Predictors for High Microsatellite Instability in Patients with Colorectal Cancer Fulfilling the Revised Bethesda Guidelines

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Abstract. *Background: The revised Bethesda guidelines (rBG) are generally used for screening of Lynch syndrome, and few researchers have investigated the associations between microsatellite instability (MSI) status and each item of the rBG. Patients and Methods: This retrospective study included patients with colorectal cancer who were classified into those fulfilling the rBG (Bethesda group) and those not (control group). The breakdown of each item in the rBG and predictors of high MSI (MSI-H) were determined in the Bethesda group. Results: Of 809 consecutive patients, 161 (19.9%) were found to fulfil the rBG criteria. As a predictor of MSI-H, items 2 or 5 of the rBG showed a sensitivity of 93.3%. Item 5 and right-sided tumour location were independent predictors of MSI-H in patients fulfilling the rBG (odds ratio(OR)=4.49 and 25.1; p=0.0260 and <0.0001, respectively). Conclusion: Item 5 of the rBG and right-sided tumour location are significant predictors of MSI-H.*

Lynch syndrome (LS) is a common autosomal dominant disorder that accounts for approximately 1-3% cases of colorectal cancer (CRC) (1). In 1991, the Amsterdam criteria were originally designed to select families appropriate for enrolment in research projects aimed at identifying the genetic causes of hereditary CRC. In 1999, these criteria were extended to extra-colonic cancer associated with LS (2). However, even the revised Amsterdam II criteria have relatively low sensitivity for diagnosing LS (2, 3). On the other hand, in 1997, the National Cancer Institute hosted an international workshop to develop criteria and identify patients with CRC who should be offered microsatellite instability (MSI) testing due to an increased risk for LS, and concurrently suggested the Bethesda guidelines with less stringent criteria than the Amsterdam criteria (4). These guidelines queried the patient's medical and familial history of LS-related tumours and early age of onset. In 2004, these guidelines were revised to achieve higher specificity (5).

Some studies recommended universal testing of MSI or immunostaining for mismatch repair proteins in all patients with newly diagnosed CRC. However, universal screening is not cost-effective in clinical practice (6). In general, the preselection of patients with LS based on clinical criteria seems to be a more cost-effective approach (7). At present, the Japanese guidelines for hereditary CRC recommend the use of the revised Bethesda guidelines (rBG) for the primary screening of LS (Table I), and patients who fulfil the rBG have been recommended for testing for MSI (8). The proportion of patients fulfilling the rBG was 16-47% in all types of CRC in previous studies (6, 9, 10). Furthermore, in other studies, the proportion of patients with high MSI (MSI-H) was 12-18% in patients fulfilling the rBG, and the proportion of those with LS was 2-4% in patients fulfilling the rBG (11-14). In this study, we aimed to assess the relationship between each item of the rBG and MSI-H status to seek a more cost-effective approach for selection of patients with MSI-H tumours. In particular, we identified the independent factors predictive of MSI-H among patients fulfilling the rBG.

Patients and Methods

The medical records of patients with CRC who underwent surgical resection between January 2014 and December 2017 were retrospectively collected from the database of the Department of Surgical Oncology at the University of Tokyo, Japan. All surgically resected specimens were histopathologically reviewed. Tumour staging was determined according to the criteria of the Union for International Cancer Control (UICC) TNM classification (15). Patients were categorized into two groups: those fulfilling the rBG

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Table I. *The number and proportion of cases fulfilling the revised Bethesda guidelines.**

Item	Revised Bethesda Guidelines Description	This study, N (%) (N=161)
1	Colorectal cancer diagnosed in a patient under 50 years of age.	68 (42.2)
2	Presence of synchronous, metachronous colorectal, or other LS-associated tumours, regardless of age.	65 (40.4)
3	Colorectal cancer with the MSI-H histology diagnosed in a patient under 60 years of age.	3 (1.9)
4	Colorectal cancer diagnosed in one or more first-degree relatives with an LS-related tumour, with one being diagnosed under 50 years of age.	6 (3.7)
5	Colorectal cancer diagnosed in two or more first-or second-degree relatives with LS-related tumours, regardless of age.	47 (28.6)

LS, Lynch syndrome; MSI-H, high microsatellite instability. *Modified from (5).

(Bethesda group), and those not (control group) (Table I). Our focus was on the Bethesda group, and the proportion of patients fulfilling each criterion of the rBG in this group was evaluated. We also assessed the combination of each item for high sensitivity for prediction of MSI-H. MSI status was assessed using five microsatellite loci: *BAT25, BAT26, MONO27, NR21,* and *NR24* for patients in the Bethesda group. The locations of tumours and MSI status in the Bethesda group were evaluated and stratified by the item number of the rBG. The sensitivity and specificity for prediction of MSI-H were calculated using numbers of patients fulfilling each rBG. Additionally, we assessed the number of patients required in order to identify one patient with MSI-H and the proportion of patients with MSI-H who would be missed for each item of the rBG. Moreover, independent factors predicting MSI-H were sought in the Bethesda group. Tumour location and MSI status were evaluated in association with each rBG item and their combination.

This study was approved by the Ethics Committees of the University of Tokyo [no. 3252-(6)]. The research was conducted according to the principles of the Declaration of Helsinki.

Statistical analyses. Differences in the categorical variables between the Bethesda and control groups were examined using the Chi-squared or Fisher test, as appropriate. The independent predictive factors for MSI-H were also assessed using logistic regression analysis. Values of $p<0.05$ were considered statistically significant. All statistical analyses were conducted using JMP software version 10 (SAS Institute Inc., Cary, NC, USA).

Results

A total of 809 patients were included in this study: 161 (19.9%) patients fulfilling the rBG comprised the Bethesda group and 648 (80.1%) patients formed the control group. Clinicopathological characteristics of the Bethesda and control groups were compared (Tables I and II). The proportion of patients fulfilling items 1 to 5 of the rBG were 42.2%, 40.4%, 1.9%, 3.7%, and 28.6%, respectively. Patients in the Bethesda group were significantly younger than those in the control group since the rBG includes CRC diagnosis in patients younger than 50 years. Tumour location by each rBG item and combinations of rBG are presented in Table III and MSI status in Table IV.

Table II. *Clinicopathological features of all cases of colorectal cancer included in this study.*

	Bethesda group N=161 (19.9%)	Control group N=648 (80.1%)	p-Value
Age (years)			
Median (range)	59 (30-88)	67 (5-94)	<0.0001*
Gender, N (%)			
Male	98 (60.9)	372 (57.4)	0.4220
Female	63 (39.1)	276 (42.6)	
CEA level (ng/ml)			
Median (range)	3.9 (0.8-331)	4.4 (0.3-4256)	0.2141
Location, N (%)			
Right	38 (23.6)	193 (29.8)	0.1270
Left	123 (76.4)	455 (70.2)	
Histopathology, N (%)			
Well/mod	147 (91.3)	605 (93.4)	0.3715
Other	14 (8.7)	43 (6.6)	
T-Stage, N (%)			
1	40 (24.8)	119 (18.4)	0.1527
2	30 (18.6)	142 (21.9)	
3	49 (30.4)	238 (36.7)	
4	42 (26.1)	149 (23.0)	
LN metastasis, N (%)			
Absent	89 (55.3)	385 (59.0)	0.3874
Present	72 (44.7)	265 (41.0)	
Lymphatic invasion, N (%)			
Absent	108 (67.1)	465 (71.8)	0.2466
Present	53 (32.9)	183 (28.2)	
Venous invasion, N (%)			
Absent	76 (47.2)	265 (40.9)	0.1481
Present	85 (52.8)	383 (59.1)	
Stage			
I	45 (28.0)	186 (28.7)	0.2796
II	41 (25.5)	155 (23.9)	
III	58 (36.0)	203 (31.3)	
IV	17 (10.6)	104 (16.1)	

CEA, Carcinoembryonic antigen; LN metastasis, lymph node metastasis; Muc, mucinous carcinoma; Mod, moderately differentiated adenocarcinoma; Wel, well differentiated adenocarcinoma. *Significance accepted at $p<0.05$.

Table III. *Relationship between tumour location and each item of the revised Bethesda guidelines in the Bethesda group.*

Revised Bethesda Guidelines		Overall	Right	Left	
Item	Response	N=161	38 (23.6%)	121 (66.4%)	*p*-Value
1	Yes	68 (42.2%)	6 (8.8%)	62 (91.2%)	<0.0001*
	No	93 (57.8%)	32 (34.4%)	61 (65.6%)	
2	Yes	65 (40.4%)	18 (27.7%)	47 (72.3%)	0.3170
	No	96 (59.6%)	20 (20.8%)	76 (79.2%)	
3	Yes	3 (1.9%)	1 (33.3%)	3 (66.7%)	0.7000
	No	158 (98.1%)	37 (23.4%)	121 (76.6%)	
4	Yes	6 (3.7%)	3 (50.0%)	3 (50.0%)	0.1521
	No	155 (96.3%)	35 (22.6%)	120 (77.4%)	
5	Yes	47 (28.6%)	17 (36.1%)	30 (63.9%)	0.0187*
	No	114 (71.4%)	21 (18.4%)	93 (81.6%)	
1 or 2	Yes	129 (80.1%)	24 (18.6%)	105 (81.4%)	0.0043*
	No	32 (19.9%)	14 (43.8%)	18 (56.3%)	
1 or 5	Yes	101 (62.7%)	21 (20.8%)	80 (79.2%)	0.2795
	No	60 (37.3%)	17 (28.3%)	43 (71.7%)	
2 or 5	Yes	109 (67.1%)	34 (31.2%)	75 (68.8%)	0.0007*
	No	52 (32.9%)	4 (7.7%)	48 (92.3%)	

*Significance accepted at *p*<0.05.

Table IV. *Relationship between the microsatellite instability status and each item of the revised Bethesda guidelines in the Bethesda group.*

Revised Bethesda Guidelines		Total	MSS	MSI-H	*p*-Value	Sensitivity	Specificity	NPR	Patients with MSI-H missed, N (%)
Item	Response	N=161	146 (90.7%)	15 (9.3%)		NA	NA	10.7	NA
1	Yes	68 (42.2%)	63 (92.7%)	5 (7.3%)	0.4585	33.3	56.8	13.6	10 (66.7%)
	No	93 (57.8%)	83 (89.3%)	10 (10.7%)					
2	Yes	65 (40.4%)	59 (90.8%)	6 (9.2%)	0.9753	40	59.6	10.8	9 (60%)
	No	96 (59.6%)	87 (90.6%)	9 (9.4%)					
3	Yes	3 (1.9%)	3 (100%)	0 (0%)	0.4414	0	97.9	-	15 (100%)
	No	158 (98.1%)	143 (90.5%)	15 (9.5%)					
4	Yes	6 (3.7%)	5 (83.3%)	1 (16.7%)	0.5639	6.7	96.6	6	14 (93.3%)
	No	155 (96.3%)	141 (91.0%)	14 (9.0%)					
5	Yes	47 (28.6%)	38 (80.9%)	9 (20.1%)	0.0089*	60	74.0	5.2	6 (40%)
	No	114 (71.4%)	108 (94.8%)	6 (5.2%)					
1 or 2	Yes	129 (80.1%)	119 (92.3%)	10 (7.7%)	0.1961	66.7	18.5	12.9	5 (33.3%)
	No	32 (19.9%)	27 (84.4%)	5 (15.6%)					
1 or 5	Yes	101 (62.7%)	90 (89.1%)	11 (10.9%)	0.3620	73.3	38.4	9.2	4 (26.7%)
	No	60 (37.3%)	56 (93.3%)	4 (0.7%)					
2 or 5	Yes	109 (67.1%)	95 (87.1%)	14 (12.9%)	0.0121*	93.3	34.9	7.8	1 (6.7%)
	No	52 (32.9%)	51 (98.1%)	1 (1.9%)					

MSS, Microsatellite stable; MSI-H, high microsatellite instability; NA, not applicable; NPR, number of patients required to identify one patient with MSI-H. *Significance accepted at *p*<0.05.

In this study, there were a few patients fulfilling items 3 (1.9%) and 4 (3.7%) of the rBG. In terms of tumour location, the proportion of patients with left-sided tumours was higher than that of those with right-sided tumours among patients fulfilling item 1 and items 1 or 2. By contrast, the proportion with right-sided tumours was higher than that of left-sided tumours among those fulfilling item 5 and items 2 or 5 (Table III).

Table V. *Logistic regression analysis for predicting high microsatellite instability (MSI-H) for patients in the Bethesda group.*

		Univariate analysis	Multivariate analysis		
Factor	Subgroup	*p*-Value	OR	95% CI	*p*-Value
General variables					
Gender	Male *vs.* female	0.8185			
CEA (ng/ml)	<5.0 *vs.* ≥5.0	0.5275			
Cancer-related variables					
Tumour location	Left *vs.* right	<0.0001*	25.1	4.99-126	<0.0001*
Pathology	Well/mod *vs.* poor/muc/sig	0.0887	4.06	0.76-21.6	0.0883
T Factor	T1,2 *vs.* T3,4	0.5472			
N Factor	N0 *vs.* N1	0.3749			
Lymphatic invasion	Absent *vs.* present	0.4450			
Venous invasion	Absent *vs.* present	0.8143			
M Factor	M0 *vs.* M1	0.1189			
rBG item					
1	Yes *vs.* no	0.0406*	2.83	0.60-13.3	0.1886
2	Yes *vs.* no	0.5873			
3	Yes *vs.* no	0.4626			
4	Yes *vs.* no	0.1215			
5	Yes *vs.* no	0.0350*	4.49	1.20-16.8	0.0260*

CEA, Carcinoembryonic antigen; CI, confidence interval; MSI-H, microsatellite instability-high; Muc, mucinous carcinoma; sig, signet ring cell carcinoma; Mod, moderately differentiated adenocarcinoma; OR, odds ratio; rBG, Revised Bethesda Guidelines; Well, well-differentiated adenocarcinoma. *Significance accepted at *p*<0.05.

Table VI. *The predictors for high microsatellite instability (MSI-H) for patients in the Bethesda group.*

	Total	MSS	MSI-H	*p*-Value	Sensitivity	Specificity	NPR	Patients with MSI-H missed, N (%)
All cases	N=161	N=146	N=15		NA	NA	10.7	NA
Right-sided tumour	38 (23.6%)	26 (68.4%)	12 (31.6%)	<0.0001*	80.0	82.2	3.2	3 (20.0%)
Left-sided tumour	123 (76.4%)	120 (97.6%)	3 (2.4%)		20.0	17.8	41	12 (80.0%)
Patients fulfilling rBG items 2 or 5	N=109	N=95	N=14					
Right-sided tumour	34 (31.2%)	22 (64.7%)	12 (35.3%)	<0.0001*	85.7	76.8	2.8	2 (14.3%)
Left-sided tumour	75 (68.8%)	73 (97.3%)	2 (2.7%)		14.3	23.2	37.5	12 (85.7%)

MSS, Microsatellite stable; MSI-H, high microsatellite instability; rBG, Revised Bethesda Guidelines; NPR, number of patients required to identify one patient with MSI-H. *Significance accepted at *p*<0.05.

The number of patients presenting with MSI-H was 15 (9.3%) in the Bethesda group. The proportion of patients with MSI-H was higher among those fulfilling item 5 and those fulfilling items 2 or 5 (Table IV). Regarding the number of patients required to identify one patient with MSI-H, the combination of item 2 or 5 required eight patients, and missed only one patient among all 15 patients with MSI-H. In the logistic regression analysis, independent factors predictive of MSI-H included fulfilment of rBG item 5 and right-sided tumour (odds ratio=4.49 and 25.1, *p*=0.0260 and <0.0001, respectively) (Table V). In terms of correlation between MSI

and tumour location, the number of right-sided tumours with MSI-H was higher than that of left-sided tumours with MSI-H. Additionally, with regard to fulfilling either rBG item 2 or 5, which had the highest sensitivity for prediction of MSI-H, details of which are presented in Table VI.

Discussion

In this study, we investigated each rBG item for association with MSI-H. The logistic regression analysis revealed that right-sided tumour and rBG item 5 were independent factors

associated with MSI-H. The number of patients required to identify one patient with MSI-H was 11 in the Bethesda group, including three in the group with right-sided tumour and three among those fulfilling item 2 or 5 and with right-sided tumour location, which missed only 14.3% of the patients with MSI-H (Table VI). These results do not necessarily reflect the trends in the general population, since we did not conduct universal screening. Considering the cost-effectiveness of identifying patients with MSI-H, MSI testing may be better confined to patients with right-sided colonic tumour or those fulfilling r BG items 2 or 5. This result suggests that care should be taken while collecting familial and past medical histories of patients regarding the presence of LS-associated tumours.

The findings of our study are consistent with those of a previous study reporting that tumours with MSI-H tended to be located in the right-sided colon (8). Our finding that familial history, as in item 5, was associated with MSI-H was likely due to selection bias because we included only patients fulfilling the rBG criteria. Two previous studies assessed each item of the rBG. Rodriguez *et al*. reported that item 1 of the rBG was independently associated with the presence of an *MSH2/MLH1* germline mutation (16). Jung *et al*. reported that items 1 and 2 were independently associated with a deficient DNA mismatch repair gene (3). We did not investigate germline mutations, since the germline mutation test is not covered under medical insurance in Japan. However, mismatch repair gene mutations should ideally be investigated in patients with MSI-H.

Regarding tumour location by rBG item, tumours of patients fulfilling item 1 were more frequently located in the left side of the colorectum and those of patients fulfilling item 5 were more frequently located in the right side than those of patients not fulfilling it. Tumours of patients fulfilling either items 1 or 2 were located in the left side of the colorectum, and those of patients fulfilling either items 2 or 5 were located in the right side. These findings may be due to the relationships between items 1 or 5 and respective tumour locations.

This study had a few limitations. Firstly, we did not investigate germline mutations of mismatch repair genes in this study. There were two types of MSI-H-associated CRC: LS and sporadic; sporadic MSI-H-associated CRC is attributed to methylation of the *MLH1* gene. We were unable to differentiate these two types. Secondly, the cohort of this study included only patients surveyed with the rBG, not all cases of CRC, since we focused on each item of the rBG among patients fulfilling the rBG. In a previous Japanese study, the proportion of patients with MSI-H ranged from 4.3% to 8.3% (17-19). Although identifying patients with MSI-H in the control group would have been ideal, analysis of those not fulfilling the rBG criteria was beyond the scope of this study. Thirdly, there were few patients who met

criteria 3 or 4 of the rBG. In some cases, eliciting the exact age of the family members at the time of cancer diagnosis was difficult, and due to the retrospective nature of this study, some pathologists might not pay attention to the MSI-H histology, such as Crohn's-like reaction. This tendency was also reported in a previous study and may be a limitation of the rBG in clinical screening (3).

In conclusion, in patients with CRC who fulfil the rBG, item 5 and right-sided tumours are factors predictive of MSI-H.

Acknowledgements

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