# Diagnostic Performance of Four-Panel Immunohistochemistry for Detecting Mismatch Repair Deficiency in Korean Patients with Colorectal Cancer

Donghyoun Lee *

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*Article*

# Diagnostic Performance of Four-Panel Immunohistochemistry for Detecting Mismatch Repair Deficiency in Korean Patients with Colorectal Cancer

**Donghyoun Lee**

Department of Surgery, Jeju National University Hospital, Jeju National University School of Medicine, 15, Aran 13-gil, Jeju-si, Jeju-do, 63241 Republic of Korea; dlee@jejunu.ac.kr

**Abstract**

We conducted this single-center, retrospective study to assess a concordance rate between the microsatellite instability (MSI) analysis and the immunohistochemistry (IHC) in a cohort of Korean patients with hereditary non-polyposis colorectal carcinoma (HNPCRC). A total of 251 patients ($n$ = 251) were included in the current study, who comprise 149 men (59.4%) and 102 women (40.6%) and whose mean age was 64.6 ± 11.5 years old. In our series, MSI-high (MSI-H) and microsatellite-stable (MSS) were identified in 17 (6.7%) and 234 patients (93.3%), respectively. Concordance analysis showed a strong agreement between MSI status and IHC expression of mismatch repair (MMR) proteins. Of the 17 patients with MSI-H, 16 (94.11%) had a loss of expression of ≥ one MMR protein in the IHC findings, while one patient (5.9%) with MSI-H retained an intact expression of all four MMR proteins. Moreover, of the 234 patients with MSS, four (1.71%) had a loss of expression of ≥ one MMR protein in the IHC findings. Of the 20 patients with a loss of expression of ≥ one MMR protein in the IHC findings, 16 (80%) and four (20%) were found to have MSI-H and MSS, respectively. By contrast, 231 patients retained an intact expression of all four MMR proteins, with only one case (0.4%) being MSI-H and the remaining 98.3% ($n$ = 230) MSS. There were distinct associations between the pattern of IHC expression of MMR proteins and microsatellite status. The most frequent abnormal expression patterns include mutL homolog 1 (MLH1) (-) postmeiotic segregation increased 2 (PMS2) (-) ($n$ = 7), all of which were MSI-H, and mutS homologs 2 (MSH2) (-) mutS homologs 6 (MSH6) (-) ($n$ = 7), with six patients with MSI-H and one with MSS. PMS2 (-) alone was observed in three patients, one and two of whom were MSI-H and MSS, respectively. MSH6 (-) alone was observed in one patient with MSS. Finally, there were two patients with PMS2 (-) and MSH6 (-), both classified as MSI-H. The current study indicates the high concordance between IHC and MSI testing in HNPCRC. But this deserves further large-scale, prospective studies.

**Keywords:** colon; colorectal neoplasms; DNA mismatch repair; microsatellite instability; immunohistochemistry

## 1. Introduction

Colorectal cancer (CRC) is the second most common cause of cancer-related death in the Western countries. Its multifactorial etiology involved both genetic and environmental factors [1]. The most common form of hereditary CRC is hereditary non-polyposis colorectal carcinoma (HNPCRC), also known as Lynch syndrome (LS), which accounts for 1-6% of total cases of CRC [2,3]. HNPCRC is a disease entity that is characterized as familial clustering of colorectal and other types of malignancies [4,5]. From perspectives of molecular diagnostics, it is defined as a cancer-predisposing syndrome that is secondary to a deleterious germline mutation in one of a set of DNA mismatch repair (MMR) genes, such as mutL homolog 1 (MLH1), mutS homologs 2 and 6 (MSH2 and MSH6) and postmeiotic

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segregation increased 2 (PMS2) [6,7]. Thus, patients with HNPCC carry one mutated copy of the gene in all their tissues, in whom a somatic mutation or loss of the second normal allele in colorectal or other epithelial tissues inactivates the gene and then impairs mismatch repair function. This eventually leads to the development of tumor. To date, detection of the deleterious germline mutation has been employed to establish a diagnosis of HNPCRC [8,9].

Three major mechanisms are involved in the pathogenesis of HNPCRC. These include chromosomal instability (CIN) (75%), an epigenetic modification of DNA methylation, also known as CpG island methylator phenotype (CIMP), (20%) and the microsatellite instability (MSI) or deficiency in DNA mismatch repair system (dMMR) (approximately 15%) [10]. Microsatellites, also known as short tandem repeats, are DNA sequences distributed throughout the genome (coding or non-coding sequences) with a repetitive structure of nucleotides. These repetitive structures are vulnerable to replication errors in the presence of dMMR. An accumulation of errors in the sequence of these microsatellites is termed as the microsatellite instability (MSI); it serves as an indicator of the impairment in the DNA MMR system [11].

Mutation screening is both time-consuming and expensive due to the heterogeneity of the spectrum of the MMR genes. Over the past decades, considerable efforts have been made to screen a high-risk group of patients. This has eventually led to the development of the most efficient workup algorithm [12–14]. As a result, family history, tumor pathological characteristics, tumor DNA MSI and tumor MMR protein detection by immunohistochemistry (IHC) have been used as popular screening tools [14]. Of these, the MSI analysis and the IHC have been compared for their usefulness according to a review of literatures [15,16].

Given the above background, we conducted this single-center, retrospective study to assess a concordance rate between the MSI analysis and the IHC in a cohort of Korean patients with HNPCRC.

## 2. Patients and Methods

### *2.1. Study Patients and Setting*

We conducted this single-center, retrospective study in a consecutive series of the patients with CRC undergoing colorectal resection at our medical institution during a 2-year period between January 2015 and December 2016.

Inclusion criteria for the current study are as follows:

(1) Korean men or women aged 20 years or older
(2) The patients with a diagnosis of HNPCRC
(3) The patients undergoing colorectal resection
(4) The patients with available medical records.

Exclusion criteria for the current study are as follows:

(1) The patients undergoing either of both the MSI and the IHC
(2) The patients undergoing multiple colorectal resection due to synchronous CRC
(3) The patients with recurred CRC
(4) The patients lost to follow-up.

The current study was approved by the Institutional Review Board (IRB) of our medical institution (IRB approval # 2016-07-002-002). It followed the applicable laws, regulations and ethics guidelines. A written informed consent was waived because of its retrospective nature.

### *2.2. Patient Evaluation and Criteria*

Baseline characteristics and clinicopathological variables of the patients were obtained from surgical and pathology reports; these include age, sex, concurrent chemoradiotherapy, the length of follow-up period, tumor-node-metastasis (TNM) stage, histologic type, the number of metastatic and harvested lymph nodes and lymphovascular, perineural and venous invasions. TNM staging was determined according to the American Joint Committee on Cancer (AJCC) staging system (8th edition) [17]. Histologic types were categorized as well-differentiated, moderately-differentiated,

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poorly-differentiated adenocarcinoma, or medullary carcinoma. All the pathological results were reviewed by independent four board-certified specialists in gastrointestinal pathology, all of whom were blinded to details of the patient characteristics.

### *2.3. MSI Analysis*

The panel contains five quasi-monomorphic mononucleotides, such as big adenine tract (BAT)-25, BAT-26, NR21, NR24 and NR27, as previously described [18]. Results of the MSI analysis were categorized into (1) MSI-high (MSI-H) (≥ 3 markers), (2) low (MSI-L) (≤ 2 markers) and (3) microsatellite-stable (MSS) (no markers).

### *2.4. IHC*

IHC was performed using MLH1 [M1, ready-to-use (RTU), Ventana Medical Systems, Inc.; Roche Diagnostics, Basel, Switzerland], MSH6 (44, RTU; Ventana Medical Systems, Inc.; Roche Diagnostics), PMS2 (EPR3947, RTU; Ventana Medical Systems, Inc.; Roche Diagnostics) and MSH2 (G219-1129, RTU; Ventana Medical Systems, Inc.; Roche Diagnostics). The tissue full sections were dewaxed in xylene three times. Then, endogenous peroxidase activity was blocked with 0.5% $H_2O_2$ for 20 min. This was followed by rehydration with graded alcohol (100%, 95% and 80%) and distilled waters. After a 30-min blocking with universal blocking serum (Ventana Medical Systems, Tucson, AZ), the samples were incubated with the primary antibody at 4 °C for 30 min with BenchMark XT automated staining system (Ventana Medical systems). The tissue sections were incubated with a biotinylated secondary antibody (Ventana Medical Systems, Tucson, AZ) for 30 min each and then counterstained with hematoxylin (Ventana Medical Systems, Inc.) [19]. The IHC findings were classified as an intact nuclear expression and a loss of nuclear expression defined as that of any of four proteins. In cases of disagreement, the tissue sections were re-evaluated [20].

### *2.5. Statistical Analysis*

All statistical analyses were performed using the STATA 13 (Stata Corporation, College Station, Texas, USA). Descriptive statistics were used to summarize baseline characteristics of the patients. Continuous variables were expressed as mean ± standard deviation or median with interquartile range, as appropriate. Categorical variables were expressed as the frequency with percentage. Group comparisons between MSI-H and MSS patients were performed using the $\chi^2$ test or Fisher's exact test for categorical variables, and Student's *t*-test or Mann-Whitney U test for continuous variables. Concordance between MSI status and IHC findings was evaluated by calculating concordance rates with corresponding 95% confidence intervals (CIs).

## 3. Results

### *3.1. Baseline Characteristics of the Patients*

A total of 251 patients ($n$ = 251) were included in the current study, who comprise 149 men (59.4%) and 102 women (40.6%) and whose mean age was 64.6 ± 11.5 years old. Moreover, 27 patients (10.8%) had received CCRT. Regarding microsatellite status, 17 patients (6.7%) were classified as MSI-H, whereas 234 patients (93.3%) were MSS. The distribution of TNM stage showed that 27.9% were stage I, 25.5% stage IIa, 7.2% stage IIb, 8.7% stage IIIa, 14.3% stage IIIb, 19.2% stage IIIc and 4.4% stage IV. Histologically, the majority of tumors were moderately-differentiated adenocarcinomas (88%), followed by well-differentiated (6.8%) and poorly differentiated adenocarcinomas (4.8%). One case (0.3%) was diagnosed as medullary carcinoma. The mean number of harvested lymph nodes was 27.0 ± 13.4, with a mean of 2.31 ± 4.7 metastatic nodes. Lymphatic, venous and perineural invasion were seen in 31.5%, 10.0% and 16.7% of cases, respectively. The median follow-up duration was 657.2 ± 263.3 days. There were no patients who met the Amsterdam criteria for Lynch syndrome, although

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1.6% fulfilled the revised Bethesda guidelines. Their baseline characteristics are represented in Table 1.

**Table 1. Baseline characteristics of the patients (*n* = 251).**

| Variables | Values | |
|---|---|---|
| **Age (years old)** | 64.6 ± 11.5 | |
| **Sex** | | |
| Men | 149 (59.4%) | |
| Women | 102 (40.6%) | |
| **CCRT** | 27 (10.8%) | |
| **Microsatellite status** | | |
| MSI-H | 17 (6.7%) | |
| MSS | 234 (93.3%) | |
| **TNM stage** | | |
| I | 70 (27.9%) | |
| IIa | 46 (18.3%) | 64 (25.5%) |
| IIb | 18 (7.2%) | |
| IIIa | 22 (8.7%) | 106 (42.2%) |
| IIIb | 36 (14.3%) | |
| IIIc | 48 (19.2%) | |
| IV | 11 (4.4%) | |
| **Histologic type** | | |
| Well-differentiated adenocarcinoma | 17 (6.8%) | |
| Moderately-differentiated adenocarcinoma | 221 (88%) | |
| Poorly-differentiated adenocarcinoma | 12 (4.8%) | |
| Medullary carcinoma | 1 (0.3%) | |
| **Results of lymph node evaluation** | | |
| Metastatic lymph node | 2.31 ± 4.7 | |
| Harvested lymph node | 27 ± 13.4 | |
| **Histopatholigical findings of tumor cells** | | |

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| | | |
|---|---|---|
| | Lymphatic invasion | 79 (31.5%) |
| | Venous invasion | 25 (10%) |
| | Perineural invasion | 42 (16.7%) |
| **Median length of FU period (days)** | | 657.2 ± 263.3 |
| **Eligibility for diagnostic criteria** | | |
| | Amsterdam criteria | 0 (0.0%) |
| | Bethesda guidelines | 4 (1.6%) |

**Abbreviations:** CCRT, concurrent chemoradiotherapy; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS, microsatellite-stable; TNM, tumor-node-metastasis; FU, follow-up. Values are mean ± standard deviation, median ± standard deviation or the number of the patients with percentage, where appropriate.

*3.2. Concordance Rates Between the MSI and the ICH Findings*

In our series, MSI-H and MSS were identified in 17 (6.7%) and 234 patients (93.3%), respectively. Concordance analysis showed a strong agreement between MSI status and IHC expression of MMR proteins. Of the 17 patients with MSI-H, 16 (94.11%) had a loss of expression of ≥ one MMR protein in the IHC findings, while one patient (5.9%) with MSI-H retained an intact expression of all four MMR proteins. Moreover, of the 234 patients with MSS, four (1.71%) had a loss of expression of ≥ one MMR protein in the IHC findings (Table 2).

**Table 2. Concordance rates between immunohistochemical and microsatellite instability findings.**

| **Variables** | **Values** | |
|---|---|---|
| | ***n* (%)** | **95% CI** |
| **MSI-H (*n* = 17)** | | |
| Loss of expression of ≥ one MMR protein in the IHC findings | 16 (94.11%) | 0.81-1.06 |
| Intact expression of all four proteins in the IHC findings | 1 (5.89%) | 0.065-0.18 |
| **MSS (*n* = 234)** | | |
| Loss of expression of ≥ one MMR protein in the IHC findings | 4 (1.71%) | 0.003-0.33 |
| Intact expression of all four proteins in the IHC findings | 230 (98.3%) | 0.96-0.99 |
| **Loss of expression of ≥ one MMR protein in the IHC findings (*n* = 20)** | | |

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| | | | |
|---|---|---|---|
| | MSI-H | 16 (80%) | 0.60-0.99 |
| | MSS | 4 (20%) | 0.007-0.39 |
| **Intact expression of all four proteins in the IHC findings (*n* = 231)** | | | |
| | MSI-H | 1 (0.4%) | 0.033-0.094 |
| | MSS | 230 (98.29%) | 0.88-0.95 |

**Note:** n, the number of the patients; CI, confidence interval. **Abbreviations:** MSI-H, microsatellite instability-high; MMR, mismatch repair; IHC, immunohistochemical; MSS, microsatellite-stable.

Of the 20 patients with a loss of expression of ≥ one MMR protein in the IHC findings, 16 (80%) and four (20%) were found to have MSI-H and MSS, respectively. By contrast, 231 patients retained an intact expression of all four MMR proteins, with only one case (0.4%) being MSI-H and the remaining 98.3% (*n* = 230) MSS (Table 2).

*3.3. Associations Between the Pattern of IHC Expression of MMR Proteins and Microsatellite Status*

There were distinct associations between the pattern of IHC expression of MMR proteins and microsatellite status. The most frequent abnormal expression patterns include MLH1 (-) PMS2 (-) (*n* = 7), all of which were MSI-H, and MSH2 (-) MSH6 (-) (*n* = 7), with six patients with MSI-H and one with MSS. PMS2 (-) alone was observed in three patients, one and two of whom were MSI-H and MSS, respectively. MSH6 (-) alone was observed in one patient with MSS. Finally, there were two patients with PMS2 (-) and MSH6 (-), both classified as MSI-H (Table 3).

**Table 3. The pattern of immunohistochemical expression of mismatch repair proteins and microsatellite status.**

| **The pattern of IHC expression of MMR proteins** | **Values** | |
|---|---|---|
| | **MSI-H** | **MSS** |
| **≥ one MMR protein (-) (*n* = 20)** | 16 | 4 |
| **MLH1 (-) PMS2 (-) (*n* = 7)** | 7 | 0 |
| **PMS2 (-) alone (*n* = 3)** | 1 | 2 |
| **MSH2 (-) MSH6 (-) (*n* = 7)** | 6 | 1 |
| **MSH6 (-) alone (*n* = 1)** | 0 | 1 |
| **MSH6 (-) PMS2 (-) (*n* = 2)** | 2 | 0 |

**Abbreviations:** IHC, immunohistochemical; MMR, mismatch repair; MSI-H, microsatellite instability-high; MSS, microsatellite-stable.

## 4. Discussion

The phenomenon of DNA MSI arises from failure in DNA mismatch repair as soon as DNA replication occurs [21]. HNPCC has been studied, which has led to the recognition of MSI as the phenotypic expression of a germline mutation in ≥ 1 of genes involved in DNA mismatch repair [22–24]. But the MSI is not confined to cases of HNPCRC; it is observed in up to 15% of total patients with CRC who had no family history [25–27].

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In this retrospective cohort of 251 patients with CRC, we observed a high concordance between IHC for MMR proteins and MSI testing. Specifically, a loss of expression of ≥ one MMR protein had a strong correlation with MSI-H status, whereas an intact expression of MMR protein was highly predictive of MSS. This concordance emphasizes the usefulness of IHC as a practical, first-line approach for assessing MMR deficiency in CRC.

In our cohort, the overall concordance rates of 94.1% for MSI-H and 98.3% for MSS are in agreement with previous published studies, which have demonstrated sensitivity and specificity exceeding 90% for IHC relative to MSI testing [28,29]. However, we observed a small subset of discordant cases-MSI-H tumors with an intact expression of MMR protein and MSS tumors with a loss of MMR expression. Such discrepancies may arise due to rare missense mutations that result in a nonfunctional but antigenically intact MMR protein, technical variability in IHC interpretation, or intratumoral heterogeneity [30,31]. These findings emphasize the importance of a complementary testing strategy in selected cases.

The prevalence of MSI-H tumors in the current study was 6.7%, slightly lower than the 10-15% typically reported in Western populations [32], but consistent with findings in Asian cohorts [33–35]. MSI-H CRC is often associated with distinct clinicopathological features, including proximal colon predominance, poor differentiation, mucinous histology and a prominent lymphocytic infiltrate [32]. In our cohort, most tumors were moderately-differentiated adenocarcinomas (88%), but MSI-H cases were frequently linked to combined loss of MLH1/PMS2 or MSH2/MSH6, reflecting the dimeric nature of MMR proteins [36–38].

Extensive evidence indicates that MSI-H tumors show a favorable prognosis, particularly in stage II/III CRC. According to a systematic review of the previous published studies, MSI-H status was associated with significantly improved overall survival and reduced recurrence risk as compared with MSS tumors [39]. Moreover, it was also reported that patients with MSI-H CRC derive limited benefit from adjuvant 5-fluorouracil (5-FU) therapy, suggesting that chemotherapy strategies should be tailored based on MSI status [40].

Beyond the prognostic value of MSI-H status, it has emerged as a predictive biomarker for immune checkpoint inhibitor (ICI) therapy. According to a previous landmark study, PD-1 blockade achieved durable responses in patients with MSI-H metastatic CRC, leading to the approval of pembrolizumab and nivolumab in this setting [41]. Consequently, routine MSI/IHC testing is now recommended for all CRC patients, not only for identifying Lynch syndrome carriers but also for optimizing treatment strategies [42]. Given the relatively small proportion of MSI-H tumors in our cohort, universal testing is particularly critical to avoid missing these patients who may benefit substantially from immunotherapy.

Our results cannot be generalized; there are three limitations of the current study. First, the retrospective and single-center design may limit generalizability. Second, our study lacked survival and recurrence outcome data, preventing direct validation of prognostic implications within this cohort. Although survival data were not available in our cohort, however, the predominance of stage II/III cases highlights the clinical importance of MSI testing for prognostication and therapeutic decision-making. Third, discordant cases were not further analyzed using next-generation sequencing, which could have clarified the underlying molecular mechanisms.

## 5. Conclusions

The current study indicates the high concordance between IHC and MSI testing in HNPCRC. But this deserves further large-scale, prospective studies.

**Author Contributions:** Conceptualization, D.L.; data curation, D.L.; formal analysis, D.L.; investigation, D.L.; methodology, D.L.; supervision, D.L.; writing—original draft, D.L.; writing—review and editing, D.L.

**Institutional Review Board Statement:** The current study was approved by the Institutional Review Board (IRB) of our medical institution (IRB approval # 2016-07-002-002). It followed the applicable laws, regulations and ethics guidelines. A written informed consent was waived because of its retrospective nature.

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**Conflicts of Interest:** The author has nothing to declare in relation to this work.

## References

1. Kinzler, K.W.; Vogelstein, B. Lessons from hereditary colorectal cancer. *Cell* **1996,** *87,* 159–170.
2. Aaltonen, L.A.; Salovaara, R.; Kristo, P.; Canzian, F.; Hemminki, A.; Peltomäki, P.; Chadwick, R.B.; Kääriäinen, H.; Eskelinen, M.; Järvinen, H.; Mecklin, J.P.; de la Chapelle, A. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N. Engl. J. Med.* **1998,** *338,* 1481–1487.
3. de Jong, A.E.; van Puijenbroek, M.; Hendriks, Y.; Tops, C.; Wijnen, J.; Ausems, M.G.; Meijers-Heijboer, H.; Wagner, A.; van Os, T.A.; Bröcker-Vriends, A.H.; Vasen, H.F.; Morreau, H. Microsatellite instability, immunohistochemistry, and additional PMS2 staining in suspected hereditary nonpolyposis colorectal cancer. *Clin. Cancer Res.* **2004,** *10,* 972–980.
4. Steinke, V.; Engel, C.; Büttner, R.; Schackert, H.K.; Schmiegel, W.H.; Propping, P. Hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch syndrome. *Dtsch. Arztebl. Int.* **2013,** *110,* 32–38.
5. Jass, J.R. Hereditary non-polyposis colorectal cancer: The rise and fall of a confusing term. *World J. Gastroenterol.* **2006,** *12,* 4943–4950.
6. Heinen, C.D. Mismatch repair defects and Lynch syndrome: The role of the basic scientist in the battle against cancer. *DNA Repair (Amst.)* **2016,** *38,* 127–134.
7. Shin, Y.K.; Heo, S.C.; Shin, J.H.; Hong, S.H.; Ku, J.L.; Yoo, B.C.; Kim, I.J.; Park, J.G. Germline mutations in MLH1, MSH2 and MSH6 in Korean hereditary non-polyposis colorectal cancer families. *Hum. Mutat.* **2004,** *24,* 351.
8. Chao, E.C.; Lipkin, S.M. Molecular models for the tissue specificity of DNA mismatch repair-deficient carcinogenesis. *Nucleic Acids Res.* **2006,** *34,* 840–852.
9. Armaghany, T.; Wilson, J.D.; Chu, Q.; Mills, G. Genetic alterations in colorectal cancer. *Gastrointest. Cancer Res.* **2012,** *5,* 19–27.
10. Tariq, K.; Ghias, K. Colorectal cancer carcinogenesis: A review of mechanisms. *Cancer Biol. Med.* **2016,** *13,* 120–135.
11. Fishel, R. Mismatch repair. J. Biol. Chem. 2015, 290, 26395–26403.
12. Umar, A.; Boland, C.R.; Terdiman, J.P.; Syngal, S.; de la Chapelle, A.; Rüschoff, J.; Fishel, R.; Lindor, N.M.; Burgart, L.J.; Hamelin, R.; Hamilton, S.R.; Hiatt, R.A.; Jass, J.; Lindblom, A.; Lynch, H.T.; Peltomäki, P.; Ramsey, S.D.; Rodriguez-Bigas, M.A.; Vasen, H.F.; Hawk, E.T.; Barrett, J.C.; Freedman, A.N.; Srivastava, S. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J. Natl. Cancer Inst.* **2004,** *96,* 261–268.
13. Hampel, H.; Frankel, W.L.; Martin, E.; Arnold, M.; Khanduja, K.; Kuebler, P.; Nakagawa, H.; Sotamaa, K.; Prior, T.W.; Westman, J.; Panescu, J.; Fix, D.; Lockman, J.; Comeras, I.; de la Chapelle, A. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N. Engl. J. Med.* **2005,** *352,* 1851–1860.
14. Vasen, H.F.; Möslein, G.; Alonso, A.; Bernstein, I.; Bertario, L.; Blanco, I.; Burn, J.; Capella, G.; Engel, C.; Frayling, I.; Friedl, W.; Hes, F.J.; Hodgson, S.; Mecklin, J.P.; Møller, P.; Nagengast, F.; Parc, Y.; Renkonen-Sinisalo, L.; Sampson, J.R.; Stormorken, A.; Wijnen, J. Guidelines for the clinical management of Lynch syndrome (hereditary non-polyposis cancer). *J. Med. Genet.* **2007,** *44,* 353–362.
15. Shia, J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I: The utility of immunohistochemistry. *J. Mol. Diagn.* **2008,** *10,* 293–300.
    Zhang, L. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part II: The utility of microsatellite instability testing. *J. Mol. Diagn.* **2008,** *10,* 301–307.
16. Amin, M.B.; Greene, F.L.; Edge, S.B.; Compton, C.C.; Gershenwald, J.E.; Brookland, R.K.; Meyer, L.; Gress, D.M.; Byrd, D.R.; Winchester, D.P. The eighth edition AJCC cancer staging manual: Continuing to build a

 doi:10.20944/preprints202604.0464.v1



bridge from a population-based to a more personalized approach to cancer staging. *CA Cancer J. Clin.* **2017,** *67,* 93–99.

17. Buhard, O.; Cattaneo, F.; Wong, Y.F.; Yim, S.F.; Friedman, E.; Flejou, J.F.; Duval, A.; Hamelin, R. Multipopulation analysis of polymorphisms in five mononucleotide repeats used to determine the microsatellite instability status of human tumors. *J. Clin. Oncol.* **2006,** *24,* 241–251.
18. Tóth, C.; Sükösd, F.; Valicsek, E.; Herpel, E.; Schirmacher, P.; Tiszlavicz, L. Loss of CDX2 gene expression is associated with DNA repair proteins and is a crucial member of the Wnt signaling pathway in liver metastasis of colorectal cancer. *Oncol. Lett.* **2018,** *15,* 3586–3593.
19. McCarthy, A.J.; Capo-Chichi, J.M.; Spence, T.; Grenier, S.; Stockley, T.; Kamel-Reid, S.; Serra, S.; Sabatini, P.; Chetty, R. Heterogeneous loss of mismatch repair protein expression: A challenge for immunohistochemical interpretation and microsatellite instability evaluation. *J. Pathol. Clin. Res.* **2019,** *5,* 115–129.
20. Modrich, P. Mechanisms and biological effects of mismatch repair. *Annu. Rev. Genet.* **1991,** *25,* 229–253.
21. Ionov, Y.; Peinado, M.A.; Malkhosyan, S.; Shibata, D.; Perucho, M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* **1993,** *363,* 558–561.
22. Thibodeau, S.N.; Bren, G.; Schaid, D. Microsatellite instability in cancer of the proximal colon. *Science* **1993,** *260,* 816–819.
23. Peltomäki, P.; Aaltonen, L.A.; Sistonen, P.; Pylkkänen, L.; Mecklin, J.P.; Järvinen, H.; Green, J.S.; Jass, J.R.; Weber, J.L.; Leach, F.S.; et al. Genetic mapping of a locus predisposing to human colorectal cancer. *Science* **1993,** *260,* 810–812.
24. Kim, H.; Jen, J.; Vogelstein, B.; Hamilton, S.R. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *Am. J. Pathol.* **1994,** *145,* 148–156.
25. Risio, M.; Reato, G.; di Celle, P.F.; Fizzotti, M.; Rossini, F.P.; Foà, R. Microsatellite instability is associated with the histological features of the tumor in nonfamilial colorectal cancer. *Cancer Res.* **1996,** *56,* 5470–5474.
26. Thibodeau, S.N.; French, A.J.; Cunningham, J.M.; Tester, D.; Burgart, L.J.; Roche, P.C.; McDonnell, S.K.; Schaid, D.J.; Vockley, C.W.; Michels, V.V.; Farr, G.H.; O'Connell, M.J. Microsatellite instability in colorectal cancer: Different mutator phenotypes and the principal involvement of hMLH1. *Cancer Res.* **1998,** *58,* 1713–1718.
27. Shia, J.; Ellis, N.A.; Paty, P.B.; Nash, G.M.; Qin, J.; Offit, K.; Zhang, X.M.; Nafa, K.; Guillem, J.G.; Wong, W.D.; Gerald, W.L.; Klimstra, D.S. Value of histopathology in predicting microsatellite instability in hereditary and sporadic colorectal cancer. *Am. J. Surg. Pathol.* **2003,** *27,* 1407–1417.
28. Hampel, H.; Frankel, W.; Panescu, J.; Lockman, J.; Sotamaa, K.; Fix, D.; Comeras, I.; La Jeunesse, J.; Nakagawa, H.; Westman, J.A.; Prior, T.W.; Clendenning, M.; Penzone, P.; Lombardi, J.; Dunn, P.; Cohn, D.E.; Copeland, L.; Eaton, L.; Fowler, J.; Lewandowski, G.; Vaccarello, L.; Bell, J.; Reid, G.; de la Chapelle, A. Screening for Lynch syndrome among endometrial cancer patients. *Cancer Res.* **2006,** *66,* 7810–7817.
29. van Lier, M.G.; Wagner, A.; van Leerdam, M.E.; Biermann, K.; Kuipers, E.J.; Steyerberg, E.W.; Dubbink, H.J.; Dinjens, W.N. A review on the molecular diagnostics of Lynch syndrome: A central role for the pathology laboratory. *J. Cell Mol. Med.* **2010,** *14,* 181–197.
30. Chen, W.; Hampel, H.; Pearlman, R.; Jones, D.; Zhao, W.; Alsomali, M.; Knight, D.; Frankel, W.L. Unexpected expression of mismatch repair protein is more commonly seen with pathogenic missense mutations in Lynch syndrome. *Hum. Pathol.* **2020,** *103,* 34–41.
31. Boland, C.R.; Goel, A. Microsatellite instability in colorectal cancer. *Gastroenterology* **2010,** *138,* 2073–2087.e3.
32. Mei, W.J.; Mi, M.; Qian, J.; Xiao, N.; Yuan, Y.; Ding, P.R. Clinicopathological characteristics of high microsatellite instability/mismatch repair-deficient colorectal cancer: A narrative review. *Front. Immunol.* **2022,** *13,* 1019582.
33. Oh, J.R.; Kim, D.W.; Lee, H.S.; Lee, H.E.; Lee, S.M.; Jang, J.H.; Kang, S.B.; Ku, J.L.; Jeong, S.Y.; Park, J.G. Microsatellite instability testing in Korean patients with colorectal cancer. *Fam. Cancer* **2012,** *11,* 459–466.
34. Cui, M.; Li, P.; Mao, Y.; Zhang, L.; Xia, P.; Liu, E.; Wang, W.; Zhang, J.; Jiang, G.; Li, W. Implication of microsatellite instability in a Chinese cohort of human cancers. *Cancer Manag. Res.* **2020,** *12,* 10287–10295.

 doi:10.20944/preprints202604.0464.v1




35. Aiyer, K.T.S.; Doeleman, T.; Ryan, N.A.; Nielsen, M.; Crosbie, E.J.; Smit, V.T.H.B.M.; Morreau, H.; Goeman, J.J.; Bosse, T. Validity of a two-antibody testing algorithm for mismatch repair deficiency testing in cancer: A systematic review and meta-analysis. *Mod. Pathol.* **2022,** *35,* 1775–1783.
36. Abildgaard, A.B.; Nielsen, S.V.; Bernstein, I.; Stein, A.; Lindorff-Larsen, K.; Hartmann-Petersen, R. Lynch syndrome, molecular mechanisms and variant classification. *Br. J. Cancer* **2023,** *128,* 726–734.
37. Rosty, C.; Clendenning, M.; Walsh, M.D.; Eriksen, S.V.; Southey, M.C.; Winship, I.M.; Macrae, F.A.; Boussioutas, A.; Poplawski, N.K.; Parry, S.; Arnold, J.; Young, J.P.; Casey, G.; Haile, R.W.; Gallinger, S.; Le Marchand, L.; Newcomb, P.A.; Potter, J.D.; DeRycke, M.; Lindor, N.M.; Thibodeau, S.N.; Baron, J.A.; Win, A.K.; Hopper, J.L.; Jenkins, M.A.; Buchanan, D.D. Germline mutations in PMS2 and MLH1 in individuals with solitary loss of PMS2 expression in colorectal carcinomas. *BMJ Open* **2016,** *6,* e010293.
38. Popat, S.; Hubner, R.; Houlston, R.S. Systematic review of microsatellite instability and colorectal cancer prognosis. *J. Clin. Oncol.* **2005,** *23,* 609–618.
39. Ribic, C.M.; Sargent, D.J.; Moore, M.J.; Thibodeau, S.N.; French, A.J.; Goldberg, R.M.; Hamilton, S.R.; Laurent-Puig, P.; Gryfe, R.; Shepherd, L.E.; Tu, D.; Redston, M.; Gallinger, S. Tumor microsatellite instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N. Engl. J. Med.* **2003,** *349,* 247–257.
40. Le, D.T.; Uram, J.N.; Wang, H.; Bartlett, B.R.; Kemberling, H.; Eyring, A.D.; Skora, A.D.; Luber, B.S.; Azad, N.S.; Laheru, D.; Biedrzycki, B.; Donehower, R.C.; Zaheer, A.; Fisher, G.A.; Crocenzi, T.S.; Lee, J.J.; Duffy, S.M.; Goldberg, R.M.; de la Chapelle, A.; Koshiji, M.; Bhaijee, F.; Huebner, T.; Hruban, R.H.; Wood, L.D.; Cuka, N.; Pardoll, D.M.; Papadopoulos, N.; Kinzler, K.W.; Zhou, S.; Cornish, T.C.; Taube, J.M.; Anders, R.A.; Eshleman, J.R.; Vogelstein, B.; Diaz, L.A. PD-1 blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.* **2015,** *372,* 2509–2520.
41. Overman, M.J.; Lonardi, S.; Wong, K.Y.M.; Lenz, H.J.; Gelsomino, F.; Aglietta, M.; Morse, M.A.; Van Cutsem, E.; McDermott, R.; Hill, A.; Sawyer, M.B.; Hendlisz, A.; Neyns, B.; Svrcek, M.; Moss, R.A.; Ledeine, J.M.; Cao, Z.A.; Kamble, S.; Kopetz, S.; André, T. Durable clinical benefit with nivolumab plus ipilimumab in mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J. Clin. Oncol.* **2018,** *36,* 773–779.