either low-grade (no increase or mild/ patchy increase in inflammatory cells) or high-grade (prominent inflammatory reaction forming a band at the invasive margin or florid cup-like infiltrate at the invasive edge with destruction of cancer cell islands) (Klintrup *et al*, 2005; Roxburgh *et al*, 2009). Tumour stroma percentage was graded as either low ($\leqslant$50%) or high ($>$50%) based on previously derived thresholds (Mesker *et al*, 2007; Park *et al*, 2014).

Tumour-infiltrating T-lymphocyte density at the invasive margin and within the cancer cell nests was assessed using immunohistochemistry as previously described (Richards *et al*, 2014b). Briefly, tumour sections were stained for $CD3^+$ (mature T lymphocyte), $CD8^+$ (cytotoxic T-lymphocyte), $CD45R0^+$ (memory T lymphocyte) and $FOXP3^+$ (regulatory T lymphocyte), and the density of each lymphocyte subset within each compartment graded semi-quantitatively as low (absent or weak) or high (moderate or strong). Investigators blinded to clinicopathological and outcome data performed all assessments with co-scoring by two investigators for immunohistochemistry staining and the KM grade in 100 cases and TSP in 30 cases, to ensure consistency of scoring.

Assessment of the systemic inflammatory responses. Pre-operative C-reactive protein (CRP), serum albumin and differential white cell count measured within 30 days before surgery were recorded prospectively. On the basis of previously derived thresholds, neutrophil count $>7.5\times10^9\,l^{-1}$, lymphocyte count $>4\times10^9\,l^{-1}$ and platelet count $>400\times10^9\,l^{-1}$ were considered elevated (Watt *et al*, 2015a). The modified Glasgow Prognostic Score (mGPS) was calculated as previously described (Park *et al*, 2016); patients with a normal CRP ($\leqslant10\,mg\,l^{-1}$) were allocated a score of 0, an elevated CRP ($>10\,mg\,l^{-1}$) alone a score of 1 and an elevated CRP and low albumin ($<35\,g\,l^{-1}$) a score of 2. The neutrophil : platelet score (NPS) was calculated as previously described (Watt *et al*, 2015b); patients with a normal platelet count and neutrophil count were allocated a score of 0, either a neutrophil count $>7.5\times10^9\,l^{-1}$ or platelet count $>400\times10^9\,l^{-1}$ a score of 1 and those with both an elevated neutrophil and platelet count a score of 2.

Assessment of MMR status. Previously constructed TMAs, comprising four 0.6-mm cores of formalin-fixed paraffin-embedded cancer tissue per patient, were used to assess MMR

status (Roxburgh *et al*, 2013). Tissue microarray slides were placed in a ThermoFisher pH 9 PT module solution (Thermo Fisher Scientific Inc., Waltham, MA, USA) at room temperature. Slides were then heated in the PT module to a temperature of 96 °C for 20 min and allowed to cool. Using the ThermoFisher autostainer, slides were incubated in peroxidase block for 5 min and rinsed with TBS before incubating in UV protein blocker for 5 min and rinsing once again with TBS solution. Slides were then incubated in primary antibody for 20 min at a concentration of 1 : 100 for MLH1 and MSH6, and 1 : 50 for MSH2 and PMS2 (product codes: M3640, M3646, M3639 and M3647, respectively; Dako UK Ltd, Cambridgeshire, UK). Following this incubation period, slides were rinsed with TBS and Quanto Amplifier (Thermo Fisher Scientific Inc.) was applied to slides for 10 min followed by a further wash with TBS. Quanto Polymer was then added for 10 min followed by a TBS wash. DAB Quanto substrate was then added for 5 min, slides washed in TBS, counterstained in haematoxylin, blued in Scotts' tap water, dehydrated through a series of graded alcohols and cover slips applied with DPX mounting medium.

Mismatch repair protein expression was established by a single observer (AGP) blinded to clinical outcomes using UK NEQAS scoring guidelines (Arends *et al*, 2008). Appendix and normal colon were used as positive controls and positive staining within intra-tumoural immune cells serving as an internal positive control. An observer blinded to clinical outcome (JHP) scored 10% of cores. Expression was reported as MMR proficient (tumour cell nuclear expression with positive immune cell expression) or MMR deficient (absent tumour nuclear expression with normal immune cell expression). The use of multiple TMA cores per patient has been shown to be comparable to the use of full sections, even in the presence of known intra-tumoural heterogeneity of protein expression (Zhang *et al*, 2003). In the present study, four cores were examined per patient for each MMR protein; TMA assessment of MLH1 and MSH2 using three cores per patient has previously been shown to be comparable to full section analysis (Jourdan *et al*, 2003).

Statistical analysis. The relationship between MMR status, clinicopathological characteristics and the local and systemic inflammatory responses was examined using the χ^2 method for linear trend for categorical variables and Mann–Whitney *U*-test for continuous variables. The relationship between MMR status, local and systemic inflammatory characteristics associated with MMR status and survival was examined by Kaplan–Meier log-rank survival analysis and Cox proportional hazards regression using a multivariate backwards conditional model to calculate hazard ratios and 95% confidence intervals. Variables with a $P \leqslant 0.05$ on univariate analysis were entered into a multivariate model. A *P*-value $\leqslant 0.05$ was considered statistically significant. All analyses were performed using SPSS version 22.0 (IBM SPSS, Armonk, NY, USA). The West of Scotland Research Ethics Committee approved the study and tissue for analysis of MMR status was obtained from the National Health Service Greater Glasgow and Clyde Tissue Biorepository.

RESULTS

A total of 228 patients who underwent elective, potentially curative resection of stage I–III CRC were included. Almost two thirds of patients were older than 65 years at the time of surgery and 53% were male. Pathological assessment confirmed Stage I disease in 16 patients (7%), stage II disease in 111 patients (49%) and stage III disease in 101 patients (44%). Sixty-six patients (29%) received adjuvant therapy; 1 patient with stage I disease, 15 patients with stage II disease and 50 patients with stage III disease received adjuvant therapy. Mismatch repair deficiency was identified in 35 patients (15%); the frequency of aberrant MMR protein expression in patients with dMMR CRC is displayed in Table 1.

Table 1. Pattern of aberrant MMR protein expression

Aberrant protein expression	Number of patients
MLH1/PMS2	17
MSH6/MSH2	8
PMS2	7
MSH6	1
PMS2/MSH6	1
PMS2/MSH6/MSH2	1

Abbreviations: CRC = colorectal cancer; dMMR = mismatch repair deficient; MMR = mismatch repair. Pattern of aberrant MMR protein expression in patients undergoing elective, potentially curative resection of dMMR I–III CRC.

Mismatch repair status and clinicopathological characteristics. The relationship between MMR status and clinicopathological characteristics is displayed in Table 2. Patients with dMMR CRC were more likely to have a colonic primary and poor tumour differentiation (both $P < 0.05$). In addition, although not associated with T stage, dMMR status was associated with an increased rate of peritoneal involvement ($P < 0.05$). Detection of dMMR did not differ with year of diagnosis ($P = 0.290$). Furthermore, the age of patients with dMMR CRC did not differ significantly from those with MMR-competent cancer ($P = 0.707$). As such, it is unlikely that a significant proportion of included patients had Lynch syndrome cancer.

Mismatch repair status and the tumour microenvironment. The relationship between MMR status and the tumour microenvironment is displayed in Table 3. Patients with dMMR CRC had an increased density of $CD3^+$ ($P < 0.01$), $CD45R0^+$ ($P < 0.05$) and $CD8^+$ ($P = 0.071$) T lymphocytes within the cancer cell nests. Although not reaching statistical significance, patients with dMMR CRC were less likely to have a high TSP (15% *vs* 28%, $P = 0.118$). The density of $FOXP3^+$ T lymphocytes within the cancer cell nests, density of T lymphocytes at the invasive margin nor the KM grade showed significant association with MMR status.

Mismatch repair status and systemic inflammatory responses. The relationship between MMR status and host systemic inflammatory responses is displayed in Figure 1 and Table 4. Patients with dMMR CRC had a higher median pre-operative CRP ($P < 0.001$) and neutrophil count ($P < 0.05$), and showed a trend towards a higher median platelet count ($P = 0.091$). Serum albumin concentrations and circulating lymphocyte count did not differ with MMR status. Patients with dMMR CRC were more likely to have a neutrophil count $> 7.5 \times 10^9 l^{-1}$ ($P < 0.01$) and platelet count $> 400 \times 10^9 l^{-1}$ ($P < 0.05$). In addition, both the mGPS and NPS were more likely to be elevated in patients with dMMR CRC (both $P < 0.01$).

Mismatch repair status and survival. The relationship between MMR status, characteristics of the local and systemic inflammatory responses significantly associated with MMR status and cancer-specific survival was subsequently examined (Table 5). The median follow-up of survivors was 143 months (range 87–206 months) with 66 cancer-specific deaths and 5-year cancer-specific survival of 76%. On multivariate survival analysis, dMMR was not significantly associated with cancer-specific survival ($P = 0.790$), whereas the density of $CD3^+$ T lymphocytes within the cancer cell nests ($P < 0.001$), mGPS ($P < 0.01$) and NPS ($P < 0.05$) were independently associated with survival. When analysis was restricted to patients with stage II/III disease only, cancer cell nest

Table 2. Relationship between MMR status and clinicopathological characteristics

Host characteristics	All $n=228$ (%)	MMR competent $n=193$ (%)	dMMR $n=35$ (%)	*P*-value
Age (years)				
<65	83 (36)	71 (37)	12 (34)	0.707
65–74	73 (32)	62 (32)	11 (32)	
>75	72 (32)	60 (31)	12 (34)	
Sex				
Male	108 (47)	92 (48)	16 (46)	0.832
Female	120 (53)	101 (52)	19 (54)	
Diagnosis year				
1997–2002	142 (62)	123 (64)	19 (54)	0.290
2003–2007	86 (38)	70 (36)	16 (46)	
Adjuvant therapy				
No	162 (71)	135 (70)	27 (77)	0.389
Yes	66 (29)	58 (30)	8 (23)	
Tumour characteristics				
Tumour site				
Colon	151 (66)	122 (63)	29 (83)	0.024
Rectum	77 (34)	71 (37)	6 (17)	
TNM stage				
I	25 (11)	21 (11)	4 (11)	0.037
II	141 (62)	124 (64)	17 (49)	
III	62 (27)	48 (25)	14 (40)	
T stage				
1–2	127 (55)	105 (54)	22 (63)	0.160
3	77 (34)	65 (34)	12 (34)	
4	24 (11)	23 (12)	1 (3)	
N stage				
0	16 (7)	14 (7)	2 (6)	0.539
1	111 (49)	91 (47)	20 (57)	
2	101 (44)	88 (46)	13 (37)	
Differentiation				
Moderate/well	200 (88)	173 (90)	27 (77)	0.039
Poor	28 (12)	20 (10)	8 (23)	
Venous invasion				
Absent	148 (65)	123 (64)	25 (71)	0.381
Present	80 (35)	70 (36)	10 (29)	
Margin involvement				
Absent	215 (94)	182 (94)	33 (94)	0.997
Present	13 (6)	11 (6)	2 (6)	
Peritoneal involvement				
Absent	165 (72)	145 (75)	20 (57)	0.029
Present	63 (28)	48 (25)	15 (43)	
Tumour perforation				
Absent	223 (98)	188 (97)	35 (100)	0.337
Present	5 (2)	5 (3)	0 (0)	

Abbreviations: CRC = colorectal cancer; dMMR = mismatch repair deficient; MMR = mismatch repair; TNM, tumour, node, metastasis. The relationship between MMR status and clinicopathological characteristics of patients undergoing elective, potentially curative resection of stage I–III CRC.

Table 3. Relationship between MMR status and tumour microenvironment

Tumour microenvironment	All $n=228$ (%)	MMR competent $n=193$ (%)	dMMR $n=35$ (%)	*P*-value
KM grade				
Weak	77 (34)	63 (33)	14 (40)	0.398
Strong	151 (66)	130 (67)	21 (60)	
CD3 margin density (215)				
Low	118 (55)	100 (55)	18 (56)	0.867
High	97 (45)	83 (45)	14 (44)	
CD3 cancer cell nest density (224)				
Low	146 (65)	130 (69)	16 (46)	0.009
High	78 (35)	59 (31)	19 (54)	
CD8 margin density (216)				
Low	127 (59)	105 (57)	22 (67)	0.319
High	9 (41)	78 (43)	11 (33)	
CD8 cancer cell nest density (222)				
Low	161 (72)	140 (75)	21 (60)	0.071
High	61 (28)	47 (25)	14 (40)	
CD45R0 margin density (217)				
Low	112 (52)	96 (53)	6 (47)	0.564
High	105 (48)	87 (47)	18 (53)	
CD45R0 cancer cell nest density (224)				
Low	160 (71)	141 (75)	19 (54)	0.015
High	64 (29)	48 (25)	16 (46)	
FOXP3 margin density (216)				
Low	126 (58)	104 (57)	22 (65)	0.413
High	90 (42)	78 (43)	12 (35)	
FOXP3 cancer cell nest density (219)				
Low	110 (50)	92 (50)	18 (53)	0.731
High	109 (50)	93 (50)	16 (47)	
TSP (225)				
Low	166 (74)	138 (72)	28 (85)	0.118
High	59 (26)	54 (28)	5 (15)	

Abbreviations: CRC = colorectal cancer; dMMR = mismatch repair deficient; KM = Klintrup–Mäkinen; MMR = mismatch repair; TSP = tumour stroma percentage. The relationship between MMR status and tumour microenvironment of patients undergoing elective, potentially curative resection of stage I–III CRC.

$CD3^+$ T-lymphocyte density ($P<0.001$), mGPS and NPS (both $P<0.05$) remained associated with survival independent of MMR status ($P=0.833$).

As cancer cell nest density of $CD3^+$ T lymphocytes, mGPS and NPS were all associated with survival independent of MMR status, the relationship between these characteristics and cancer-specific survival of patients with MMR-competent CRC was subsequently examined (Figure 2). Five-year cancer-specific survival was stratified from 94% to 67% by cancer cell nest $CD3^+$ T-lymphocyte density ($P<0.001$), from 83% to 46% by mGPS ($P=0.002$) and from 78% to 60% by NPS ($P=0.054$).

DISCUSSION

The present study describes the distinct tumour and host phenotypic characteristics associated with MMR deficiency in patients undergoing elective, potentially curative resection of CRC. Patients with dMMR CRC were more likely to have a high density of T lymphocytes within the tumour microenvironment and evidence of an elevated host systemic inflammatory response as evidenced by components of the differential white cell count and serum acute phase proteins. Furthermore, these characteristics were associated with cancer-specific survival independent of MMR status. Taken together with the previous literature (Ogino *et al*, 2009; Deschoolmeester *et al*, 2010; Huijbers *et al*, 2013; Vayrynen *et al*, 2014; Park *et al*, 2015b), this provides further evidence that the prognostic benefit associated with dMMR CRC is not necessarily independent of such characteristics.

Patients with dMMR CRC were more likely to have a high density of intratumoural $CD3^+$, $CD8^+$ and $CD45R0^+$ T lymphocytes; however, dMMR status did not appear to influence $FOXP3^+$ T-regulatory lymphocyte density. Furthermore, it was of interest that the inflammatory cell infiltrate at the invasive margin, as measured by either T-lymphocyte density or KM grade, did not differ with MMR status. Given that the KM grade is reflective of

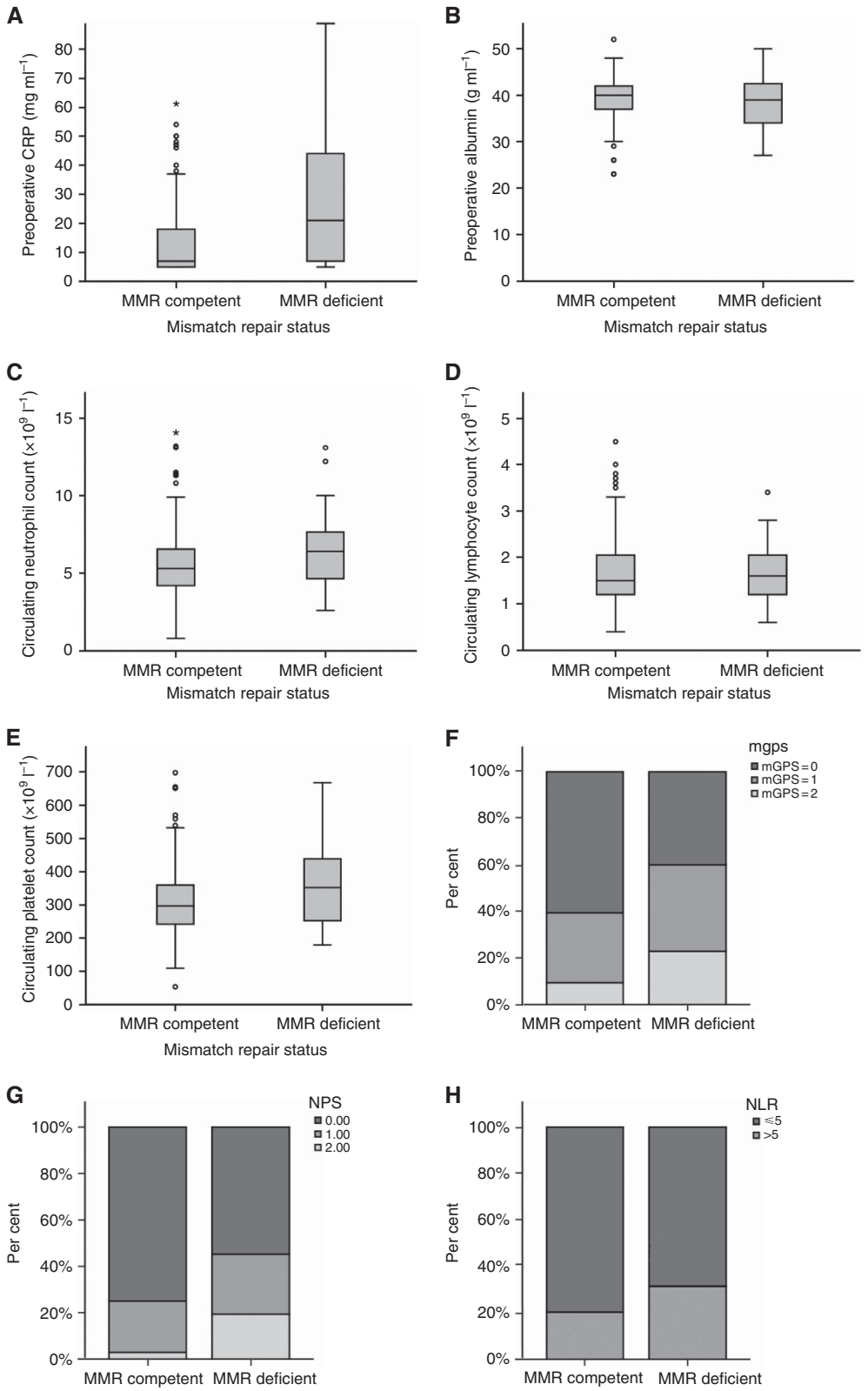


Figure 1. Relationship between MMR status and host systemic inflammatory responses. The relationship between MMR status and host systemic inflammatory responses in patients undergoing elective, potentially curative resection of stage I–III CRC (**A**) serum CRP ($P<0.001$), (**B**) serum albumin ($P=0.258$), (**C**) circulating neutrophil count ($P=0.032$), (**D**) circulating lymphocyte count ($P=0.669$), (**E**) circulating platelet count ($P=0.091$), (**F**) mGPS ($P=0.007$), (**G**) NLS ($P=0.001$), and (**H**) neutrophil : lymphocyte ratio (NLR; $P=0.145$). Boxplots represent median value and interquartile range.

components of both adaptive and innate local immune responses (Vayrynen *et al*, 2013; Park *et al*, 2015a), the present study would favour an association between dMMR status and development primarily of a co-ordinated, adaptive intratumoural immune response. Indeed, this is consistent with recent work addressing the nature of the immune microenvironment in patients with

Table 4. Relationship between MMR status and systemic inflammatory responses

Systemic inflammatory responses	All $n=228$ (%)	MMR competent $n=193$ (%)	dMMR $n=35$ (%)	*P*-value
Serum CRP				
$mg\,l^{-1}$	8 (6–20)	7 (5–18)	21 (7–48)	<0.001
Serum albumin				
$g\,l^{-1}$	40 (36–42)	40 (37–42)	39 (34–43)	0.258
Modified Glasgow Prognostic Score				
0	131 (58)	117 (61)	14 (40)	0.007
1	71 (31)	58 (30)	13 (37)	
2	26 (11)	18 (9)	8 (23)	
Neutrophil count (227)				
$\times 10^9\,l^{-1}$	5.4 (4.3–6.7)	5.3 (4.2–6.6)	6.4 (4.6–7.7)	0.032
Lymphocyte count (227)				
$\times 10^9\,l^{-1}$	1.5 (1.2–2.1)	1.5 (1.2–2.1)	1.6 (1.2–2.1)	0.891
Platelet count (207)				
$\times 10^9\,l^{-1}$	300 (245–369)	296 (242–360)	352 (251–441)	0.091
Neutrophil count (227)				
$\leqslant 7.5\times 10^9\,l^{-1}$	192 (85)	168 (87)	24 (69)	0.004
$>7.5\times 10^9\,l^{-1}$	35 (15)	24 (13)	11 (31)	
Lymphocyte count (227)				
$\leqslant 4\times 10^9\,l^{-1}$	171 (83)	191 (99)	35 (100)	0.669
$>4\times 10^9\,l^{-1}$	36 (17)	1 (1)	0 (0)	
Platelet count (207)				
$\leqslant 400\times 10^9\,l^{-1}$	226 (99)	150 (85)	21 (68)	0.018
$>400\times 10^9\,l^{-1}$	1 (1)	26 (15)	10 (32)	
NLR (227)				
⩽5	177 (78)	153 (80)	24 (69)	0.145
>5	50 (22)	39 (20)	11 (31)	
NPS (207)				
0	149 (72)	132 (75)	17 (55)	0.001
1	47 (23)	39 (22)	8 (26)	
2	11 (5)	5 (3)	6 (19)	

Abbreviations: CRC = colorectal cancer; CRP = C-reactive protein; dMMR = mismatch repair deficient; mGPS = modified Glasgow Prognostic Score; MMR = mismatch repair; NLR = neutrophil : lymphocyte ratio; NPS = neutrophil : platelet score. The relationship between MMR status and systemic inflammatory responses of patients undergoing elective, potentially curative resection of stage I–III CRC.

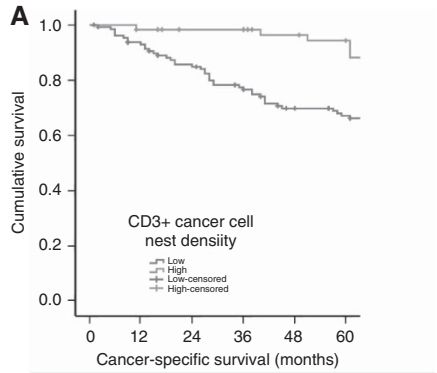
dMMR CRC (De Smedt *et al*, 2015; Maby *et al*, 2015). De Smedt *et al* (2015) recently reported that MSI-associated colon cancers primarily elicited an intratumoural, lymphocytic inflammatory response with little change in the peritumoural generalised inflammatory infiltrate. Second, Maby *et al* (2015) reported that an increased burden of MSI-associated frameshift mutations predominantly favoured tumour infiltration by $CD8^+$ T lymphocytes but not $FOXP3^+$ T lymphocytes. Taken together with these prior studies, the present results further support the role of dMMR/MSI status in promoting tumour infiltration by a co-ordinated, adaptive anti-tumour lymphocytic response (Llosa *et al*, 2015).

An unexpected finding was an association between dMMR status and the presence of an elevated systemic inflammatory response. In particular, dMMR status was associated with an elevated CRP, neutrophil count and platelet count, as well prognostic scores derived from these markers. Of interest however, and consistent with recent work by Pine *et al* (2015), neither circulating lymphocyte count nor neutrophil : lymphocyte ratio were associated with MMR status. Although Pine *et al* (2015) hypothesised that the peritumoural lymphocytosis associated with dMMR CRC may translate into an increase in circulating lymphocyte count, the results of the present study more closely reflect our understanding of the nature of the systemic inflammatory response in cancer. However, whereas the presence of a conspicuous inflammatory cell infiltrate within the tumour microenvironment primarily reflects the presence of an adaptive, anti-tumour immune response, it is increasingly appreciated that cancer-associated perturbances of the systemic inflammatory response primarily reflects upregulation of mediators of innate

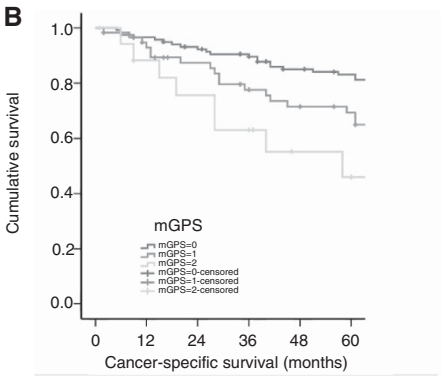
Table 5. Relationship between tumour microenvironment and systemic inflammatory response characteristics

	Multivriate analysis HR (95% CI)	*P*-value
All patients ($n=228$)		
CD3 cancer cell nest density (low/high)	0.28 (0.14–0.57)	<0.001
CD45R0 cancer cell nest density (low/high)	0.69 (0.28–1.72)	0.430
mGPS (0/1/2)	1.59 (1.12–2.27)	0.010
NPS (0/1/2)	1.47 (1.01–2.14)	0.042
MMR status (competent/deficient)	0.69 (0.31–1.54)	0.367
Stage II/Stage III only ($n=212$)		
CD3 cancer cell nest density (low/high)	0.30 (0.15–0.61)	0.001
CD45R0 cancer cell nest density (low/high)	0.77 (0.30–1.95)	0.578
mGPS (0/1/2)	1.52 (1.06–2.19)	0.023
NPS (0/1/2)	1.46 (1.01–2.13)	0.047
MMR status (competent/deficient)	0.71 (0.32–1.58)	0.399

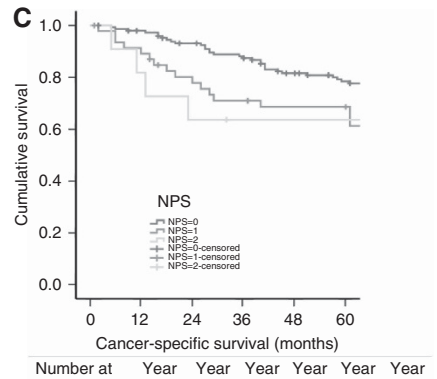
Abbreviations: CRC = colorectal cancer; CI = confidence interval; HR = hazard ratio; mGPS = modified Glasgow Prognostic Score; MMR = mismatch repair; NPS = neutrophil : platelet score. The relationship between tumour microenvironment and systemic inflammatory response characteristics associated with MMR status and cancer-specific survival of patients undergoing elective, potentially curative resection of stage I–III CRC.



Number at risk	Year 0	Year 1	Year 2	Year 3	Year 4	Year 5
High density	59	57	54	54	49	47
Low density	130	117	105	93	80	74



Number at risk	Year 0	Year 1	Year 2	Year 3	Year 4	Year 5
mGPS 0	117	112	106	101	91	87
mGPS 1	58	52	45	39	35	32
mGPS 2	18	14	12	10	6	5



Number at risk	Year 0	Year 1	Year 2	Year 3	Year 4	Year 5
NPS 0	132	125	117	109	95	87
NPS 1	39	34	28	25	23	23
NPS 2	5	4	3	3	3	3

Figure 2. Relationship between tumour and host characteristics. The relationship between tumour and host characteristics associated with survival independent of MMR status and cancer-specific survival of patients undergoing elective, potentially curative resection of MMR competent, stage I–III CRC (**A**) cancer cell nest $CD3^+$ T-lymphocyte density ($P<0.001$), (**B**) mGPS ($P=0.002$) and (**C**) NPS ($P=0.054$).

immunity, which in turn promote tumour progression and dissemination (McAllister and Weinberg, 2014). As such, it would be expected that any association between tumour characteristics and the systemic inflammatory response would be reflected by changes in markers of innate immunity, such as circulating CRP concentrations and neutrophil and platelet counts.

The mechanism underlying an association between systemic inflammation and MMR status is not clear. Although dMMR/MSI-associated tumours may be more likely to express an 'inflammatory response'-type gene signature (Missiaglia *et al*, 2014), another possible explanation is that the presence of a chronic systemic inflammatory response may predispose patients to sporadic development of dMMR tumours (Boland and Goel, 2010; Fuseya *et al*, 2012). For example, the pro-inflammatory cytokine interleukin-6 has previously been implicated in the initiation of MMR defects in colon cancer cell lines (Tseng-Rogenski *et al*, 2015) and a similar relationship between systemic inflammation and MMR status has been observed in patients with gynaecological malignancies (Fuseya *et al*, 2012). Furthermore, despite dMMR tumours eliciting a profound anti-tumour lymphocytic immune response, it has recently been shown that this is counterbalanced by upregulation of multiple immune checkpoints (Llosa *et al*, 2015). Indeed, whether the systemic inflammatory response reflects underlying immune checkpoint activation, or may be indicative of an activated common upstream precursor, such as the JAK/STAT3 pathway, would be of considerable interest (Pardoll, 2012).

On multivariate survival analysis, characterisation of host local and systemic inflammatory responses was a stronger predictor of survival than assessment of MMR status, and showed prognostic value in patients with MMR competent CRC, consistent with previous reports (Ogino *et al*, 2009; Sinicrope *et al*, 2009; Dahlin *et al*, 2011; Vayrynen *et al*, 2013; Vayrynen *et al*, 2014; Park *et al*, 2015b). Furthermore, a considerable proportion of patients with MMR-competent CRC had a high density of intraepithelial T lymphocytes. Given that assessment of MMR status alone would have failed to identify these patients, it is clear that combined assessment of host local and systemic inflammatory response, in conjunction with MMR status and standard pathological staging could potentially lead to better risk stratification of patients following potentially curative resection of CRC.

The present study is perhaps limited by its use of immunohistochemistry to identify loss of MMR activity rather than genetic sequencing for microsatellite instability. Indeed, not all MSI pathway tumours will be identifiable by loss of MMR proteins (Shia, 2008). Immunohistochemical detection of MLH1 and MSH2 however has an acceptable sensitivity and specificity for microsatellite instability screening (Lindor *et al*, 2002) and this is further improved by the use of the additional markers, PMS2 and MSH6, as used in the present study (Shia, 2008). In addition, previous studies have found that immunohistochemical assessment of MMR status using TMA sections is comparable to full-section analysis (Hendriks *et al*, 2003; Jourdan *et al*, 2003). Whereas prior studies have recommended the use of three cores per tumour (Jourdan *et al*, 2003), the present analysis was performed using four cores for each protein. Furthermore, although the use of older, archival tissue can influence the results of immunohistochemistry, there was no difference in the frequency of detection of MMR deficiency with year of surgery, suggesting that this was not an issue in the present study. Finally, manual semi-quantitative assessment of the local inflammatory cell infiltrate was presently employed; however, this has been shown to have excellent inter-operator agreement (Richards *et al*, 2014a) and correlates strongly with automated digital assessment (Forrest *et al*, 2014; De Smedt *et al*, 2015).

In summary, the present study further highlights the complexities of the relationship between the local and systemic tumour environment and MMR status in patients with CRC. Furthermore, these results confirm the importance of the tumour microenvironment and host inflammatory responses, in addition to the intrinsic properties of tumour cells, in determining outcome of patients with CRC.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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