Original Article

Validation of microsatellite instability histology scores with Bethesda guidelines in hereditary nonpolyposis colorectal cancer

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ABSTRACT

Aims: Hereditary nonpolyposis colorectal cancer (HNPCC) is a subgroup of colorectal cancer (CRC) which should be differentiated because of the high risk for additional cancers and risk evaluation for other family members, especially for CRC. It is not practical to perform genetic testing for all CRC patients; therefore, various prediction modalities, for example, Bethesda guideline (BG) were studied in the literature. We aimed to assess the association of microsatellite instability (MSI), histology scores, and BG for predicting HNPCC risk.

Subjects and Methods: Data were collected from CRC patients between 2009 and 2012. A total of 127 patients were retrospectively reviewed for BG status and the MSI scores, MsPath, and PathScore.

Statistical Analysis Used: Definitive statistical methods (mean, standard deviation, median, frequency, and percentage) were used to evaluate the study data. Comparison used Student's *t*-test, Continuity (Yates) correction, Fisher-Freeman-Halton test, Pearson correlation, and receiver operating characteristics curve analysis.

Results: Patients who were detected as Bethesda-positive had significantly higher MsPath and PathScore scores ($P = 0.001$ and $P = 0.007$, respectively). According to the cut-off value of 2.8 and 2.9 for MsPath and PathScore, respectively, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 90%, 43%, 22.8%, 95.8%, and 50.4% for MsPath, and 55%, 83.2%, 37.9%, 90.8%, and 78.7% for PathScore, respectively.

Conclusions: The MSI scoring systems, MsPath, and PathScore, are reliable systems and effectively correlated with BG for predicting patients who need advanced analysis techniques because of the risk of HNPCC.

KEY WORDS: Cancer genetics, colorectal cancer, pathology

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy worldwide and one of the most common causes of cancer-related death.[1] Hereditary nonpolyposis colorectal cancer (HNPCC) syndrome (Lynch syndrome) is the most common form of CRC associated with genetic predisposition. It is an autosomal dominant disease characterized by neoplastic lesions characterized by early age at onset and microsatellite instability (MSI).[2] HNPCC is due to defective mismatch repair (MMR) proteins. Although there are four main MMR genes (MLH1, MSH2, MSH6, and PMS2), MLH1 and MSH2 are defective in most HNPCC patients.[3,4]

The International Collaborative Group has advised clinical criteria for predicting patients at risk for HNPCC. These so-called Amsterdam criteria included a family history of CRC with regard to the number of affected relatives, degree of relationship, and age at diagnosis. Because of the relatively narrow features of Amsterdam criteria, the Bethesda guidelines (BGs) were developed, and extended criteria for hereditary CRC including a tool to predict the risk of developing HNPCC could be offered.[5]

Genetic testing can be used to identify MMR gene mutations for HNPCC patients.[3,4] However, it should be offered to suspected patients selectively

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due to its cost and time-consuming evaluation. Before recommending genetic testing, the Amsterdam criteria and Bethesda guideline should be used.[5-7] However, problems may appear due to missing information in the presence of scattered family members preventing the optimum use of these criteria. Hence, researchers have proposed MSI histology to identify candidates who are most likely HNPCC carriers.[8]

Microsatellites are tandem-repeated nucleotide sequences that are especially common in HNPCC patients with frameshift mutations and base-pair substitutions. This form of genetic destabilization is most commonly caused by the loss of a DNA MMR function. It is referred to as an MSI pathway, and the term "high-frequency–MSI (MSI-H) phenotype" is used for tumors with this defect.[9-11] In literature, MSI scoring systems such as the MsPath score and PathScore that are based on clinicopathologic features including age at diagnosis below 50 years, a proximal tumor location, and histologic features such as tumor-infiltrating lymphocytes, mucinous histology, differentiation, and Crohn's-like reaction have been used to standardize the prediction of HNPCC patients. They have been used to identify the probability of MSI-H presence in CRCs. Cut-off levels of 5.6 and 4.3 are recommended for the MsPath score and PathScore, respectively, to predict MSI-H with 95% specificity.[12,13]

Immunohistochemistry testing can also be used during pathological evaluation to detect MSI. Immunohistochemistry of tumor specimens searches for the presence/absence of MMR genes. However, due to a lack of technical expertise on immunohistochemistry, these MSI scoring systems have been offered to predict HNPCC.[12-14]

Consequently, the diagnosis of HNPCC usually includes a review of family cancer history, and if possible, detailed pathologic (e.g., MSI histology, immunohistochemistry) or genetic tests.

Although BG and MSI histology can be used to predict the risk of developing HNPCC, the association between BG and MSI histologic status has not yet been studied and has not been adequately elucidated in the literature. To evaluate that, we designed novel method to get family history rigorously. Then, we assessed the degree of association and predictive power of MSI histology scores in relation to BG.

SUBJECTS AND METHODS

Patients with primary CRC who underwent surgery between 2009 and 2012 were evaluated retrospectively using a prospective database. Patients with preoperative chemoradiotherapy or treated with local excision were excluded from this study. The study protocol was approved by the local Ethical Committee.

The patients were evaluated in terms of demographic characteristics, tumor location, tumor stage (according to the tumor-node-metastasis [TNM] classification), and histopathological examinations including MSI scores. Tumors of the cecum and ascending and transverse colon were considered to be right-sided. Those in the descending, sigmoid, and the rectum were considered to be left-sided. Tumors with two or more distinct primary tumors were noted as synchronous.

We interviewed with three to five family members of each patient within two generation with the aid of family registry system. The pedigrees of all patients were meticulously investigated and assessed according to the published BG criteria [Table 1].[5] The results were expressed as BG-positive or -negative if any or none of the BG criteria were met, respectively. The MSI scores of all patients were evaluated based on the MsPath and PathScore scoring systems [Table 2].[12-14]

We next evaluated the relationship among BG, MSI scores, MsPath, and PathScore. Discriminatory power or the ability to identify the likelihood of the patients with positive BG, sensitivity, and specificity, and corresponding cut-off points was determined using receiver operating characteristics (ROC)

Table 1: Bethesda guideline criteria

Individuals with cancer in families meeting the Amsterdam criteria
At least 3 relatives with histologically confirmed CRC, 1 of whom is a first-degree relative of the other 2; familial adenomatous polyposis should be excluded
At least 2 successive generations involved
At least 1 of the cancers diagnosed before the age of 50
Individuals with 2 or more HNPCC related cancers, including synchronous and metachronous CRCs or associated extracolonic cancers
An individual and a first-degree relative with either CRC and/or HNPCC related extracolonic cancer; and/or a colorectal adenoma
One of the cancers diagnosed at age <45 years, and the adenoma diagnosed at age <40 years
Individuals with CRC or endometrial cancer <45 years of age
Individuals with right-sided undifferentiated (solid or cribriform) CRC on histology diagnosed <45 years of age
Individuals with signet ring type CRC <45 years of age
Individuals with colonic adenomas <40 years of age

CRC=Colorectal cancer, HNPCC=Hereditary nonpolyposis colorectal cancer

Table 2: MsPath and PathScore scoring system

Feature	Criteria	MsPath	PathScore
Tumor locations	Right-sided	1.6	0.8
Age	<50	0.7	1.1
Grade	Well differentiated	0	1.2
	Moderately differentiated	0	0
	Poorly differentiated	0.6	1.2
Histologic type	Mucinous differentiated		0.5
	Mucinous carcinoma >50%, or signet ring, or medullary carcinoma	1.1	
Dirty necrosis	Absent	0	0.6
Crohn-like reaction	Present	0.5	0.8
Tumor infiltrating lymphocytes ≥1	Present	2.1	
Tumor infiltrating lymphocytes >2	Present		1.3

Total scores are calculated by the sum of score of positive criteria curve analysis. Statistical analyses were performed with IBM SPSS 22 (version 22.0, IBM, New York, USA) program. Definitive statistical methods (mean, standard deviation, median, frequency, and percentage) were used to evaluate the study data. Comparison used Student's *t*-test, continuity (Yates) correction, Fisher-Freeman-Halton test, Pearson correlation, and ROC curve analysis. The results were evaluated within the 95% confidence interval and $P < 0.05$ was recognized as statistically significant.

RESULTS

During the study period, we enrolled 138 patients with CRC. Eleven patients (preoperative chemoradiotherapy [$n = 9$], local excision [$n = 2$]) were excluded from the study. Therefore, 127 patients were enrolled in total. The mean age of the patients was 62.90 ± 12.53 years with a male-to-female ratio of 1.3:1.

A total of 20 patients (15.7%) were considered to be positive according to BG. Three positive scores were seen in seven patients, two positive scores were seen in two patients, and one positive score was seen in 11 patients. The remaining 107 patients (84.3%) were BG-negative. Analysis of BG results with age and tumor location showed a significant difference; the BG-positive patients were younger and had more synchronous tumors ($P = 0.001$ and $P = 0.001$, respectively), but there was no difference with regard to gender or TNM stages [Table 3]. There were 31 (24.4%) and 91 (71.6%) patients with right-sided and left-sided tumors, respectively. The cohort included five patients (4%) with synchronous CRC. Among five patients (4%) with positive familial history for CRC, four of them were BG-positive. Characteristics of the BG-positive patients are shown in Table 4.

Patients who identified as BG-positive had significantly higher MsPath and PathScore scores than that of BG-negative ones ($P = 0.001$ and $P = 0.007$, respectively) [Table 5]. Both the MsPath and PathScore values correlated positively with the BG scores with r values of 33.6% and 32.4%, and P values of 0.001 and 0.001, respectively [Table 6]. A cut-off level of 2.8 was determined for MsPath to predict BG status with a sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of 90%, 43%, 22.8%, 95.8%, and 50.4%, respectively. The area under the curve was 0.725 [Table 7 and Figure 1]. A cut-off level of 2.9 was determined for the PathScore to predict the BG status with a sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of 55%, 83.2%, 37.9%, 90.8% and 78.7%, respectively. The area under curve was 0.690 [Table 7 and Figure 1].

Table 3: Characteristics of patients with colorectal cancer (*n*=127)

Characteristic	BG-positive (*n*=20)	BG-negative (*n*=107)	*P**
Age (mean±SD)	49.45±13.62	65.42±10.63	0.001†
Gender, male/female (ratio)	11/9 (1.2)	61/46 (1.3)	1.00
Tumor TNM stage			
Stage 1, *n* (%)	1 (16.7)	5 (83.3)	0.59
Stage 2, *n* (%)	7 (12.7)	48 (87.3)	
Stage 3, *n* (%)	7 (15.2)	39 (84.8)	
Stage 4, *n* (%)	5 (25)	15 (75)	
Tumor location			
Right-sided, *n* (%)	2 (6.5)	29 (93.5)	0.001†
Left-sided, *n* (%)	13 (14.3)	78 (85.7)	
Synchronous, *n* (%)	5 (100)	0 (0)	

*Student's *t*-test, Continuity (Yates) correction and Fisher-Freeman-Halton test, †$P<0.01$. SD=Standard deviation, BG=Bethesda guideline, TNM=Tumor-node-metastases

DISCUSSION

The HNPCC assessment tools are the Amsterdam criteria, BG, revised BG, MSI histology, MSI scoring systems, MSI immunochemistry, and genetic analysis. Each has pros and cons described in the literature.[2,3,5-7] The Amsterdam criteria and BG are a set of diagnostic criteria used by clinicians to identify families that are likely to have HNPCC. If any of these criteria is fulfilled, the immunohistochemical staining for the MMR-genes, MSI histology, and genetic testing is recommended.

Preoperative use of these guidelines gives the opportunity to decide whether extended surgery should be pursued in HNPCC patients. However, the failure to assess the full family history due to scattered family members and a lack of awareness of this important hereditary syndrome are the major causes of under-diagnoses. This puts patients and their families at risk of CRC and associated extracolonic cancers. In clinical practice, the postoperative analysis of MSI histology is inconsistently performed regardless of the BG criteria. Therefore, affected HNPCC patients are left uninformed of their increased genetic risk, and family members who are possible gene carriers miss the opportunity to actively engage in the recommended screening program.[13,15,16]

The International Collaborative Group on HNPCC recommends a screening program for gene-positive families or individuals

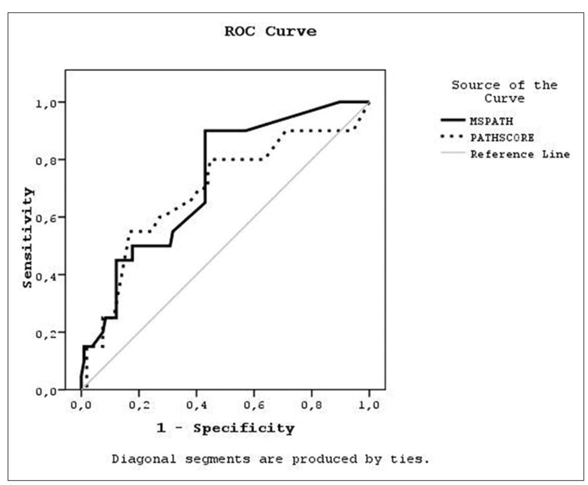

Figure 1: Cut-off analysis of microsatellite instability scores (MsPath, PathScore)

Table 4: Characteristics of Bethesda guideline positive patients

Patient	Age	Sex	Family history	Tumor location	TNM/stage	BG score	MsPath	PathScore
1	46	Male	No	Synchronous	T3N0, T3N0M0/2	3+	4.4	3.7
2	31	Female	No	Left	T2N1M0/3	3+	5	5
3	42	Female	No	Right	T3N1M0/3	3+	6	3.8
4	44	Male	No	Right	T4N2M0/3	3+	6.1	4.9
5	45	Male	Yes	Synchronous	T3N0, T2N0M0/2	3+	2.8	1.6
6	38	Male	Yes	Left	T3N1M0/3	3+	4.4	3.2
7	44	Male	No	Left	T4N2M0/3	3+	4.5	2.9
8	39	Female	Yes	Left	T4N1M1/4	2+	3.9	2.9
9	55	Female	Yes	Left	T3N0M0/2	2+	3.2	3.3
10	71	Male	No	Synchronous	T3N0, T2N0M1/4	1+	3.2	2.4
11	44	Female	No	Left	T3N2M1/4	1+	2.8	1.1
12	44	Male	No	Left	T3N2M1/4	1+	2.8	1.1
13	56	Male	No	Left	T3N0M0/2	1+	2.1	0
14	40	Female	No	Left	T3N1M0/3	1+	3.3	1.9
15	83	Male	No	Synchronous	T3N0, T1N0M0/2	1+	5.3	2.1
16	44	Male	No	Left	T4N0M0/2	1+	4.4	3
17	73	Female	No	Synchronous	T2N0, T2N0M0/1	1+	5.9	4.7
18	39	Female	No	Left	T4N1M0/3	1+	2.8	1.7
19	66	Male	No	Left	T3N0M0/2	1+	2.1	0
20	45	Female	No	Left	T4N1M1/3	1+	2.8	2.9

BG=Bethesda guideline, TNM=Tumor-node-metastases

Table 5: The relationship between Bethesda guideline and microsatellite instability scoring systems, MsPath, and PathScore

	BG (mean±SD (median))		P*
	Positive (n=20)	Negative (n=107)	
MsPath score	3.89±1.28 (3.60)	2.87±1.27 (2.60)	0.001†
PathScore score	2.61±1.45 (2.90)	1.75±1.26 (1.30)	0.007†

*Student's t-test, †P<0.01. SD=Standard deviation, BG=Bethesda guideline

Table 6: Correlation between Bethesda guideline and microsatellite instability scores

	BG scores	
	r	P*
MsPath	0.336	0.001†
PathScore	0.324	0.001†

*Pearson correlation, †P<0.01. BG=Bethesda guideline

with a high risk based on family history. This program was started 25 years or 10 years before the age of the youngest member of the family at the time of CRC diagnosis. Applying colonoscopy each year after reaching the age of 30 complements existing evaluations that are performed every 2 years including endometrial aspiration biopsy, transvaginal ovarian ultrasonography, Papanicolaou stain, and two-handed pelvic examination.[7,11]

Deficiency in MMR proteins in HNPCC reveals the presence of MSI-H. In general, HNPCC patients present with MSI-H. The MSI-H is detected in approximately 86% of HNPCC cases, but are also in 10–15% of sporadic CRC patients due to hypermethylation of the MLH1 promoter region. The clinicopathologic and prognostic characteristics of MSI-positive tumors are quite different than those with low MSI or stable microsatellites. Proximal CRCs show more frequent MSI-H disruptions than distal CRCs. CRC patients with MSI-H had poor differentiation, mucinous cell type, and peritumoral lymphocytic infiltration. They are usually diploid, unlike microsatellite-stable tumors that are commonly aneuploid. CRC with MSI-H are also associated with a large-sized primary tumor, but a more favorable stage distribution. A longer survival is expected in patients with CRCs that exhibit MSI-H than patients with cancers exhibiting microsatellite stability in similar stages.[17,18]

MSI testing is an important tool to identify patients with hereditary CRC. MSI status also plays an important role in the responsiveness of CRC to certain chemotherapies.[18,19] This testing can be performed by immunohistochemical staining for the MMR genes. However, the availability of this method may be low in some centers. This problem is overcome by MSI histology and scoring systems such as MsPath and PathScore.[13] Indeed, in this study, the BG criteria were rigorously investigated and revealed to understand BG status. We analyzed BG status by MSI scores, MsPath, and PathScore scoring systems. The BG-positive patients showed significantly higher scores for MsPath and PathScore. We could not perform MSI analysis by polymerase chain reaction (PCR) or immunohistochemistry for MMR protein expression in our laboratory at the time of this study because of technical deficiencies and lack of infrastructure. Furthermore, we did not confirm the results with genetic analysis in the patients with BG-positive or MSI scores at cut-off levels. Future studies are needed for this confirmation. This can be the main limitation of the study; however, the aim of the study was to evaluate the association of BG status and MSI histology scores. Nevertheless, after learning from this study, we designed a new study with MSI immunochemistry, scoring systems, and genetic analysis.

There is evidence suggesting that people who have family members with a history of CRC develop CRC earlier than the general population. The risk of CRC development in a 40-year-old individual with a family history of CRC is

Table 7: Statistical evaluation of Bethesda guideline criteria, and microsatellite instability scoring systems, MsPath, and PathScore, at new cut-off levels

	BG-positive (*n*=20)	BG-negative (*n*=107)	*P**	SEN	SPE	PPV	NPV	ACC
MsPath >2.8, *n* (%)	18 (90)	46 (43)	0.001†	90	43	22.8	95.8	50.4
PathScore >2.9, *n* (%)	11 (55)	18 (16.8)	0.001†	55	83.2	37.9	90.8	78.7
MsPath >2.8 or PathScore >2.9, *n* (%)	18 (90)	48 (44.9)	0.001†	90	55.1	22.3	96.7	60.6

*Fisher's exact test, †*P*<0.05. SEN=Sensitivity, SPE=Specificity, PPV=Positive predictive value, NPV=Negative predictive value, ACC=Accuracy, BG=Bethesda guideline

comparable to that of a 50-year-old individual in the general population. The prevalence of CRC is twice as high in these individuals. This increase becomes more significant during the early years of life. If a person's relative has developed CRC at an early age, then the risk for this person is even higher. Nevertheless, there is no evidence indicating that carcinomas develop more rapidly in patients with a family history of CRC.[20]

In this study, the mean age of all CRC patients was 62.9 years. Five patients had a family history of CRC, and the mean age of diagnosis was 48.2 years. Four of the five patients with a family history of CRC were BG-positive. The most common tumor location in the patients with a family history was the left colon. This was consistent with the literature. In the US, 15–20% of all CRC cases have a family history of CRC. The HNPCC constitutes 20–35% of these cases, but only 4–7% of all cases.[20,21] In the present report, 4% of CRC patients had a family history. This difference in the percent with family history may be caused by geographic variations in the Turkish population.

At the time of the original Bethesda criteria (1997), no consensus was reached about which microsatellite markers would define the MSI. This was discussed at a subsequent workshop in 2002 and was included as a part of the revised BG in 2004. The revised BG expanded the inclusion criteria and widely supports the use of MSI in HNPCC screening. The revised BG system diagnoses CRC via MSI-H histology in patients younger than 60. The MSI-H histology changes defined in the revised BG show the presence of tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet ring differentiation, or medullary growth pattern.[22] In this study, we used the original BG to compare and assess the validation with MSI scores because the revised BG includes some MSI histology features. However, the remaining features in MSI histology have a more decisive effect on MSI scores. Our hypothesis was that more MSI histology features would be more effective at MSI-H prediction than the revised BG and the original BG. We found that MSI scores are competent to BG at cut-off levels of 2.8 and 2.9 for MsPath and PathScore, respectively. The MsPath score values at 2.8 and PathScore values at 2.9 had a negative predictive value of 95.8% and 90.8% for BG, respectively. In light of these data, patients with MsPath and PathScore values at these levels had a 95.8% and 90.8% chance of being negative for BG, respectively. According to studies about MSI scoring systems, an MsPath score of 2.8 and a PathScore of 2.9 had a probability of 18.1% and 15.7% for MSI-H presence, respectively.[12,14]

Our initial hypothesis was that combined use of two systems, BG and MSI scoring system, can enhance the power of prediction. We designed a new study for combined methods confirmed by genetic analysis. However, the possibility of missing family information limited the use of BG for the study of this hypothesis. Therefore, we tried to predict BG with MSI histology in this study. We determined cut-off levels of 2.8 and 2.9 for MsPath and PathScore, respectively, which are suitable for BG association. Although this contribution has to be proved by genetic analysis with new studies, at these cut-off levels, MSI scoring systems offer an enhancement of combined (BG and MSI histology) methods for prediction of MSI-H presence even without disturbed to get inaccessible family history.

CONCLUSION

We highly recommend evaluating the MSI histology scoring systems including MsPath or PathScore as tools to predict candidates for genetic testing with a comparable result to BG while overcoming limitations of BG. These may be especially applicable in centers with technical deficiencies and lack of infrastructure for PCR or immunohistochemistry related to MMR protein expression.

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Conflicts of interest
There are no conflicts of interest.

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