Clinical and pathological tools for identifying microsatellite instability in colorectal cancer

Aim To assess practical accuracy of revised Bethesda criteria (BGrev), pathological predictive model (MsPath), and histopathological parameters for detection of high-frequency of microsatellite instability (MSI-H) phenotype in patients with colorectal carcinoma (CRC).

Method Tumors from 150 patients with CRC were analyzed for MSI using a fluorescence-based pentaplex polymerase chain reaction technique. For all patients, we evaluated age, sex, family history of cancer, localization, tumor differentiation, mucin production, lymphocytic infiltration (TIL), and Union for International Cancer Control stage. Patients were classified according to the BGrev, and the groups were compared. The utility of the BGrev, MsPath, and clinical and histopathological parameters for predicting microsatellite tumor status were assessed by univariate logistic regression analysis and by calculating the sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values.

Results Fifteen out of 45 patients who met and 4 of 105 patients who did not meet the BGrev criteria had MSI-H CRC. Sensitivity, specificity, PPV, and NPV for BGrev were 78.9%, 77%, 30%, and 70%, respectively. MSI histology (the third BGrev criterion without age limit) was as sensitive as BGrev, but more specific. MsPath model was more sensitive than BGrev (86%), with similar specificity. Any BGrev criterion fulfillment, mucinous differentiation, and right-sided CRC were singled out as independent factors to identify MSI-H colorectal cancer.

Conclusion The BGrev, MsPath model, and MSI histology are useful tools for selecting patients for MSI testing.
Microsatellite instability (MSI) is a hallmark of mismatch repair (MMR) deficiency in hereditary nonpolyposis colorectal cancer (HNPCC). Because these carcinomas were observed to develop in the absence of polyposis, the term HNPCC was used instead of Lynch syndrome (LS). Moreover, HNPCC corresponds to at least two different entities, LS and type X familial colorectal cancer (1). LS is always characterized by germline defect of mismatch repair system and is associated with an increased lifetime risk for cancer, predominantly colorectal and endometrial cancer.

Testing for MSI is an important tool for identification of patients with hereditary colorectal cancer, because approximately 90% of HNPCC-associated colorectal tumors are characterized by MSI (2). Clinical criteria facilitated the identification of the molecular basis of HNPCC (3). Because the original criteria (Amsterdam criteria) were considered to be too restrictive (4,5), extended criteria were established (Amsterdam II criteria), which took into account other types of HNPCC-associated cancer, such as cancer of the endometria, small bowel, ureter, and renal pelvis (6-8). The use of the Amsterdam criteria achieved the original purpose of classifying HNPCC families but their limited sensitivity hampered decisions about which patients should undergo genetic testing. In 1996, an international workshop on HNPCC hosted by the National Cancer Institute outlined a set of recommendations, known as the Bethesda guidelines, for the identification of individuals with HNPCC who should be tested for MSI and/or genetic testing (4). The Bethesda guidelines initially proved to be too sensitive, because it is important not to miss MSI-H cases.

The Bethesda guidelines initially proved to be too sensitive (4,5), extended criteria were established (Amsterdam II criteria), which took into account other types of HNPCC-associated cancer, such as cancer of the endometria, small bowel, ureter, and renal pelvis (6-8). The use of the Amsterdam criteria achieved the original purpose of classifying HNPCC families but their limited sensitivity hampered decisions about which patients should undergo genetic testing. In 1996, an international workshop on HNPCC hosted by the National Cancer Institute outlined a set of recommendations, known as the Bethesda guidelines, for the identification of individuals with HNPCC who should be tested for MSI and/or genetic testing (4). The Bethesda guidelines initially proved to be too sensitive, because it is important not to miss MSI-H cases.

The revised Bethesda guidelines (BGrev) was used instead of Lynch syndrome (LS). Moreover, HNPCC corresponds to at least two different entities, LS and type X familial colorectal cancer (1). LS is always characterized by germline defect of mismatch repair system and is associated with an increased lifetime risk for cancer, predominantly colorectal and endometrial cancer.

The aim of the study was to evaluate clinical and pathological parameters in a regional cohort of Serbian unselected colorectal cancer patients who were tested for MSI in tumor tissue. We analyzed practical validity of revised Bethesda criteria, MSI histology, and MsPath model (9) for detection of MSI-H phenotype in CRC, by determination of sensitivity, specificity, positive (PPV), and negative (NPV) predictive value.

### METHODS

One hundred and fifty primary colorectal carcinomas were randomly selected for MSI testing and excised surgically at the Clinic for Digestive Surgery, Clinical...
Centre of Serbia, Belgrade, from January 2007-September 2010. The study was approved by the ethics committee of the Clinical Centre. Patients treated by preoperative radiotherapy or chemotherapy, those with inflammatory bowel disease, or a known history of familial adenomatous polyposis were excluded. Family history data were obtained through an interview with each patient at hospital admission. Patients who fulfilled the Amsterdam criteria were also excluded. Fresh representative tissue samples from all 150 tumors were immediately frozen at -80°C and tested for MSI. The genomic DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol (10).

Five mononucleotide markers, BAT-25, BAT-26, NR-21, NR-22, and NR-24, were coamplified in a single pentaplex polymerase chain reaction (PCR) mix containing QIAGEN Multiplex PCR Kit, five fluorescent primers set in a final concentration of 0.25 μmol/L for each primer, and 100 ng of DNA, in the previously described conditions (11). The size of PCR products and the corresponding fluorescent label Gene Scan 500LIZ Size Standard were analyzed in ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using Gene Mapper Software, version 3.7. The size of PCR products and the corresponding fluorescent labels were chosen so as to allow simultaneous analysis of normal-sized alleles, with the smaller-sized alleles containing deletions typically seen in MSI-H tumors. Tumors were classified as MSI-H if three or more out of five markers showed MSI and as microsatellite stable/with low frequency of microsatellite instability (MSS/MSI-L) if none or fewer than three markers showed MSI. Patients were divided into two groups based on revised Bethesda criteria (BGrev + patients who fulfilled any of the criteria and BGrev- patients who did not fulfill any of the criteria). The following pathological parameters were examined independently by an experienced pathologist: mucin production graded as present or none, tumor-infiltrating lymphocytes (TILs) graded also as present (at least 5 per high power field) or none, and tumor differentiation graded as poor, moderate, and good. Sensitivity, specificity, PPV, NPV of BGrev, MSI histology, MsPath model, and pathological parameters (presence of any mucin, TILs, poor differentiation) for detecting MSI-H CRC were calculated.

Statistical analysis

Results are expressed as mean±standard deviation for parametric data and counts for non-parametric data. Statistical analysis was performed between groups using independent samples t-test to analyze numerical parameters (of normally distributed variables), while asymptotic χ2 and χ2 likelihood ratio tests were used for non-parametric data. Univariate logistic regression analysis was performed to identify significant predictors of MSI status and to calculate the odds ratio (OR). The utility of different parameters at predicting MSI status was compared by assessing the sensitivity, specificity, positive (PPV) and negative (NPV) predictive values, which were calculated using standard definition (12). Statistical analysis was performed with SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). All P values lower than 0.05 were considered as significant.

RESULTS

A total of 150 patients were enrolled (60 women, 90 men; mean age at diagnosis, 61 ± 10.3 years). Forty seven patients had left-sided colorectal cancer (in the descending and sigmoid colon), 28 had right-sided colorectal cancer (in the transverse colon, ascending colon, or cecum), and 75 had rectal cancer. The cohort included synchronous or metachronous colorectal cancer in 5 patients.

| Table 2. Number of patients who met the revised Bethesda criteria and microsatellite instability status of these patients |
|---------------------------------------------------------------|
| Number of patients                                           |
| High frequency of microsatellite instability                  |
| Microsatellite stable/low frequency of microsatellite instability |

Statistical analysis

Results are expressed as mean±standard deviation for parametric data and counts for non-parametric data. Statistical analysis was performed between groups using independent samples t-test to analyze numerical parameters (of normally distributed variables), while asymptotic χ2 and χ2 likelihood ratio tests were used for non-parametric data. Univariate logistic regression analysis was performed to identify significant predictors of MSI status and to calculate the odds ratio (OR). The utility of different parameters at predicting MSI status was compared by assessing the sensitivity, specificity, positive (PPV) and negative (NPV) predictive values, which were calculated using standard definition (12). Statistical analysis was performed with SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). All P values lower than 0.05 were considered as significant.

RESULTS

A total of 150 patients were enrolled (60 women, 90 men; mean age at diagnosis, 61 ± 10.3 years). Forty seven patients had left-sided colorectal cancer (in the descending and sigmoid colon), 28 had right-sided colorectal cancer (in the transverse colon, ascending colon, or cecum), and 75 had rectal cancer. The cohort included synchronous or metachronous colorectal cancer in 5 patients.

| Table 2. Number of patients who met the revised Bethesda criteria and microsatellite instability status of these patients |
|---------------------------------------------------------------|
| Number of patients                                           |
| High frequency of microsatellite instability                  |
| Microsatellite stable/low frequency of microsatellite instability |

Statistical analysis

Results are expressed as mean±standard deviation for parametric data and counts for non-parametric data. Statistical analysis was performed between groups using independent samples t-test to analyze numerical parameters (of normally distributed variables), while asymptotic χ2 and χ2 likelihood ratio tests were used for non-parametric data. Univariate logistic regression analysis was performed to identify significant predictors of MSI status and to calculate the odds ratio (OR). The utility of different parameters at predicting MSI status was compared by assessing the sensitivity, specificity, positive (PPV) and negative (NPV) predictive values, which were calculated using standard definition (12). Statistical analysis was performed with SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). All P values lower than 0.05 were considered as significant.
Forty five of the 150 patients with colorectal cancer (30%) fulfilled at least one of the five revised Bethesda criteria (Table 1), whereas 105 patients (70%) fulfilled none. The most commonly fulfilled criteria were B1 and B3 (Table 2). The distribution of sex, Union for International Cancer Control (UICC) tumor stage, and pathological grading were similar in the patients who did and did not fulfill the revised Bethesda criteria (Table 3).

Microsatellite instability was detected in 19 of the 150 patients with colorectal cancer (12.6%). The results obtained with the individual microsatellite markers are summarized in Figure 1. The mononucleotide repeat markers BAT26 and BAT25 were most sensitive. Fifteen of the 45 patients who met the revised Bethesda criteria (30%) and 4 of the 105 patients who met none of the revised Bethesda criteria (3.4%) had a tumor with microsatellite instability. In the group of patients with synchronous or metachronous colorectal cancer, one patient had MSI-H, one had MSI-L (BAT25), and 3 had MSS CRC. Characteristics of patients with CRC and MSI are shown in Tables 3,4,5.

The sensitivity of the revised Bethesda criteria to detect microsatellite instability in our cohort was 79% (confidence interval [CI], 54% to 93%), and the specificity was 77% (CI, 68.7% to 83.7%). Positive and negative predictive value was 30% and 70%, respectively. When the age limit of 45 years was used in the first criterion of BGrev (instead 50 years), the specificity was 89%, while sensitivity, PPV, and NPV did not significantly change. We estimated the utility of the B3 criterion, MSI histology (B3 criterion without age limit, defined by presence of TILs, Crohn’s-like lymphocytic reaction, mucinous/signet ring differentiation, or medullar growth pattern), TILs, mucinous and poor tumor differentiation, family history of cancers (B4, B5 criteria together) for identification MSI-H tumors. Specificity, sensitivity, PPV, and NPV for these parameters are given in the Table 6. MsPath model with recommended cut-off score of 1.0 identified 84% of our MSI-H tumors (patients below the age of 60 years). The sensitivity and specificity were 86% and 85%, respectively.

| TABLE 3. Patients’ clinical characteristics, according to fulfillment (BGRev+) or nonfulfillment (BGRev-) of at least one criterion of the revised Bethesda guidelines |
|-----------------|-----------------|-----------------|
| Variable        | BGRev- (n = 105)| BGRev+ (n = 45) |
| Women/men       | 43/62           | 17/28           |
| Mean age ± standard deviation (range) | 65.1 ± 10.3 | 51.6 ± 9.9 |
| Localization:   |                 |                 |
| rectum          | 52              | 23              |
| left colon      | 34              | 13              |
| right colon     | 19              | 9               |
| Patients with synchronous or metachronous colorectal cancer | 0 | 5 |

| TABLE 4. Patients’ histopathological characteristics and microsatellite instability (MSI) status, according to fulfillment (BGRev+) or nonfulfillment (BGRev-) of at least one criterion of the revised Bethesda guidelines |
|-----------------|-----------------|-----------------|
| Variable        | BGRev- (n = 105)| BGRev+ (n = 45) |
| Tumor infiltrating lymphocytes: | | |
| 1 (present)     | 38              | 18              |
| 2 (absent)      | 67              | 27              |
| Differentiation: | | |
| 1 (undifferentiated and poorly differentiated) | 11 | 4 |
| 2 (moderately differentiated) | 45 | 20 |
| 3 (well differentiated) | 49 | 21 |
| Mucin production: | | |
| present         | 77              | 21              |
| absent          | 28              | 24              |
| MSI status:     | | |
| High frequency of microsatellite instability | 4 | 15 |
| Microsatellite stable/low frequency of microsatellite instability | 101 | 30 |
Eight clinicopathological features were included into univariate logistic regression analysis – diagnostic age lower than 60 years, male sex, right-sided colon cancer, poor differentiation, mucinous differentiation, the presence of TILs, lower disease stage (I and II UICC stage), and any BGrev criterion fulfillment. Any BGrev criterion fulfillment, mucinous differentiation, and right-sided CRC were singled out as independent factors to identify MSI-H colorectal cancer (Table 7).

Pathological parameters (tumor differentiation, mucin production, and TILs) were compared between BGrev+ and BGrev- patients. There were no significant differences between the groups, when differentiation and TILs were used as grouping criteria. Mucinous carcinomas were significantly more frequently present in BGrev+ group (odds ratio 3.14; 95% CI, 1-4.4; \( P = 0.02 \)) (Table 4).

**DISCUSSION**

In this prospective study of unselected and consecutively diagnosed CRC patients, we assessed the performance of currently used clinical guidelines (BGrev) against a molecu-

| TABLE 5. Characteristics of patients with colorectal cancer (CRC) and microsatellite instability (MSI) |
|---|---|---|---|---|---|
| Patient | Age at CRC diagnosis | Sex | Colorectal tumors location of CRC | Tumor node stage, grade | Family history of HNPCC |
| 1 | m | 44 | rectum | T2N0M0,G1 | no |
| 2 | f | 64 | ascending colon | T2N0M0,GIII | yes |
| 3 | m | 68 | rectum | T3N1M0,G1 | yes |
| 4 | m | 72 | cecum and rectum | T2N0M0,GII T3N0M0,GIII | no |
| 5 | m | 52 | cecum | T4bN2M0,GII | yes |
| 6 | m | 56 | rectum | T3N0M0,G1 | yes |
| 7 | m | 64 | cecum | T2N0M0,G1 | yes |
| 8 | f | 63 | cecum | T2N1M0,G1 | yes |
| 9 | m | 41 | rectum | T1N0M0,GIII | yes |
| 10 | m | 58 | ascending colon | T3N0M0,G1 | no |
| 11 | m | 66 | descending colon | T1N0M0,G1 | yes |
| 12 | m | 57 | rectum | T3N1M0,G1 | no |
| 13 | m | 57 | ascending colon | T3N1M0,GII | no |
| 14 | m | 59 | sigmoid colon | T4aN0M0,G1 | no |
| 15 | m | 59 | descending colon | T2N0M0,G1 | yes |
| 16 | f | 70 | transverse colon | T3N0M0,G1 | no |
| 17 | f | 76 | ascending colon | T4bN1M1,GII | no |
| 18 | m | 71 | rectum | T3N1M0,G1 | no |
| 19 | m | 68 | ascending colon | T3N0M0,G1 | no |

*HNPCC – hereditary nonpolyposis colorectal cancer.

| TABLE 6. Sensitivity, specificity, positive and negative predictive value of histological and clinical features in predicting microsatellite instability (MSI) |
|---|---|---|---|---|
| Variable | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
| B3 criterion | 0.44 | 0.91 | 0.13 | 0.87 |
| MSI histology | 0.79 | 0.85 | 0.20 | 0.80 |
| MsPath score* | 0.86 | 0.85 | 0.18 | 0.82 |
| Tumor infiltrating lymphocytes | 0.52 | 0.65 | 0.37 | 0.63 |
| Any mucinous differentiation | 0.63 | 0.69 | 0.34 | 0.65 |
| Poor differentiation | 0.27 | 0.89 | 0.13 | 0.88 |
| Family history of cancers (B4,B5) | 0.64 | 0.92 | 0.13 | 0.87 |
| Revised Bethesda guidelines | 0.78 | 0.77 | 0.3 | 0.7 |
| Revised Bethesda guidelines (modified B1, age limit <45 y) | 0.78 | 0.89 | 0.19 | 0.80 |

*Cut-off value = 1.
lar tumor marker (MSI). The frequency of high microsatellite instability phenotype in our cohort was 12.6%. Nearly 80% of MSI-H CRCs were identified using BGrev, and the specificity of MsPath model and MSI histology was higher than that of MSI-H CRCs were identified using BGrev, resulting in a sensitivity and specificity of 66.7% and 50.9%, respectively. The sensitivity of MSI histology in the same study (19) was 82.5%, while the specificity was lower, 27.1%. In our cohort, MSI histology had lower sensitivity (78.9%) and higher specificity (84.7%) than BGrev. In 2007, Jenkins et al (9) published the MsPath model for predicting MSI in CRC. This model did not include family history, but included histology features described in the B3 criterion (Table 1) with tumor localization and age at diagnosis. MsPath model is only applied to patients diagnosed before the age of 60 years. MsPath score ≥1.0 had a sensitivity of 93% and a specificity of 55% for MSI-H tumors (9). Applied MsPath model with recommended cut-off score of 1.0 in our cases had an increased sensitivity of 86%. The specificity of MsPath model and BGrev in our cohort was similar. Differences, especially in specificity, between our and other studies can be explained by the fact that our cohort was unselected. A large number of patients with MSI-H CRCs are older than 60 years and they would be missed by this model. In our study, more than half patients with MSI-H tumor were older than 60 years. It is likely that these cases are sporadic MSI-H CRCs. In sporadic MSI-H CRC, MSI occurs due to MLH1 genes promoter methylation. Five patients with MSI-H CRC were between 50 and 60 years and they all met B3. The possibility of LS diagnosis in these cases is higher. Moreover, the analysis of the MMR defect discriminating sporadic from hereditary MSI-H cases needs to be developed in order to be able to recognize Lynch cases in Serbia.

An important finding was that the specificity of revised Bethesda criteria could be increased using the lower age limit in the B1 criterion (<45 years instead <50 years) as used in the previous Bethesda criteria. Twenty one patients in our cohort were younger than 50 years. Among these patients, only two (10%) had MSI-H tumors and both were younger than 45 years. When the age limit in the B1 criterion was 45 years, BGrev were more specific. However, this observation is only hypothetical and is not applicable in practice as long as germline mutation is not determined to confirm the final diagnosis of LS.

Regarding the patients presenting with synchronous or metachronous colon tumors, four patients were MSS and only one was MSI-H, and this is another reason for the low PPV and higher specificity of the BGrev in our study population (great number of false positives). Another study (20) concluded that tumor localization, rather than MSI histology, might have a key role in

| Variable | Odds ratio | 95% confidence interval | P |
|----------|------------|------------------------|---|
| Male sex | 2.59       | 0.8-8.2                | 0.125 |
| Age less than 60 y | 1.74 | 0.4-3.6 | 0.344 |
| Right-sided CRC | 8.5 | 2.7-26.7 | 0.001 |
| Poor and well differentiation | 2.18 | 0.8-7.3 | 0.181 |
| Any mucinous differentiation | 3.272 | 1.2-8.7 | 0.040 |
| TIL>5/HPF | 2.21 | 0.8-5.4 | 0.158 |
| Lower disease stage | 1.06 | 0.3-2 | 0.918 |
| (UICC stage; I and II) | | | |
| Any BGrev criterion fulfillment | 6.99 | 2.5-19.9 | 0.001 |

**Abbreviations:** CRC – colorectal cancer; TIL – tumor infiltrating lymphocytes; HPF – high-powered field; UICC – Union for International Cancer Control staging; BGrev – Revised Bethesda guidelines.

In the early 1990s, the genetic defect responsible for LS was identified as a germline mutation in one of the DNA MMR genes with the consequence of a microsatellite instability phenotype (13). Introducing the MSI determination as an initial screening test for CRC enables the molecular detection of LS in large populations. MSI-H has been shown to have a dominant impact on the global molecular phenotype in CRC. MSI-H CRC shows distinct clinicopathological features, including both better prognosis (14,15) and reduced response to 5-fluorouracil/leucovorin (5-FU) adjuvant chemotherapy (16,17). Moreover, patients with MSS tumors (especially stage III cancers) seem to benefit most from adjuvant SFU chemotherapy. Conversely, patients with MSI tumors and more specifically those with stage II cancer, do not seem to benefit from adjuvant chemotherapy (16,17). Both the MSI test and immunostaining have been shown to be highly effective for selecting patients who should be tested for hMSH2/hMLH1 germline mutations (18). In this study, we did not include patients who fulfilled the Amsterdam criteria for HNPPC, because in our and other opinions (2,6), patients with CRCs belonging to HNPPC families should be proceeded immediately to MMR gene mutation analysis. These patients do not need the MSI analysis.

In a recent study (19), the usefulness of BGrev for MSI prediction was assessed with and without B3. In this cohort, 2/3 patients with MSI-H were identified by the BGrev, resulting in a sensitivity and specificity of 66.7% and 50.9%, respectively. The sensitivity of MSI histology in the same study (19) was 82.5%, while the specificity was lower, 27.1%. In our cohort, MSI histology had lower sensitivity (78.9%) and higher specificity (84.7%) than BGrev. In 2007, Jenkins et al (9) published the MsPath model for predicting MSI in CRC. This model did not include family history, but included histology features described in the B3 criterion (Table 1) with tumor localization and age at diagnosis. MsPath model is only applied to patients diagnosed before the age of 60 years. MsPath score ≥1.0 had a sensitivity of 93% and a specificity of 55% for MSI-H tumors (9). Applied MsPath model with recommended cut-off score of 1.0 in our cases had an increased sensitivity of 86%. The specificity of MsPath model and BGrev in our cohort was similar. Differences, especially in specificity, between our and other studies can be explained by the fact that our cohort was unselected. A large number of patients with MSI-H CRCs are older than 60 years and they would be missed by this model. In our study, more than half patients with MSI-H tumor were older than 60 years. It is likely that these cases are sporadic MSI-H CRCs. In sporadic MSI-H CRC, MSI occurs due to MLH1 genes promoter methylation. Five patients with MSI-H CRC were between 50 and 60 years and they all met B3. The possibility of LS diagnosis in these cases is higher. Moreover, the analysis of the MMR defect discriminating sporadic from hereditary MSI-H cases needs to be developed in order to be able to recognize Lynch cases in Serbia.
detecting CRC with MSI-H phenotype. These authors suggested that in a future revision of criteria for MSI testing, tumor localization should play as great a role as age at the onset of the CRCs, and the Bethesda criteria should be broadened to include patients 51-60 years old with proximal colon cancer. Our findings support this claim.

We found that identification of mucinous histology, even if seen only focally, was significant, independent predictor of MSI-H CRC. Others (19,21) have reported similar findings. In one study (22), the specificity of TILs for MSI-H CRC reached 98.2%, while the sensitivity was low (33.3%). Our results showed lower TILs specificity and higher sensitivity. In our study, TILs was not an independent predictive factor for MSI-H CRC. Results from other studies (19,23,24) were different. Tumor differentiation was another feature that was not an independent predictor of MSI-H in our study but was in others (25-27). Greenenson et al (21) considered well and poor differentiated tumors together, which indicated an increased likelihood of MSI. We assessed the grade using both of these models, and no significance was found. Poor differentiation proved to be a specific parameter for MSI-H CRC. Proximal tumors were more likely to show MSI-H phenotype than distal tumors, which concurs with the results from other studies (28,29).

The main limitation of our study was the inability to analyze germline mutations of MMR genes for MSI-H tumors. Another limitation was the fact that only routine histopathological parameters that were included in the BGrev and MsPath model (9) were analyzed, so we were not able to examine the validity of newer proposed models for the prediction of MSI-H (19,21). Also, the selection of patients based on their age was not made, so the validity of clinical and histopathological parameters can be reliably observed.

In conclusion, BGrev are useful to select patients at risk for hereditary cancer, by increasing the detection rate of microsatellite instability as MSI histology. MsPath model proved to be more sensitive than BGrev, with similar specificity. Moreover, BGrev, MsPath model, and MSI histology are useful tools for selecting patients with CRC for MSI testing.

Funding This work was supported by Ministry of Science; Republic of Serbia: Grant No 41033.

Ethical approval received from the local ethics committee of the Clinical Centre of Serbia.

Declaration of authorship ZK contributed to the conception of the study; acquisition and interpretation of data; drafted the manuscript; and gave the final approval for publication. SW and JA contributed to all stages of the manuscript. ID contributed to acquisition data and statistical analysis. DB contributed to acquisition of patients’ data; revised the manuscript. PS and NJ contributed to acquisition of data. SD contributed to design of the study and interpretation of data; revised the manuscript; and gave the final approval for publication.

Competing interests All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

References
1 Colas C, Coulet F, Svreck M, Collura A, Flejou JF, Duval A, et al. Lynch or not Lynch? Is that always a question? Adv Cancer Res. 2012;113:121-66. Medline:22429854 doi:10.1016/B978-0-12-394280-7.00004-X
2 Lynch HT, Smyrk TC. Identifying hereditary nonpolyposis colorectal cancer. N Engl J Med. 1998;338:1537-8. Medline:9593794 doi:10.1056/NEJM199805213382109
3 Umbr A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96:261-8. Medline:14970275 doi:10.1093/jnci/djh034
4 Rodriguez-Bigas MA, Boland CR, Hamilton SR, Henson DE, Jass JR, Khan PM, et al. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. J Natl Cancer Inst. 1997;89:1758-62. Medline:9392616 doi:10.1093/jnci/89.23.1758
5 Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPPC, Lynch syndrome) proposed by the International Collaborative group on HNPPC. Gastroenterology. 1999;116:1453-6. Medline:10348829 doi:10.1016/S0016-5085(99)70516-X
6 Wijnen JT, Vasen HF, Khan PM, Zwinderman AH, van der Klift H, Mulder A, et al. Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. N Engl J Med. 1998;339:511-8. Medline:9709044 doi:10.1056/NEJM19980203390804
7 Syngal S, Fox EA, Li C, Dovidio M, Eng C, Kolodner RD, et al. Interpretation of genetic test results for hereditary nonpolyposis colorectal cancer: implications for clinical predisposition testing. JAMA. 1999;282:247-53. Medline:10422993 doi:10.1001/jama.282.3.247
8 Cravo ML, Fidalgo PO, Lage PA, Albuquerque CM, Chaves PP, Claro I, et al. Validation and simplification of Bethesda guidelines for identifying apparently sporadic forms of colorectal carcinoma with microsatellite instability. Cancer. 1999;85:779-85. Medline:10019754 doi:10.1002/(SICI)1097-0142(19990215)85:4<c:779-AID-CNCR4>3.0.CO;2-C
9 Jenkins MA, Hayashi S, O’Shea AM, Burgart LJ, Smyrk TC, Shimizu D, et al. Colon Cancer Family Registry. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability:
a population-based study. Gastroenterology. 2007;133:48-56. Medline:17631130 doi:10.1053/j.gastro.2007.04.044
10 Huijsmans CJ, Damen J, van den Linden JC, Soeveldkou PH, Hermans MH. Comparative analysis of four methods to extract DNA from paraffin-embbeddend tissue effect on downstream molecular applications. BMC Res Notes. 2010;3:239. Medline:20840759 doi:10.1186/1756-0500-3-239
11 Suraweera N, Duval A, Reperant M, Vaury C, Furlan D, Leroy K, et al. Evaluation of tumor microsatellite instability and BRAF mutation (V600E) in quasimonomorph mononucleotide repeats and pentaplex PCR. Gastroenterology. 2002;123:1804-11. Medline:12454837 doi:10.1053/gast.2002.37070
12 Knottenreus JA, van Weel C, Muris JWM. Evidence base of clinical diagnostic evaluation of diagnostic procedures. BMJ; 2002;324:477-80. Medline:11859054 doi:10.1136/bmj.324.7335.477
13 Bronner CE, Baker SM, Morrison PT, Warren LG, Lescoe MK, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature. 1994;368:258-61. Medline:8145827 doi:10.1038/368258a0
14 Banerjea A, Hands RE, Powar MP, Bustin SA, Dorudi S. Microsatellite and chromosomal stable colorectal cancers demonstrate poor immunogenicity and early disease recurrence. Colorectal Dis. 2009;11:601-8. Medline:18637931 doi:10.1111/j.1463-1318.2008.01639.x
15 Markovic S, Antic J, Dragicevic N, Hamelin R, Krivokapic Z. High-frequency microsatellite instability and BRAF mutation (V600E) in unselected Serbian patients with colorectal cancer. J Mol Histol. 2012;43:137-43. Medline:22210186 doi:10.1002/sjhm.710
16 Gologan A, Krasinskas A, Hunt J, Thull DL, Farkas L, Sepulveda AR. Performance of the revised Bethesda guidelines for identification of colorectal carcinomas with a high level of microsatellite instability. Arch Pathol Lab Med. 2005;129:1390-7. Medline:16253017
17 Kawabata Y, Tomita N, Monden T, Ohue M, Ohnishi T, Sasaki H, et al. A histology-based model for predicting microsatellite testing. Gastroenterology. 2006;130:3219-26. Medline:17049839 doi:10.1010/jci.2009.07.1825
18 Gruber SB. New developments in Lynch syndrome (hereditary nonpolyposis colorectal cancer) and mismatch repair gene testing. Gastroenterology. 2006;130:577-87. Medline:16472609 doi:10.1053/j.gastro.2006.01.031
19 Hyde A, Fontaine D, Stuckless S, Green R, Pollett A, Simms M, et al. A histology-based model for predicting microsatellite instability in colorectal cancers. Am J Surg Pathol. 2010;34:1820-9. Medline:21107088 doi:10.1097/PAS.0b013e3181f6a912
20 Urso E, Pucciarelli S, Agostini M, Maretto I, Mescoli C, Bertorelle R, et al. Proximal colon cancer in patients aged 51–60 years of age should be tested for microsatellites instability: A comment on the Revised Bethesda Guidelines. Int J Colorectal Dis. 2008;23:801-6. Medline:18446350 doi:10.1002/si008-004-0484-2
21 Greenson JK, Huang SC, Herron C, Moreno V, Bonner JD, Tomsho LP, et al. Pathologic predictors of microsatellite instability in colorectal cancer. Am J Surg Pathol. 2009;33:126-33. Medline:18830122 doi:10.1097/PAS.0b013e318171e2b1
22 Gologan A, Krasinskas A, Hunt J, Thull DL, Farkas L, Sepulveda AR. Pathologic predictors of microsatellite instability in colorectal cancer. Am J Surg. 2009;293:126-33. Medline:19017435 doi:10.1053/j.ajgs.2008.04.014
23 Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. Cancer. 2001;91:2417-22. Medline:1143533 doi:10.1002/1097-0142(20001615)91:12<2417::AID-CNCR1276>3.0.CO;2-U
24 Kakar S, Saime A, Lawrence JB, Thomas CS. Mucinous carcinoma of the colon: correlation of loss of mismatch repair enzymes with clinicopathologic features and survival. Mod Pathol. 2004;17:696-700. Medline:15017435 doi:10.1038/modpathol.3800093
25 Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med. 2000;342:69-77. Medline:10632174 doi:10.1056/NEJM200001133420201
26 Gafa R, Maestri I, Matteuzzi M, Santini A, Ferretti S, Cavazzini L, et al. Sporadic colorectal adenocarcinomas with high-frequency microsatellite instability. Cancer. 2000;89:2025-37. Medline:11066042 doi:10.1002/1097-0142(20001115)89:10<2025::AID-CNCR1>3.0.CO;2-S
27 Kawabata Y, Tomita N, Monden T, Ohue M, Ohnishi T, Sasaki M, et al. Molecular characteristics of poorly differentiated adenocarcinoma and signet-ring-cell carcinoma of colorectum. Int J Cancer. 1999;84:33-8. Medline:9988229 doi:10.1002/(SICI)1097-0215(19990219)84:1<33::AID-IJC7>3.0.CO;2-Z
28 Young J, Simms LA, Biden KG, Wynter C, Whitehall V, Karamatic R, et al. Features of colorectal cancers with high-level microsatellite instability occurring in familial and sporadic settings: parallel pathways of tumorigenesis. Am J Pathol. 2001;159:2107-16. Medline:11733361 doi:10.1016/S0002-9440(00)00062-3
29 Jass JR. HNPCC and sporadic MSI-H colorectal cancer: a review of the morphological similarities and differences. Fam Cancer. 2004;3:93-100. Medline:15340259 doi:10.1023/B:FAEM.0000039849.66088.b7