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# Prognosis of mucinous colon cancer is determined by histological biomarkers rather than microsatellite instability

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## Prognosis of mucinous colon cancer is determined by histological biomarkers rather than microsatellite instability

The prognostic value of microsatellite instability (MSI), as well as other histological characteristics such as lymphovascular invasion (LI), perineural invasion (PNI) and extramural vascular invasion (EMVI), is unclear in colorectal mucinous carcinoma (MC). This study aims to determine the relevance of these factors in MC patients and analyses the role of MSI in stage III MC patients treated with adjuvant chemotherapy. A cohort of 650 patients diagnosed with stages I–IV colonic MC from 2000 to 2010 was selected from PALGA, the nationwide Dutch pathology databank. Histopathology was revised and mismatch repair (MMR) status determined. Univariate and multivariate survival analyses were performed. Deficient MMR (dMMR) was found in 33% of MCs and correlated with female gender and right-sidedness, but also with lower tumour stage (stages I/II: 73.2 versus 47%; $P < 0.0001$) and the absence of EMVI (9.7 versus 23.7%; $P < 0.0001$) and PNI (5.6 versus 12.7%; $P = 0.005$). On univariate analysis OS was better for dMMR MC than for proficient MMR (pMMR) MC (median OS of 9.7 versus 5.0 years; $P = 0.009$), but MMR status was no longer a relevant prognostic factor on multivariate analysis [hazard ratio (HR) = 0.91, 95% confidence interval (CI) = 0.70–1.18]. Stage III MC patients benefited from adjuvant chemotherapy, and dMMR status was associated with better OS in this group (HR = 0.35, 95% CI = 0.13–0.94). EMVI, LI and PNI, but not MMR, status are independent prognostic factors for survival in MC patients. Stage III MC patients benefit from adjuvant chemotherapy and dMMR status is associated with improved survival when adjuvant chemotherapy is given.

Keywords: adjuvant chemotherapy, colon cancer, microsatellite instability, mismatch repair deficiency, mucinous carcinoma

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## Introduction

Colorectal cancer is the third most common malignancy worldwide and the third most frequent cause

of cancer-related mortality.[1] It is widely acknowledged that colorectal cancer forms a heterogeneous group of tumours in which histopathological characteristics are related to biological behaviour and clinical outcome. Most cases are classified as adenocarcinoma (AC) not otherwise specified (NOS) but several histopathological variants can be distinguished, of which mucinous adenocarcinoma (MC) is the most frequent (10–15%).[2] MC is characterised by a large amount of extracellular mucin that should comprise more than 50% of the tumour volume.[3] MC is more often found in young patients, females, is more commonly located in the proximal colon and is associated with Lynch syndrome and inflammatory bowel disease.[2,4] Interestingly, MC shows a relatively high frequency of microsatellite instability (MSI) as a marker of a deficient mismatch repair (dMMR) system.[5–8]

Over the years the impact of mucinous histology on prognosis has been debated. While initially MC was associated with a poor prognosis in both colon and rectal cancer patients, currently no differences in outcomes have been observed.[9–11] Initially, all MCs were considered high-grade according to the WHO classification in 2000.[12] This was changed in 2010, when only microsatellite stable (MSS) or mismatch repair proficient (pMMR) MCs were classified as high-grade.[13] Present guidelines dictate that MSI should not be used for grading, as there does not seem to be a prognostic difference between pMMR and dMMR MC,[3] although the evidence for this assumption is somewhat limited.[5,6] Clinical implications of grade are still considerable, as high grade has been considered a risk factor in stage II colon cancer and is therefore a potential indication for adjuvant chemotherapy.[14] MSI is considered an important prognostic factor with implications for neoadjuvant and adjuvant treatment decisions.[15,16] Given the high percentage of dMMR in MC (33–34%) compared with 15% in the entire colorectal cancer population, prognostic impact should be evaluated.[8,17]

While multiple histological biomarkers in AC have been identified, including intramural and extramural vascular invasion (EMVI), lymphovascular invasion (LI) and perineural invasion (PNI), this is not the case for MC.[18,19] Most large prognostic MC studies are derived from cancer registries that do not routinely register these items.[2,11] Histological studies usually include only a limited number of MC patients.

This study aims to overcome these issues by including a large cohort of MC patients with a histological review to establish the prognostic value of MSI and histological biomarkers in this group. Moreover, the relevance of MSI in stage III MC patients treated with adjuvant chemotherapy is analysed.

## Patients and methods

### PATIENT SELECTION

All patients diagnosed with colonic MC who underwent resection of the primary tumour between 2000 and 2010 in the South East of the Netherlands were selected, using the Dutch Cancer Registry (IKNL) (K16.101). No rectal cancer patients were included. Patients who received neoadjuvant therapy were excluded. This database was linked with the Dutch Pathology Registry (PALGA) (the nationwide network and registry of histopathological and cytopathological specimens in the Netherlands, with complete report coverage from 1991)[20] by an independent trusted third party (LZV2016-64). The obtained list with pathology reports was used to retrieve tissue slides and tumour blocks from pathology laboratories. The central pathology review was performed by P.Z.; in case of discrepancies, E.V.B. and I.N. were consulted until consensus was reached. After central histopathological revision of all the tumour slides, tumours were excluded if they did not classify as MC [i.e. fewer than 50% of volume contained extracellular mucin ($n$ = 202; these would be considered adenocarcinomas NOS); more than 50% of signet-ring cells ($n$ = 68; these would be considered signet-ring cell carcinomas)]. If a patient presented with multiple primary MCs for histopathological revision and outcome analyses, the tumour with the highest stage was considered. Other parameters obtained during revision were T- and N-stage, according to the 8th edition 2017 UICC staging system, and the presence of LI, EMVI and PNI on haematoxylin and eosin (H&E) evaluation, according to international definitions (Figure 1).[3] Survival data obtained from IKNL refer to all-cause survival time or time from diagnosis until 31 December 2015. Tumours were classified as right-sided if they were found from the caecum up to the splenic flexure and left-sided if they were found in the descending colon or sigmoid. The following comorbidities were registered: pulmonary disease, diabetes, dementia and cardiovascular disease. The study protocol was approved by the supervisory committee of IKNL and the scientific board and privacy committee of PALGA and performed according to the Dutch 'Federa, Human Tissue and Medical Research: Code of conduct for responsible use (2011)' regulations, not requiring patient informed consent.

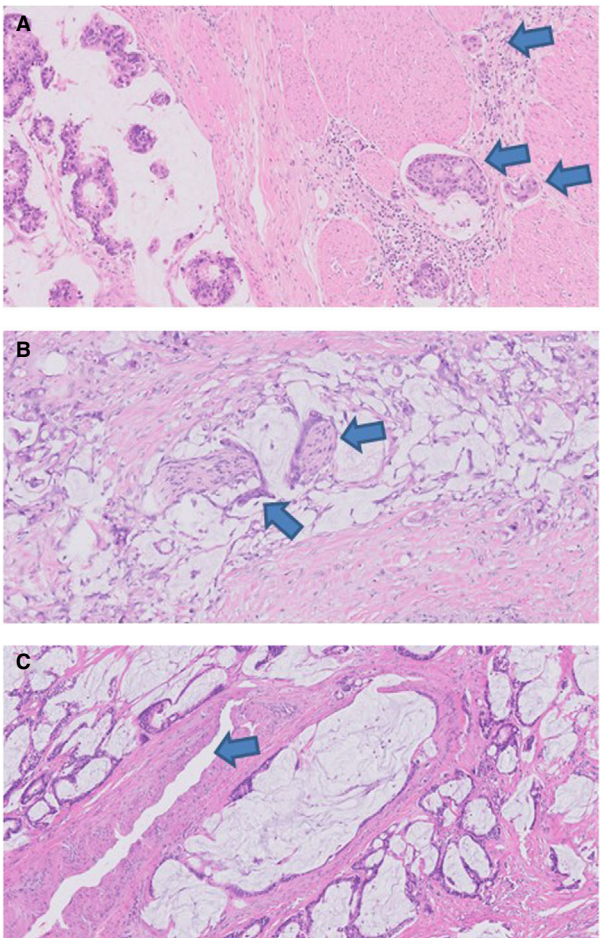



**Figure 1.** Presence of (**A**) lymphatic invasion (indicated by arrows), (**B**) perineural growth (nerves indicated by arrows) and (**C**) extramural venous invasion, with a clean orphan artery (arrow) in mucinous colon cancer on haematoxylin and eosin staining.

### IMMUNOHISTOCHEMISTRY

Formalin-fixed paraffin-embedded tumour blocks were used to create tissue microarrays (TMA) containing two to three 2-mm cores of the tumour. Immunohistochemical staining on 4 μm TMA sections was performed on the automated BOND-III stainer (Leica Biosystems, Amsterdam, the Netherlands) with alkaline antigen retrieval (solution ER2; Leica Biosystems) for the four mismatch repair proteins MLH1 (clone SO5; 1:60; Leica Biosystems), PMS2 (clone EP51; 1:30; Dako, Glostrup, Denmark), MSH2 (clone FE11; 1:15; Dako) and MSH6 (clone EP49; 1:50; Dako). dMMR, defined as unequivocal absence of one or more of the MMR stains in the cell nuclei with a positive internal control present in the same tissue section, is considered indicative of MSI. Tumours with positive nuclear staining of all four proteins were pMMR and were considered MSS.

### STATISTICS

Pearson's $\chi^2$ test was used to compare differences in the frequency distributions of categorical clinicopathological variables between dMMR and pMMR groups and a Mann–Whitney *U*-test to compare continuous variables. The primary outcome was overall survival (OS), which was defined as the interval between date of diagnosis until day of death of any cause or until date of last follow-up. Patients who were alive at the end of follow-up were censored. The log-rank test was used to compare Kaplan–Meier survival curves. Median follow-up time was calculated using the Kaplan–Meier estimate of potential follow-up.[21] Covariates with a *P*-value less than 0.25 in univariate analysis were included in multivariate analysis using Cox proportional hazards regression model. All tests were two-sided and $P < 0.05$ was considered statistically significant. Statistical analyses were performed with statistical software package SPPS version 24.0 (IBM, Armonk, NY, USA).

## Results

### PATIENTS

We included 650 MC patients (Table 1). Nine patients (1.4%) presented with multiple MCs. Patients were diagnosed at a median age of 72 years and there were slightly more female than male patients in the cohort (52%). Right-sided tumours were most frequently found (70.9%) and only 4.9% of patients were diagnosed with stage I disease.

### MMR STATUS

In 33% of MC patients the tumour showed dMMR. Eight of nine (89%) mucinous double tumours demonstrated dMMR. The majority of dMMR MCs ($n$ = 191, 88.4%) showed aberrant staining of the MLH1/PMS2 complex and 11.6% showed aberrant staining of the MSH2/MSH6 complex ($n$ = 25).

In comparison with pMMR MC, tumours with a dMMR status were associated with female gender (63.9 versus 46.1%, $P < 0.0001$) and a right-sided tumour location (94.4 versus 59.2%, $P < 0.0001$). EMVI and PNI were also less commonly found in dMMR MC tumours (9.7 versus 23.7%, $P < 0.0001$ and 5.6 versus 12.7%, $P = 0.005$, respectively). Furthermore, dMMR MCs more often presented at a

**Table 1.** Patient characteristics

| | Total | | dMMR | | pMMR | | *P*-value |
|---|---|---|---|---|---|---|---|
| | *N* = 650 | (%) | *n* = 216 | (%) | *n* = 434 | (%) | |
| Age (median, years) | 72 | | 74 | | 71 | | 0.034 |
| < 45 | 24 | 3.7 | 9 | 4.2 | 15 | 3.5 | |
| 46–60 | 103 | 15.8 | 25 | 11.6 | 78 | 18.0 | |
| 61–75 | 273 | 42.0 | 84 | 38.9 | 189 | 43.5 | |
| > 75 | 250 | 38.5 | 98 | 45.4 | 152 | 35.0 | |
| Gender | | | | | | | < 0.0001 |
| Male | 312 | 48.0 | 78 | 36.1 | 234 | 53.9 | |
| Female | 338 | 52.0 | 138 | 63.9 | 200 | 46.1 | |
| Location | | | | | | | < 0.0001 |
| Right | 461 | 70.9 | 204 | 94.4 | 257 | 59.2 | |
| Left | 189 | 29.1 | 12 | 5.6 | 177 | 40.8 | |
| Invasion depth | | | | | | | 0.013 |
| pT1 | 8 | 1.2 | 2 | 0.9 | 6 | 1.4 | |
| pT2 | 35 | 5.4 | 11 | 5.1 | 24 | 5.5 | |
| pT3 | 414 | 63.7 | 156 | 72.2 | 258 | 59.4 | |
| pT4 | 193 | 29.7 | 47 | 21.8 | 146 | 33.6 | |
| Nodal status | | | | | | | < 0.0001 |
| pN0 | 386 | 59.4 | 163 | 75.5 | 223 | 51.4 | |
| pN1 | 159 | 24.5 | 34 | 15.7 | 125 | 28.8 | |
| pN2 | 105 | 16.2 | 19 | 8.8 | 86 | 19.8 | |
| TNM stage | | | | | | | < 0.0001 |
| I | 32 | 4.9 | 11 | 5.1 | 21 | 4.8 | |
| II | 330 | 50.8 | 147 | 68.1 | 183 | 42.2 | |
| III | 179 | 27.5 | 46 | 21.3 | 133 | 30.6 | |
| IV | 109 | 16.8 | 12 | 5.6 | 97 | 22.4 | |
| LI | | | | | | | 0.057 |
| Present | 194 | 29.8 | 54 | 25.0 | 140 | 32.3 | |

LI, lymphovascular invasion; EMVI, extramural vascular invasion; PNI, perineural invasion; MMR, mismatch repair.

lower stage (stages I/II: 73.3 versus 47.0%, $P < 0.0001$) (Table 1).

## HISTOLOGICAL CHARACTERISTICS

The presence of EMVI, LI or PNI alone or combined was analysed (Figure 2). The majority of tumours did not show any of these features (59.4%), whereas LI only or EMVI only were found in 16.2 and 8.3%, respectively. In 1.8% of tumours PNI only was found. EMVI was found in combination with either LI, PNI or both in 5.8, 0.6 and 4.3%. The combination of LI and PNI was found in 3.5% of cases. Distribution of EMVI, LI and PNI varied according to MMR status,

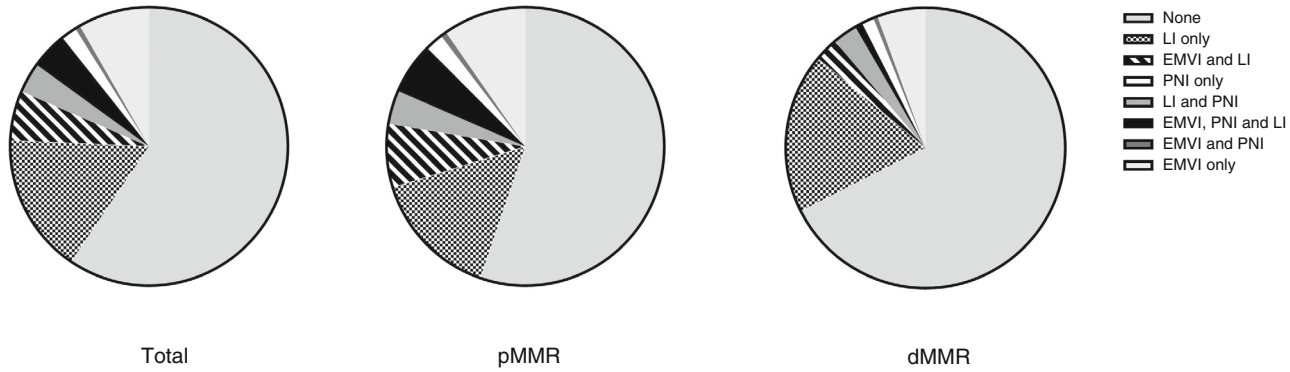



**Figure 2.** Distribution of combinations of histological characteristics in mucinous carcinoma (MC) patients according to mismatch repair (MMR) status. pMMR, proficient mismatch repair system; dMMR, deficient mismatch repair system; LI, lymphovascular invasion; EMVI, extramural venous invasion; PNI, perineural invasion.

with EMVI, LI and PNI being more frequently absent in dMMR tumours than in pMMR tumours (67.6 versus 55.3%, $P = 0.002$).

### OVERALL SURVIVAL

The median follow-up time was 68 months. Overall survival analyses demonstrated a better overall survival (OS) for dMMR tumours, with a median OS of 9.7 versus 5.0 years ($P = 0.009$) (Figure 3A). On univariate analysis dMMR status was associated with an improved OS [hazard ratio (HR) = 0.75, 95% confidence interval (CI) = 0.60–0.93], compared with pMMR status.

The presence of other histological factors such as EMVI, LI and PNI was also associated with decreased OS (Figure 3B). The absence of any of these factors demonstrated the best survival compared with presence of either EMVI, LI or PNI only. Combinations of EMVI, LI and PNI were associated with poorer survival compared with the presence of EMVI, LI or PNI only.

On multivariate analysis, the differences in OS were not confirmed for MMR status (Table 2). Higher age, advanced stage, number of comorbidities and the presence of EMVI, LI and PNI were demonstrated to be independent poor prognostic factors for OS.

### ADJUVANT CHEMOTHERAPY

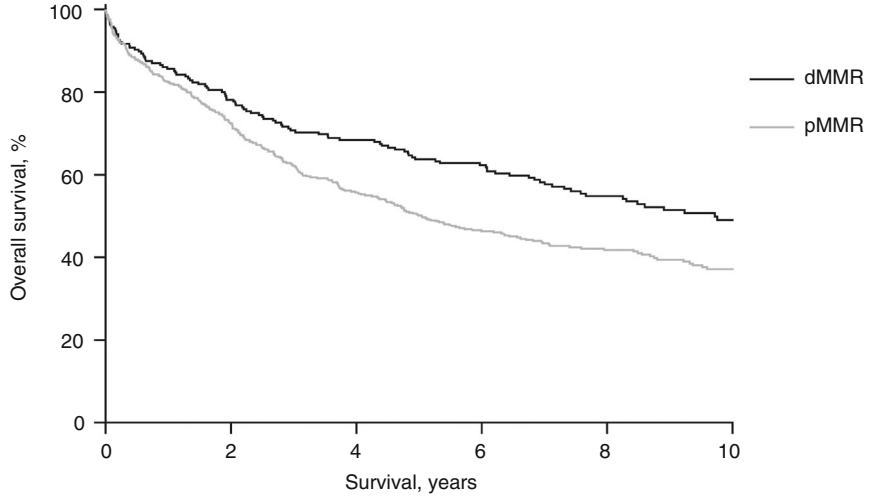
Stage III patients who received adjuvant chemotherapy were younger than patients who did not receive chemotherapy (median age = 64 versus 76 years, $P < 0.001$) and more commonly did not have any comorbidities (38.8 versus 22.4%, $P = 0.019$). There was no difference in the proportion of stage III patients with dMMR versus pMMR tumours receiving adjuvant chemotherapy (54.3 versus 58.6%, $P = 0.61$).

In patients who did not receive adjuvant chemotherapy there was no difference in OS between patients with dMMR or pMMR tumours (5-year OS = 38.1 versus 37.2%, $P = 0.904$) (Figure 4). Adjuvant chemotherapy improved 5-year OS in both dMMR (87.5%, $P < 0.0001$) and pMMR MC patients (64.1%, $P < 0.0001$). In the multivariate analysis, dMMR status was associated with an improved survival when compared with pMMR status (HR = 0.35, 95% CI = 0.13–0.94). LI and PNI, but not EMVI, were inversely correlated with prognosis in the multivariate analysis (Table 3).

## Discussion

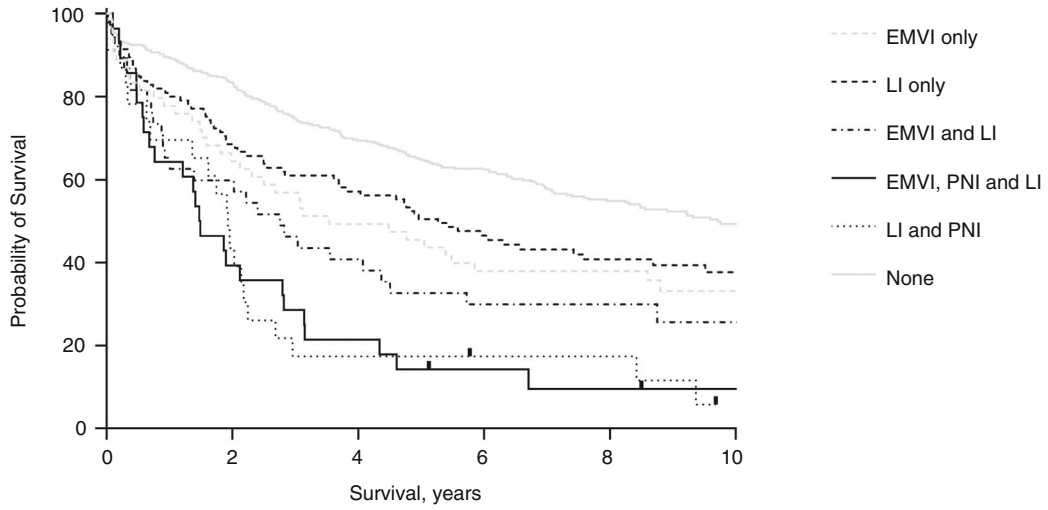
Grading of MC is controversial, and the evidence that MSI status can be used to grade MC is limited. The present study demonstrates that MMR status is not an independent prognostic factor of survival in MC on multivariate analysis. It was found that EMVI, LI and PNI are independent prognostic factors in MC patients.

As most data on MC patients are derived from population-based studies, detailed information on histopathological characteristics is generally not available. By performing a thorough histopathological re-evaluation of 650 MCs, relevant tumour characteristics could be collected. This study confirms well-known associations between dMMR status and higher age, female gender, right-sidedness and lower tumour stage.[22] The incidence of dMMR status is also in accordance with findings reported in previous studies.[8] The present cohort, therefore, is representative of MC




Patients at risk

| | 0 | 2 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|
| dMMR | 216 | 168 | 147 | 126 | 89 | 58 |
| pMMR | 434 | 356 | 241 | 184 | 121 | 77 |




Patients at risk

| | 0 | 2 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|
| None | 383 | 321 | 268 | 225 | 144 | 92 |
| LI only | 105 | 72 | 60 | 43 | 31 | 22 |
| EMVI only | 54 | 34 | 26 | 20 | 18 | 12 |
| EMVI and LI | 38 | 22 | 15 | 11 | 9 | 6 |
| EMVI, PNI and LI | 28 | 11 | 6 | 3 | 2 | 1 |
| LI and PNI | 23 | 10 | 4 | 3 | 3 | 1 |

**Figure 3. A,** Overall survival for all stages for pMMR and dMMR mucinous carcinoma patients. pMMR, proficient mismatch repair system; dMMR, deficient mismatch repair system. **B,** Overall survival for all stages according to EMVI, PNI and LI status. Only subgroups with more than 20 patients are shown in the figure. LI, lymphovascular invasion; EMVI, extramural venous invasion; PNI, perineural invasion.

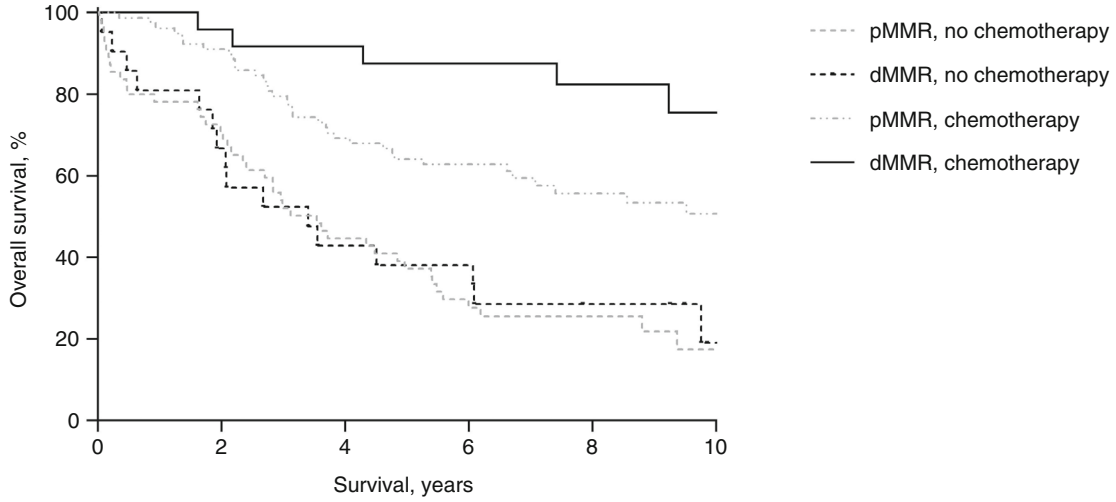
**Table 2.** Overall survival, univariate and multivariate cox regression analysis

| | Univariate | Multivariate |
|---|---|---|
| | HR (95% CI) | HR (95% CI) |
| Age (years) | | |
| < 45 | 1.00 | 1.00 |
| 46–60 | 0.81 (0.39–1.68) | 0.86 (0.40–1.84) |
| 61–75 | 1.43 (0.73–2.79) | 1.79 (0.89–3.56) |
| > 75 | 2.72 (1.39–5.30) | 3.62 (1.81–7.22) |
| Year of incidence | | |
| 1999–2002 | 1.00 | 1.00 |
| 2003–06 | 1.02 (0.79–1.31) | 1.01 (0.77–1.33) |
| 2007–10 | 0.83 (0.64–1.08) | 0.73 (0.55–0.96) |
| Gender | | |
| Male | 1.00 | – |
| Female | 0.95 (0.78–1.16) | |
| Number of comorbidities | | |
| 0 | 1.00 | 1.00 |
| 1 | 1.76 (1.37–2.26) | 1.89 (1.06–1.78) |
| 2 | 1.82 (1.34–2.48) | 1.49 (1.08–2.04) |
| 3 | 3.04 (1.48–6.27) | 1.88 (0.89–3.96) |
| TNM | | |
| Stage I | 1.00 | 1.00 |
| Stage II | 2.50 (1.23–5.08) | 1.72 (0.84–3.52) |
| Stage III | 3.12 (1.52–6.40) | 1.75 (0.83–3.69) |
| Stage IV | 10.58 (5.12–21.67) | 7.21 (3.41–15.26) |
| LI | | |
| Present | 1.83 (1.48–2.25) | 1.60 (1.25–2.06) |
| Absent | 1.00 | 1.00 |
| EMVI | | |
| Present | 1.87 (1.48–2.36) | 1.32 (1.01–1.73) |
| Absent | 1.00 | 1.00 |
| PNI | | |
| Present | 2.52 (1.89–3.35) | 1.70 (1.22–2.36) |
| Absent | 1.00 | 1.00 |
| MMR status | | |
| Deficient | 0.75 (0.60–0.93) | 0.91 (0.70–1.18) |
| Proficient | 1.00 | 1.00 |

LI, lymphovascular invasion; EMVI, extramural vascular invasion; PNI, perineural invasion; MMR, mismatch repair.

patients in general. Several limitations apply, due to the retrospective nature of this study. First, compared with the randomised controlled clinical trials on the efficacy of adjuvant chemotherapy, this study demonstrated a larger survival benefit from adjuvant chemotherapy.[23] Conceivably, patients ineligible for adjuvant treatment due to frailty of age comprise a larger share in the patient group who did not receive adjuvant chemotherapy in the current study, as illustrated by the higher age of this group. This will have influenced OS, but this bias applies to both the dMMR and pMMR subgroups, and the mean age between dMMR and pMMR patients who did not receive chemotherapy was not different (data not shown). Moreover, data on adjuvant chemotherapy were limited as the duration of treatment and the specific type of systemic therapy that was given are unknown. It is very probable that chemotherapy use did not substantially differ between the dMMR and pMMR groups because, in most cases during the given time-period, MSI status was not determined.

The prognostic effect of histopathological characteristics that have been well-established in AC patients is also seen in the current cohort of MC patients. Data concerning EMVI are in line with the findings of Park *et al.* and Langner *et al.*, who also identified venous invasion to be an independent prognostic factor in MC (but not MSI).[24,25] Others only found an association with outcome on univariate analysis, but not on multivariate analysis.[6,26,27] To the best of our knowledge, the present study is the first to show an independent prognostic effect of LI and PNI in MC patients. Song *et al.* also found LI and PNI to be independent predictors of worse survival, but their cohort of MC also included signet ring cell carcinomas, a different subtype of carcinoma with a high rate of LI and PNI and poor prognosis.[28] We found that dMMR status is associated with increased overall survival on univariate analysis, but not on multivariate analysis. This is in line with Park *et al.*, who showed that dMMR, in a cohort of 72 MC, is no longer significant




Patients at risk

| | 0 | 2 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|
| pMMR, no CT | 55 | 38 | 24 | 13 | 10 | 4 |
| dMMR, no CT | 21 | 14 | 9 | 8 | 5 | 1 |
| pMMR, CT | 78 | 71 | 54 | 42 | 25 | 19 |
| dMMR, CT | 25 | 23 | 22 | 20 | 15 | 10 |

**Figure 4.** Overall survival for stage III patients according to mismatch repair (MMR) status and adjuvant chemotherapy. pMMR, proficient mismatch repair system; dMMR, deficient mismatch repair system; CT, chemotherapy.

after inclusion of venous invasion as covariate in their multivariate survival analysis.[24]

MSI is a relative contraindication for adjuvant chemotherapy in high-risk stage II AC, as patients with MSI tumours are considered to have a better prognosis and respond less to fluoropyrimidine-based adjuvant therapy compared to MSS cases.[15,29] In contrast, the present study demonstrated a better survival in dMMR stage III MC patients treated with adjuvant chemotherapy. The reason for this difference with previous findings in dMMR AC patients is unclear, but it is not inconceivable that there are more factors influencing response to chemotherapy than MMR status only. One of the possible explanations may be the difference in the level of chromosomal instability in dMMR and pMMR MC patients, as chromosomal instability is associated with an acceleration of the development of anticancer drug resistance.[30] A previous study, including 235 pMMR AC and 29 pMMR MC patients who were treated with chemotherapy for advanced disease, demonstrated that, in MC patients, survival was indeed correlated with the level of chromosomal instability. This was not the case for AC patients. A low rate of chromosomal instability, which is associated with dMMR, was associated with better survival compared with poor survival in case of a high rate of chromosomal instability.[31] This hypothesis, however, could not be tested in the present study. It could be argued that by extrapolating these data to high-risk stage II MC patients the mere presence of MSI in those patients should not be the sole reason for withholding adjuvant chemotherapy, especially as dMMR status is not an independent predictor of survival. No specific analysis for high-risk stage II patients could be made in this study, as they did not receive adjuvant therapy in the given time-period.

In conclusion, the present study shows that dMMR status is not an independent predictor of survival in MC, due to its association with several favourable histopathological factors. Other well-known histological characteristics are as important prognostic factors in MC as they are in AC. Moreover, stage III MC patients seem to benefit from adjuvant chemotherapy, and in MC patients receiving adjuvant chemotherapy dMMR status was associated with better OS.

**Table 3.** Overall survival analysis for stage III patients receiving adjuvant chemotherapy, univariate and multivariate cox regression analysis

| | Univariate | Multivariate |
|---|---|---|
| | HR (95% CI) | HR (95% CI) |
| Age (years) | | |
| < 45 | 1.00 | 1.00 |
| 46–60 | 2.23 (0.29–17.14) | 1.68 (0.19–15.17) |
| 61–75 | 4.05 (0.55–30.11) | 4.11 (0.50–33.88) |
| > 75 | 8.65 (1.10–67.92) | 8.07 (0.91–71.78) |
| Year of incidence | | |
| 1999–2002 | 1.00 | – |
| 2003–06 | 1.25 (0.59–2.64) | |
| 2007–10 | 1.12 (0.52–2.44) | |
| Gender | | |
| Male | 1.00 | – |
| Female | 1.25 (0.69–2.24) | |
| Number of comorbidities | | |
| 0 | 1.00 | 1.00 |
| 1 | 2.19 (1.01–4.73) | 1.38 (0.61–3.15) |
| 2 | 2.49 (1.03–6.04) | 1.63 (0.63–4.22) |
| 3 | 2.83 (0.36–22.30) | 3.12 (0.34–28.41) |
| LI | | |
| Present | 1.83 (1.48–2.25) | 3.00 (1.29–6.96) |
| Absent | 1.00 | 1.00 |
| EMVI | | |
| Present | 1.74 (0.93–3.24) | 0.82 (0.30–2.21) |
| Absent | 1.00 | 1.00 |
| PNI | | |
| Present | 2.51 (1.23–5.12) | 2.88 (1.08–7.71) |
| Absent | 1.00 | 1.00 |
| MMR status | | |
| Deficient | 0.46 (0.20–1.03) | 0.35 (0.13–0.94) |
| Proficient | 1.00 | 1.00 |

LI, lymphovascular invasion; EMVI, extramural vascular invasion; PNI, perineural invasion; MMR, mismatch repair.

## Acknowledgements

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## Conflicts of interest

The authors declare they have no competing interests regarding this manuscript.

## Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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