Relationships of tumor inflammatory infiltration and necrosis with microsatellite instability in colorectal cancers

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AIM: The relationships between microsatellite instability (MSI) and survival in colorectal cancer patients are not consistent. The favorable survival of patients with MSI has been suggested to be related to pronounced inflammatory infiltration; however, the reason for non-association of MSI with survival is unclear. Our aims were to investigate the associations of inflammatory infiltration and tumor necrosis (TN) with microsatellite status and clinicopathological factors in colorectal cancer patients in whom MSI was not related to survival.

METHODS: Three hundred and one colorectal adenocarcinomas were evaluated for inflammatory infiltration and 300 for TN under light microscope.

RESULTS: Low infiltration at invasive margin ($\chi^2 = 3.94$, $P = 0.047$) and in whole tumor stroma ($\chi^2 = 3.89$, $P = 0.049$) was associated with MSI, but TN was not ($\chi^2 = 0.10$, $P = 0.75$). Low infiltration was related to advanced stage ($\chi^2 = 8.67$, $P = 0.03$), poorer differentiation ($\chi^2 = 8.84$, $P = 0.03$), DNA non-diploid ($\chi^2 = 10.04$, $P = 0.002$), higher S-phase fraction ($\chi^2 = 11.30$, $P = 0.004$), positive p53 expression ($\chi^2 = 7.94$, $P = 0.01$), and worse survival ($P = 0.03$ for both univariate and multivariate analyses). Abundant TN was related to advanced stage ($\chi^2 = 17.74$, $P = 0.001$) and worse survival ($P = 0.02$ for univariate, and $P = 0.05$ for multivariate analysis).

CONCLUSION: The result that high inflammatory infiltration was not related to MSI might help explain the non-association of MSI with survival in colorectal cancer patients.

Key words: Inflammatory infiltration; Necrosis; Microsatellite instability; Prognosis; Colorectal cancer

INTRODUCTION

Colorectal cancer arises through at least two distinct genetic pathways in its carcinogenesis: microsatellite instability (MSI) and chromosomal instability. MSI refers to genome-wide alteration in repetitive DNA sequence caused by deficiencies in DNA mismatch repair machinery, which accounts for about 10-15% of sporadic colorectal cancers and nearly all hereditary non-polyposis colorectal cancers. Studies have shown that colorectal cancers with MSI are likely to be characterized by more frequent right-sided location, poor differentiation, mucinous/signet-ring cell carcinoma, and intense peri- and intra-tumoral inflammatory reaction. Patients with MSI tumors appear to have a favorable prognosis compared with those with microsatellite stability tumors. The favorable prognosis associated with MSI has been suggested to be related to an enhanced inflammatory infiltration in the tumors, although the mechanism behind this phenomenon is unclear.

However, several studies including our previous two studies have reported the lack of association between MSI and survival either in entire group of colorectal cancer patients or in subgroup with stage II colorectal cancers. The reason for the non-association remains unclear. To our knowledge, no one has studied the association of inflammatory infiltration with microsatellite status in the patients in whom MSI is not related to survival. A recent study showed that necrosis in tumor was related to MSI-H in colorectal cancers. Tumor necrosis (TN) is a common feature of solid tumors associated with a poor clinical outcome due to rapid tumor growth without sufficient blood supply. However, the association of TN with microsatellite status has not been well studied. Therefore, it is interesting to evaluate the relationship of inflammatory infiltration and TN with MSI in colorectal cancers in which MSI was not related to survival. Meanwhile, we analyzed the relationship of inflammatory infiltration and TN with clinicopathological and other variables.
MATERIALS AND METHODS
Patients
Three hundred and one primary colorectal adenocarcinomas were studied for inflammatory infiltration and 300 for TN. The patients were diagnosed at the Department of Pathology, Linköping Hospital, Linköping, and Vrinnevi Hospital, Norrköping, Sweden, between 1975 and 2001. The patient’s gender, age, tumor location, and Dukes’ stage were confirmed from surgical and/or pathological records. Tumor growth pattern and the grade of differentiation were scored by two pathologists. The mean age of the patients was 71 years (ranging from 34 to 94 years). Tumors from the ascending and transverse colon were regarded as proximal tumors, whereas tumors from descending and sigmoid colon, and the rectum were considered distal. The tumor growth pattern was divided into expansive or infiltrative type based on pattern of growth and invasiveness. Differentiation was graded as well, moderately, poorly differentiated, and mucinous/signet-ring cell carcinoma. The data on microsatellite status[13,14], DNA ploidy, S-phase fraction (SPF)[9], and p53 expression[10] were taken from previous studies carried out at our laboratory. Microsatellite status was determined by a microsatellite analysis using the Bat26 marker; 25 cases were MSI, and 152 were MSS. DNA ploidy and SPF were measured by flow cytometry; 107 cases were DNA diploid, and 118 were non-diploid; 52 were <5% SPF, 65 were 5-10%, and 72 were >10%. p53 expression was identified with immunohistochemistry by using CM1 antibody; 117 cases were p53 negative (completely negative cases plus the cases having ≥5% of p53-stained tumor cells), and 114 were positive (the cases having >5% of p53-stained tumor cells). No information was available about patients’ age in two cases, tumor site in 7, Dukes’ stage in 8, growth pattern in 21, grade of differentiation in 1, microsatellite status in 125, DNA ploidy in 75, SPF in 111, and p53 expression in 69 cases. Among 301 patients, 10 MSS and one MSI case had received adjuvant preoperative radiotherapy, one MSS case had palliative radiotherapy, two MSS had adjuvant chemotherapy, and one MSS had palliative chemotherapy. No information was available for two cases, and the rest did not receive any radiotherapy or chemotherapy. The patients were followed up until the end of October 2001, and 125 died of colorectal cancer.

Histopathological evaluation
Three to ten sections from different parts of the tumor were examined at low magnification (×10) under light microscope by two of the authors (of whom one is a pathologist) independently in a blinded fashion without knowing the clinicopathological and other data of the patients. After the first run of the scoring, approximately 20% of the cases with disagreed score were reread independently by the two authors. Finally, about 4% of the cases with discrepant scoring were discussed under a dual-headed microscope to reach agreement on the scoring. Inflammatory infiltration and TN in the margins of the sections were not included in order to avoid artifacts. Infiltrating inflammatory cells were identified as small mononuclear cells in the stroma of tumor. The distributions of the infiltration were classified into two groups by localization: (a) those presented along the invasive margin of the tumor; (b) those distributed in entire tumor. The degree of infiltration was classified as absent, sparse, moderate, and intense according to the density of inflammatory cell[21]. Necrosis was scored as absent; <10%, 10-30%, and >30% based on the percentage of necrosis in whole tumor area[22]. Since the distributions of inflammatory infiltration and NT were often heterogeneous, the entire sections were examined to assess tumor areas including high and low inflammatory infiltration and NT. If higher infiltration or NT was more than one-third of the section, it was taken into account for scoring.

Statistical analysis
The relationships of inflammatory infiltration and TN with survival were tested using Cox’s Proportional Hazard Model. Survival curves were calculated using the Kaplan-Meier method. The relationships of inflammatory infiltration and TN with other variables were tested by using the χ² test or Fisher exact test. Two-sided P values of less than 5% were considered statistically significant.

RESULTS
Inflammatory infiltration in relation to microsatellite status, clinicopathological and other variables
Among the 301 tumors studied, inflammatory cells at the invasive margin were absent in one case, sparse in 105, moderate in 131, and intense in 64 (Figure 1A); inflammatory cells in the tumors were absent in 17, sparse in 135, moderate in 110, and intense in 39 cases (Figure 1B). Absent, sparse, and moderate were combined as a low-level group, and
The relationship of inflammatory infiltration at the invasive margin or in the tumor with microsatellite status is present in Table 1. Low inflammatory infiltration either at the invasive margin or in the tumor was lightly associated with MSI ($\chi^2 = 3.94, P = 0.047$ and $\chi^2 = 3.89, P = 0.049$).

The relationship of inflammatory infiltration at the invasive margin with clinicopathological and other variables is summarized in Table 2. The degree of inflammatory infiltration was lower in tumors with Dukes’ stage D ($\chi^2 = 8.67, P = 0.03$), DNA non-diploid ($\chi^2 = 10.04, P = 0.002$), higher SPF ($\chi^2 = 11.30, P = 0.004$), p53 positive expression ($\chi^2 = 7.94, P = 0.01$), as well as poor differentiation and mucinous/signet-ring cell carcinoma ($\chi^2 = 8.84, P = 0.03$). Moreover, the patients with low inflammatory infiltration had worse survival than those with high infiltration ($P = 0.03$; Figure 2A), even after adjustment for patients’ gender, Dukes’ stage and differentiation ($P = 0.03$, data not shown). We did not find any relationship between inflammatory infiltration and patients’ gender, tumor location, or growth pattern ($P > 0.05$). There was no significant relationship of inflammatory infiltration in the tumor with the above clinicopathological and other variables ($P > 0.05$, data not shown).

**TN in relation to MSS, and clinicopathological and other variables**

Among the 300 tumors studied, necrosis was absent in 76 cases, 54 had <10%, 93 had 10-30%, and 77 had >30% of necrosis. The cases with <10% of necrosis were graded as little necrosis and the remainder as abundant necrosis (Figure 1C), based on the similarities of the clinicopathological features.

We did not find a relationship between TN and MSI ($\chi^2 = 0.10, P = 0.75$; Table 1). As shown in Table 2, the frequency of TN was increased from Dukes’ stages A to D ($\chi^2 = 17.74, P = 0.001$). TN was more frequent in moderately/poorly differentiated tumors, but was the lowest in mucinous/signet-ring cell carcinomas ($\chi^2 = 22.98, P < 0.0001$). Patients with abundant TN had worse survival than those with little TN in univariate analysis ($P = 0.02$; Figure 2B). The survival significance was borderline after adjustment for Dukes’ stage and differentiation ($P = 0.05$, data not shown). There were no associations of TN with other factors including gender, age, tumor location, growth pattern, DNA ploidy, SPF, and p53 expression ($P > 0.05$).

### Table 1 Relationship of tumor inflammatory infiltration and necrosis with microsatellite status

| Variable Category | Microsatellite stability (%) | Microsatellite instability (%) | $P$ |
|-------------------|-------------------------------|-------------------------------|-----|
| Infiltration at the invasive margin |                             |                               |     |
| Low               | 120 (79)                      | 24 (96)                       | 0.047 |
| High              | 31 (21)                       | 1 (4)                         |     |
| Infiltration in the tumor |                             |                               |     |
| Low               | 71 (46)                       | 17 (68)                       | 0.049 |
| High              | 81 (54)                       | 8 (32)                        |     |
| Necrosis          |                               |                               | 0.75 |
| Little            | 76 (51)                       | 13 (54)                       |     |
| Abundant          | 74 (49)                       | 11 (46)                       |     |

Considering the relationship of inflammatory infiltration with TN, tumors with high infiltration tended to have less necrosis although the relationship did not reach statistical significance ($\chi^2 = 3.62, P = 0.057$).

### DISCUSSION

MSI has been shown to be a favorable prognostic factor in colorectal cancer patients, even independent of tumor stage, or radiotherapy and chemotherapyna882223. The reason for this evidence has been proposed to be partly due to a stronger host response of immune system in MSI tumors than in MSS ones[1,5]. However, several other studies have reported the lack of association between MSI and survival, either in entire group of colorectal cancer patients[9,10,12] or in the subgroup with stage II colorectal cancers[13,14]. Such non-association was also shown in our previous two studies[13,14] and another Swedish study[11]. The National Cancer Institute Workshop concluded that MSI has not yet been shown conclusively to be an independent predictor of prognosis in colorectal cancer patients[15]. The reason for the non-association between MSI and survival is unclear. Subgroup variation within populations is unlikely an explanation for this evidence because the similar findings have been reported by various groups among different populations. The relationship between the degree of inflammatory infiltration and microsatellite status in the patients without association of MSI with survival has not been studied. Based on our previous findings of the non-association between MSI and

![Figure 2](image-url)  
**Figure 2** Tumor inflammatory infiltration at the invasive margin (A) and necrosis (B) in relation to survival in patients with colorectal cancer.
We observed that a low level of inflammatory infiltration either at the invasive margin or in the tumor was related to MSI phenotype in the present study. Although the statistically significant differences were borderline ($P = 0.047$ and $P = 0.049$), the results provided at least an indication that pronounced inflammatory infiltration did not always accompany MSI tumors as proposed.

It is generally accepted that the immune system represents a specific host response to tumors. The survival advantage of pronounced infiltration of all the inflammatory cells, or various subsets of inflammatory cells such as lymphocytes and macrophages, around or within colorectal tumors has been demonstrated. Anti-tumor effects of infiltrating inflammatory cells may be mediated by cytokine secretion induced by the response of inflammatory cells to tumor stimulation. The expression of two cytokines interleukin-4 (IL-4) and TN factor-α (TNF-α) in colon cancer has been found to be associated with better survival.[12,22,23] In our previous study in 438 colorectal cancers with microsatellite status,[14] 34 (8%) had received radiotherapy, 15 (3%) chemotherapy, and 7 (2%) both treatments. No information was available for 59 (13%), and the rest, 316 (73%), did not receive any adjuvant or palliative treatments. Statistical analysis showed that neither radiotherapy ($P = 0.23$) nor chemotherapy ($P = 0.84$) improved survival of the patients (unpublished data). Taken together, it seems that chemotherapy and radiotherapy were unlikely the reasons behind the non-association between MSI and survival in colorectal cancer patients. Obviously, further studies are needed to evaluate the effect of chemotherapy and radiotherapy on MSI patients.

TN is a common feature of solid tumors and caused by ischemia due to rapid tumor growth. The degree of TN reflected the level of intra-tumor hypoxia, and increased survival.[13,14] We observed that a low level of inflammatory infiltration either at the invasive margin or in the tumor was related to MSI phenotype in the present study. Although the statistically significant differences were borderline ($P = 0.047$ and $P = 0.049$), the results provided at least an indication that pronounced inflammatory infiltration did not always accompany MSI tumors as proposed.

Clinical results regarding the relationship of microsatellite status in colorectal cancer patients with adjuvant chemotherapy are fairly inconsistent. Some patients with MSI tumor had no survival benefit from chemotherapy[6,21,28] but others had.[8] Reviewing all eight previous studies, that showed the non-association between MSI and survival in colorectal cancer patients, two studies were carried out on patients without chemotherapy[9,15], and one study on patients with chemotherapy, in which no survival benefit of chemotherapy was observed in MSI patients although the benefit was found in total group of the patients or microsatellite status patients.[12]. Four studies did not provide information about chemotherapy[10,11,13,16]. In our previous study in 438 colorectal cancers with microsatellite status[14], 34 (8%) had received radiotherapy, 15 (3%) chemotherapy, and 7 (2%) both treatments. No information was available for 59 (13%), and the rest, 316 (73%), did not receive any adjuvant or palliative treatments. Statistical analysis showed that neither radiotherapy ($P = 0.23$) nor chemotherapy ($P = 0.84$) improved survival of the patients (unpublished data). Taken together, it seems that chemotherapy and radiotherapy were unlikely the reasons behind the non-association between MSI and survival in colorectal cancer patients. Obviously, further studies are needed to evaluate the effect of chemotherapy and radiotherapy on MSI patients.

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| Variable            | Category                     | Inflammatory infiltration at the invasive margin | TN                      |
|---------------------|------------------------------|-----------------------------------------------|-------------------------|
|                     |                              | $n$ | High (%) | $P$  | $n$ | Abundant | $P$  |
| Gender              | Male                         | 158 | 27 (17) | 0.06 | 158 | 85 (54)  | 0.31 |
|                     | Female                       | 143 | 37 (26) | 0.68 | 142 | 68 (48)  | 0.12 |
| Age (yr)            | ≤70                          | 138 | 31 (22) |      | 138 | 77 (56)  |      |
|                     | >70                          | 161 | 33 (21) | 0.55 | 160 | 75 (47)  | 0.75 |
| Tumor location      | Proximal                     | 109 | 25 (23) | 0.03 | 108 | 54 (50)  | 0.001|
|                     | Distal                       | 185 | 37 (20) | 0.03 | 185 | 96 (52)  |      |
| Dukes’ stage        | A                            | 47  | 11 (23) |      | 45  | 15 (33)  |      |
|                     | B                            | 102 | 26 (25) |      | 103 | 45 (44)  |      |
|                     | C                            | 94  | 22 (24) |      | 94  | 53 (56)  |      |
|                     | D                            | 50  | 3 (6)   |      | 50  | 36 (72)  |      |
| Growth pattern      | Expansive                    | 128 | 25 (20) | 0.65 | 128 | 64 (50)  | 0.38 |
|                     | Infiltration                 | 152 | 33 (22) | 0.03 | 152 | 84 (55)  | <0.0001|
| Differentiation     | Well                         | 23  | 10 (43) |      | 24  | 7 (29)   |      |
|                     | Moderately                   | 196 | 42 (21) |      | 195 | 116 (60) |      |
|                     | Poorly                       | 44  | 7 (16)  |      | 43  | 22 (51)  |      |
|                     | Mucinous+signet-ring cell    | 37  | 5 (14)  |      | 37  | 8 (22)   |      |
| DNA ploidy          | Diploid                      | 107 | 32 (30) | 0.002| 107 | 53 (49)  | 0.14 |
|                     | Non-diploid                  | 118 | 15 (13) |      | 118 | 70 (59)  |      |
| S-phase fraction    | <5%                          | 52  | 19 (37) |      | 52  | 26 (50)  | 0.24 |
|                     | 5–10%                        | 65  | 16 (25) |      | 65  | 31 (48)  |      |
|                     | >10%                         | 72  | 8 (11)  |      | 72  | 44 (61)  |      |
| p53 expression      | Negative                     | 117 | 33 (28) | 0.01 | 117 | 57 (49)  | 0.07 |
|                     | Positive                     | 114 | 15 (13) |      | 114 | 69 (61)  |      |

Table 2 Relationship of tumor inflammatory infiltration at the invasive margin and TN with clinicopathological and other variables.
hypoxia has been associated with high metastatic potential. TN has been previously demonstrated as an indicator of poor prognosis in several types of cancers, including colorectal cancer[29]. In the present study, abundant TN was related to poor survival, advanced Dukes’ stage, and moderate/poor differentiation. Unexpectedly, the lowest frequency of TN was seen in mucinous carcinomas/signet-ring cell carcinoma that had more aggressive behaviors. This was probably due to mucinous carcinomas that had a substantial amount of mucin and were less likely to have gland formation. In addition, mucinous carcinomas usually grow slowly, which requires less blood supply, and were therefore lack of TN. Recently, Greenson et al[17], reported that the absence of TN was related to MSI-H in colorectal cancers. However, we could not prove this in the present study. The varying results may be due to the different characteristics of the patients and pathological features of the tumors. Besides, it may partly depend on the classification of MSI: they divided MSS into MSI-H, and microsatellite status which included MSI-low cases. Therefore, they had a lower frequency of MSI (9.8%) than ours (14.6%).

In conclusion, the lack of association of pronounced inflammatory infiltration with MSI might be one of the explanations for non-association between MSI and survival in colorectal cancers.

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