Correlation between microsatellite instability (MSI) and 5-year survival in patients with colorectal cancer

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Abstract. The prognosis and treatment of colorectal cancer are based on its stage. The European Society for Medical Oncology published a guideline in 2012, which suggests that microsatellite instability (MSI) should be evaluated to determine the course of colorectal cancer. This study aimed to investigate MSI-high (MSI-H) as a prognostic factor for 5-year survival rates. 90 patients diagnosed with colorectal cancer who underwent resection surgery between 2008 and 2013 in Dr. Cipto Mangunkusumo Hospital, Jakarta, were included. The MSI status as a prognostic factor to determine the 5-year survival rate was analyzed after adjusting for the size and type of tumor, metastasis, and patient age. Of the 90 patients, 47 were followed up. The 5-year survival rates of patients with MSI-H were 33.3%, 22.2%, and 20% for stage II, III, and IV tumors, respectively, compared with 0%, 5%, and 0% for patients with MSI-low (MSI-L) ($p = 0.003$). Multivariate analysis showed that the hazard ratio for MSI-L was 2.421 (95% CI, 1.991–2.851) compared to MSI-H ($p = 0.004$). MSI-H is an important prognostic factor to determine the 5-year survival rate in colorectal cancer patients. It is found that patients with MSI-H have a more favorable prognosis compared with those with MSI-L.

1. Introduction

Every year, 1 million people worldwide are newly diagnosed with colorectal cancer; of these, 50% have a recurrence and die within 5 years of diagnosis [1]. The staging of the disease is still a major consideration in the treatment and prognosis of colorectal cancer. Colorectal cancer stage II is controversial owing to the variation in survival rates from 87.5% for stage IIA to 58.4% for stage IIC. The administration of adjuvant chemotherapy in patients with stage II cancer is controversial because of its side effects. In patients with stage III/Dukes C cancer (with lymph node metastasis), adjuvant chemotherapy is administered to reduce recurrence. The regimens used are fluorouracil (5-FU)/leucovorin (LV) [2]. Nowadays, adjuvant chemotherapy is being considered for use in high-risk stage II patients (T4 or intestinal obstruction, perforations, poorly differentiated tumors, lymph nodes <10/12, and histological features of vascular, lymphatic, or perineural invasion) [3-5]. Surveillance programs for the detection of local and regional recurrences or distant metastases at an early or asymptomatic stage during secondary curative surgery can be performed. However, optimal
follow-up methods (physical examination, endoscopy, laboratory tests, and radiological examination) are controversial [3-6]. Patient compliance varies among methods; for example, compliance with endoscopy is low. This may be due to the relatively high price of the test.

Microsatellite instability (MSI) and the loss of heterozygosity of the 18q chromosome, KRAS, BRAF, and TP53 are widely studied potential indicators. To date, the results show that MSI is the strongest candidate indicator for evaluating colorectal cancer. In 2012, the European Society for Medical Oncology issued a guide that suggests the examination and evaluation of MSI for determining the progression of colorectal cancer. Therefore, we conducted a study to examine the prognostic ability of MSI for determining the 5-year survival rates and responses to chemotherapy in patients with colorectal cancer [7-11]. Such a study has not been conducted in Indonesia previously.

2. Methods
This study was conducted with a longitudinal study design using secondary data from a study by Setyaningsih et al. titled “Microsatellite Instability Research through Expression of PMS2 and MSH6 and Tumor-Infiltrating Lymphocyte in Left and Right Colorectal Cancer,” medical data tracing, and direct follow-up of patients. Data were collected immediately following approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia-Dr. Cipto Mangunkusumo Hospital. Participants comprised those who met the inclusion criteria and willing to be included in the study gave written consent.

This research was conducted from July 2016 to December 2016 in Dr. Cipto Mangunkusumo Hospital. This study used histological data from previous research that was conducted at the Department of Anatomical Pathology, Faculty of Medicine Universitas Indonesia-Dr. Cipto Mangunkusumo Hospital from 2008 to 2013. Data were also taken from the medical records of patients who had been diagnosed with colorectal cancer at the Department of Surgery of Digestive Division Dr. Cipto Mangunkusumo Hospital between January 1, 2008, and December 31, 2016. The survival of patients from these two data sources was determined by following up with the patients or their families. Data processing and preparation of research reports were conducted at the Department of Surgery, Faculty of Medicine, University of Indonesia-Dr. Cipto Mangunkusumo Hospital in 2016.

The target population of this study was that of patients with colorectal cancer. The accessible populations in this study were all colorectal cancer patients at Dr. Cipto Mangunkusumo Hospital. The subjects of the study were all patients who went for treatment and underwent resection surgery at Dr. Cipto Mangunkusumo Hospital between 2008 and 2013 and met the inclusion and exclusion criteria.

All patients from the previous study were included in this study. Data taken from previous studies include age, sex, cell surface, cell variant, T stage, number of positive lymph nodes, lymph vascular invasion, metastasis, cell differentiation, and MSI status. Samples with incomplete baseline data were excluded from the study. Samples were also excluded if the carcinoembryonic antigen (CEA) levels within 4–8 weeks after curative therapy or chemotherapy were unavailable or if the patient or the family could not be contacted for surveillance.

This study used the formula of sample of research of difference of mean of two unpaired group and got sample number 54 patients because this research used unpaired numerical categorical analytical test. Then, to anticipate the possibility of the respondent drop out then the number of samples were added to 60 samples. Data were obtained from only 47 patients; 21 patients with MSI-high (MSI-H) and 26 with MSI-low (MSI-L).

A total sampling method was used, in which all patient data available from the previous studies were used in the analysis. Thus, if the number of samples taken is not sufficient, the researcher will take the entire number of the existing samples and the strength of the study will be calculated.

The data were analyzed using IBM SPSS Statistics 20 for Windows® software. Patient characteristics were compared by the chi-square test or linear associations. The 3-year survival rates were analyzed by the Kaplan–Meier method and log-rank test. The data of the deceased patients were recorded and the date and cause of death, considered as intention to treat analysis. Multivariate analysis was performed with the Cox proportional hazards model to identify prognostic factors. Significant
factors \( p < 0.05 \) in the univariate analysis were incorporated into the multivariate model using the Cox proportional hazards analysis.

3. Results

Data were obtained from only 47 patients; 21 patients with MSI-high (MSI-H) and 26 with MSI-low (MSI-L). There were no significant differences between the groups with respect to the age, sex ratio, history of chemotherapy, cancer location, or tumor differentiation \( p > 0.05 \). There were significant differences between the groups with respect to the preoperative CEA levels and TNM stage. The CEA level was lower in patients with MSI-H than in patients with MSI-L \( (22.96 \pm 36.22 \text{ vs. } 77.29 \pm 17.05 \text{ ng/mL}) \). The TNM stage was also lower in patients with MSI-H than in patients with MSI-L. Both these differences were statistically significant; \( p = 0.012 \) for preoperative CEA and \( p = 0.046 \) for the TNM stage (Table 1).

| Characteristic | MSI-H (\( n = 21 \)) | MSI-L (\( n = 26 \)) | P-value |
|----------------|---------------------|---------------------|---------|
| Age (mean ± SD (yr)) | 56.57 ± 9.56 | 53.19 ± 13.96 | 0.322 |
| Sex | | | 0.821 |
| Male | 12 (57.14%) | 14 (53.85%) | | |
| Female | 9 (42.86%) | 12 (46.15%) | | |
| Preoperative CEA level (mean ± SD (ng/mL)) | 22.96 ± 36.22 | 77.29 ± 17.05 | 0.012* |
| TNM stage | | | 0.046* |
| I | 1 (0.05%) | 0 (0%) | | |
| II | 6 (28.57%) | 3 (11.54%) | | |
| III | 9 (42.86%) | 20 (76.92%) | | |
| IV | 5 (23.81%) | 3 (11.54%) | | |
| Chemotherapy | | | 0.671 |
| Yes | 10 (47.62%) | 14 (53.85%) | | |
| No | 11 (53.38%) | 12 (46.15%) | | |
| Location | | | 0.503 |
| Proximal | 8 (38.10%) | 11 (42.31%) | | |
| Distal | 13 (61.90%) | 15 (57.69%) | | |
| Differentiation | | | 0.896 |
| Good | 10 (47.62%) | 8 (30.77%) | | |
| Moderate | 6 (28.57%) | 12 (46.15%) | | |
| Bad | 2 (9.52%) | 3 (11.54%) | | |
| Signet | 0 | 1 (3.85%) | | |
| Mucinosum | 3 (14.29%) | 2 (7.69%) | | |

CEA, carcinoembryonic antigen; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low. ; *\( p < 0.05 \)
Based on univariate analysis using the unpaired $t$-test, chi-square test, it was found that preoperative CEA, TNM stage, tumor location, tumor differentiation, and MSI level were prognostic factors for colorectal cancer. A survival plot was then developed using the Kaplan–Meier method and multivariate analysis using the Cox proportional hazards analysis method to find the hazard ratio for each factor.

Patients with high preoperative CEA (>5 ng/mL) had a 5-year survival rate of 10.3% compared with patients with low preoperative CEA (<5 ng/mL) who had a 5-year survival rate of 22.2% ($p = 0.047$, chi-square test). However, on multivariate analysis, the $p$-value was 0.239 with a hazard ratio of 1.002 (95% CI, 1.001–1.003). This suggests that CEA levels are less significant as a prognostic factor on multivariate analysis.

Among patients with TNM staging, the 5-year survival rate was 100% for stage I, 22.2% for stage II, 10.3% for stage III, and 12.5% for stage IV ($p = 0.012$, chi-square test). On multivariate analysis, the $p$-value was 0.001 with a hazard ratio of 0.822 (95% CI, 0.216–1.428) for stages I and II against stages III and IV.

Patients with proximal colorectal cancer had a 5-year survival rate of 21.1% compared with patients with distal colorectal cancer who had a 5-year survival rate of 10.7% ($p = 0.043$, chi-square test). On multivariate analysis, the $p$-value was 0.049 with a hazard ratio of 0.484 (95% CI, 0.104–0.864) for proximal cancer compared with distal cancer.

Patients with a good tumor differentiation had a 5-year survival rate of 66.6%, which was much better than the rates of 11.1% for patients with moderate differentiation, 0% for bad or signet differentiation, and 20% for mucinosum differentiation ($p = 0.032$, chi-square test). On multivariate analysis, besides the good differentiation were combined to be compared with good differentiation, a $p$-value of 0.018 was obtained with a hazard ratio of 4.459 (95% CI, 3.656–5.262) for other differentiation on good differentiation.

The 5-year survival rate was 28.6% among patients with MSI-H compared with 3.8% among patients with MSI-L ($p = 0.003$, chi-square test). On multivariate analysis, the $p$-value was 0.004 with a hazard ratio of 2.421 (95% CI, 1.991–2.851) (see Table 2).

| Table 2. Univariate and multivariate analysis of 5-year survival rates of colorectal cancer patients. |
|------------------------------------------|----------------|----------------|----------------|----------------|----------------|
| Characteristic                           | Number of cases | 5-year survival rate | $p$-value | 5-year survival rate | $p$-value |
| Age (yr)                                 |                |                    |          |                    |            |
| <55                                      | 21             | 9.5%               | 0.943    | –                  |            |
| 55–65                                    | 15             | 26.7%              |          |                    |            |
| >65                                      | 11             | 9.1%               |          |                    |            |
| Sex                                      |                |                    | 0.422    | –                  |            |
| Male                                     | 26             | 11.5%              |          |                    |            |
| Female                                   | 21             | 19.0%              |          |                    |            |
| Preoperative CEA level (ng/mL)           |                |                    | 0.047*   | 0.239              |            |
| <5                                       | 18             | 22.2%              |          | 1.002 (1.001–1.003) |            |
| ≥5                                       | 29             | 10.3%              |          | (CEA level ≥5 vs. CEA level <5) |            |
| TNM stage                                |                |                    | 0.012*   | <0.001*            |            |
| I                                        | 1              | 100%               |          | 0.822 (0.216–1.428) |            |
| II                                       | 9              | 22.2%              |          | (Stage I and II vs. stage III and IV) |            |
| III                                      | 29             | 10.3%              |          |                    |            |
| IV                                       | 8              | 12.5%              |          |                    |            |
| Chemotherapy                             |                |                    | 0.879    | –                  |            |
| Yes                                      | 24             | 16.7%              |          |                    |            |
| No                                       | 23             | 13.0%              |          |                    |            |

*p<0.05
Based on univariate and multivariate analysis, it was found that the TNM stage, tumor location, tumor differentiation, and MSI levels were prognostic factors for colorectal cancer. Subsequently, a survival plot was developed using the Kaplan–Meier method by comparing patients with MSI-H and MSI-L for all stages and for each stage.

Only one patient with MSI-H had a stage I tumor, and the survival rate was 100%. Nine patients (six with MSI-H and three with MSI-L) had stage II tumors; their 5-year survival rates were 0% for MSI-L and 33.3% for MSI-H.

There were 29 patients with stage III tumors (20 patients with MSI-L and nine with MSI-H). The 5-year survival rates were 5% for MSI-L and 22.2% for MSI-H. There were eight patients with stage IV tumors (three with MSI-L and five with MSI-H). Their 5-year survival rates were 0% for MSI-L and 20% for MSI-H.

Based on multivariate analysis with Cox proportional hazards analysis, the graphs of survival function and hazard function were plotted. The graphs showed that survival was better for patients with MSI-H than for those with MSI-L for each tumor stage.

4. Discussion
In this study, there were 47 colorectal cancer patients who met the sampling criteria, including 21 patients with MSI-H and 26 patients with MSI-L. MSI-H was found in 44% of the patients, a greater proportion than the 15%–25% found in the general population. A possible explanation for this difference is the proportion of proximal cancers in this study, which was approximately 40%. Several studies have linked MSI with proximal tumor incidence, low levels of CEA, and rarer metastases [12,13]. Studies have also linked MSI with a lower age and female gender. However, the correlation between those variables was not seen in this study. This may have been due to the small number of samples causing the strength of this study to detect these correlations to be low [14,15].

This study aimed to assess the prognostic ability of MSI to determine the 5-year survival rate in colorectal cancer patients. It found that MSI was one prognostic factor for the 5-year survival rate. In addition to MSI, other prognostic factors were found, including the TNM stage, tumor location, and tumor differentiation. CEA level was found to be a prognostic factor for survival rate based on univariate analysis but not on multivariate analysis. The results of this study, in accordance with other research results, suggest that MSI is a prognostic factor for the 5-year survival rate in patients with colorectal cancer.
Multivariate analysis found that MSI was a factor affecting survival in colorectal cancer patients. This is consistent with studies on a large population (>1,000 patients) that were conducted by Samowitz et al., which showed that MSI was an independent prognostic factor of other prognostic factors [16,17]. The 5-year survival rate was better for patients with MSI-H than for those with MSI-L for each tumor stage. For stage II, III, and IV, the 5-year survival rates of patients with MSI-H were 33.3%, 22.2%, and 20%, respectively, compared with 0%, 5%, and 0% for patients with MSI-L. The only patient with a stage I tumor had MSI-H. It should be noted that the 5-year survival in this study tended to be lower than in other studies showing international survival rates of MSI-H of >50%. This difference may be due to the limited facilities of national health services for malignant diseases in common [15,18,19].

There are some limitations of this study. It was conducted in one institution based on retrospective data. The institution is a national referral hospital, which could lead to a selection bias, as most patients are in advanced stages. We sought to reduce the bias by showing that most patient characteristics were equivalent between the two groups. However, CEA levels and tumor stages were lower in the MSI-H group than in the MSI-L group. This difference is in agreement with the results of the previous studies, suggesting that because MSI-H is a good indicator of survivability, it tends to be found in patients with lower tumor stages and lower CEA levels.

Another limitation of this study is that it could not assess the effect of chemotherapy with fluorouracil (5-FU) on patient survival because complete surveillance data could not be obtained. In addition, the status of MSI was determined with only two retrievals, i.e., PMS2 and MSH6, whereas in general, two additional increments of MLH1 and MSH2 are used.

5. Conclusions
MSI-H is an important prognostic factor for determining the 5-year survival in colorectal cancer patients. Patients with MSI-H had a better prognosis than patients with MSI-L.

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