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ORIGINAL RESEARCH

Discordance between immunochemistry of mismatch repair proteins and molecular testing of microsatellite instability in colorectal cancer

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Background: DNA mismatch repair system deficiency (dMMR) is found in 15% of colorectal cancers (CRCs). Two methods are used to determine dMMR, immunohistochemistry (IHC) of MMR proteins and molecular testing of microsatellite instability (MSI). Only studies with a low number of patients have reported rates of discordance between these two methods, ranging from 1% to 10%.

Materials and methods: Overall, 3228 consecutive patients with CRCs from two centers were included. Molecular testing was carried out using the Pentaplex panel and IHC evaluated four (MLH1, MSH2, MSH6, and PMS2; cohort 1; $n = 1085$) or two MMR proteins (MLH1 and MSH2; cohort 2; $n = 2143$). The primary endpoint was the rate of discordance between MSI and MMR IHC tests.

Results: Fifty-one discordant cases (1.6%) were initially observed. Twenty-nine out of 51 discordant cases were related to IHC misclassifications. In cohort 1, after re-reading IHC and/or carrying out new IHC, 16 discordant cases were reclassified as nondiscordant. In cohort 2, after the addition of MSH6/PMS2 IHC and re-examination, 13 were reclassified as nondiscordant. In addition, 10 misclassifications of molecular tests were identified. Finally, only 12 discordant cases (0.4%) remained: 5 were proficient MMR/MSI and 7 were dMMR/microsatellite stable.

Conclusions: Our study confirmed the high degree of concordance between MSI and MMR IHC tests. Discordant cases must be reviewed, and if needed, tests must be repeated and analyzed by an expert team.

Key words: colorectal cancer, microsatellite instability, deficient mismatch repair, immunohistochemistry, molecular biology

INTRODUCTION

Microsatellite instability (MSI) is detected in ~15% of all colorectal cancers (CRCs); 3% of them are associated with Lynch syndrome (LS) and the remaining 12% are sporadic mostly due to hypermethylation of the *MLH1* gene promoter, although other sporadic mechanisms exist.[1-3] MSI is due to a DNA mismatch repair system deficiency (dMMR). Determination of MSI has a major impact on CRC management, notably in screening for LS.[4] The dMMR/MSI phenotype has been shown to confer good prognosis in patients with nonmetastatic CRC, whereas stage II dMMR/MSI CRCs present resistance to 5-fluorouracil.[5,6] While dMMR/MSI is found in ~5% of metastatic CRCs (mCRCs), its impact on prognosis and chemosensitivity remains unclear. Nevertheless, immune checkpoint inhibitors (ICIs) are highly effective in patients with dMMR/MSI mCRC.[7-9]

Two methods are available to detect dMMR/MSI phenotype: (i) expression of MMR proteins (MLH1, MSH2, MSH6, and PMS2) in tumor tissue by immunohistochemistry (IHC) and (ii) MSI tumor DNA molecular testing by PCR. Commonly, a tumor is called dMMR if it presents complete nuclear loss of expression of at least one MMR protein, in contrast to pMMR tumor (proficient MMR). MMR IHC

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presents sensitivity between 85% and 100% and specificity between 85% and 92%.[10,11] The MMR status can be established based on the analysis of either two MMR proteins (MHL1 and MSH2 or MSH6 and PMS2) or four MMR proteins (MLH1, MSH2, MSH6, and PMS2), though IHC of the four MMR proteins is favored.[12,13] MMR function requires two by two protein heterodimerization, where MSH2 combines with MSH6 and MLH1 combines with PMS2. MSH2 and MLH1 are essential to their respective heterodimers and their loss leads to degradation of their respective partners, MSH6 and PMS2.

MSI molecular testing using PCR determines the level of instability of microsatellite markers.[14] Two panels of five consensus nucleotide repeats exist: the Bethesda panel and the Pentaplex panel.[14,15] Sensitivity of MSI testing ranges between 67% and 100% and specificity between 61% and 92% using the Bethesda panel,[16] but is improved using the Pentaplex panel (specificity 98.7% and sensitivity 95.8%).[17,18]

Some studies have shown discordance between MMR IHC and MSI molecular testing, ranging from 1% to 10%.[19-24] All of these studies used relatively old techniques for MMR/MSI determination (fewer than five microsatellite markers) and/or were conducted with a low number of patients (<1000 with CRCs). Failure in determining the MMR/MSI status has a major impact on therapeutic strategy, especially on eligibility for ICI treatment.[12,25,26] Indeed, a recent study from Cohen et al,[27] showed that one cause of resistance to ICI in mCRC can be related to an error in the determination of the MMR/MSI status, whereas other reports show responses to ICI despite MSI misdiagnosis.[28]

In a large French bicentric series, we evaluated the rate of discordance between MMR IHC and MSI molecular testing in CRC patients. We also examined the consequences of analyzing four versus two MMR proteins by IHC. Finally, we tried to better understand the causes of these discordances in view of improving the diagnosis of dMMR/MSI CRC.

MATERIALS AND METHODS

Study population

This retrospective multicenter study included all consecutive CRC cases with MSI testing in the Poitiers University Hospital (Poitiers) between January 2013 and December 2018 and MMR IHC testing in the Saint-Antoine University Hospital (Paris) between January 2006 and December 2014 ($n = 3228$). Inclusion criteria were histologically proven CRC with both molecular MSI testing and IHC of MMR proteins carried out on the same specimen (biopsy or surgical specimen).

Two cohorts, with different strategy of MMR/MSI testing, were combined. In cohort 1, from the Poitiers University Hospital, CRCs with MSI testing were identified by the Poitiers molecular genetic platform and subsequently included MSI cases for which the four MMR proteins were tested by IHC. In cohort 2, from the Saint-Antoine University Hospital, both MSI testing and IHC for MLH1 and MSH2 proteins were frequently carried out.

Nonprimary CRCs, CRCs with IHC or molecular testing failure, or CRCs with only one test (MMR IHC or MSI testing alone) were excluded (Figure 1). The study was approved by our institution's Ethics Committee (DC-2008-565).

MSI molecular testing and expression of MMR proteins

MSI was determined on tumor DNA using the five consensus mononucleotide repeats called Pentaplex panel (BAT-25, BAT-26, NR-21, NR-24, and NR-27), as recommended.[14] Tumor DNA was extracted from formalin-fixed paraffin-embedded tissue using the DNeasy Blood and Tissue DNA isolation kit (Qiagen, Hilden, Germany).

The size of the microsatellite markers was analyzed on ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA) and compared with their expected sizes. A tumor was considered MSI if three or more markers were found unstable according to the Bethesda guidelines.[29] Using a mononucleotide panel, no comparison with nontumor tissue was necessary, as recommended.[14] If none or one marker was found to be unstable, the tumor was classified microsatellite stable (MSS). However, if two markers were unstable, new testing was carried out on both tumor and nontumor tissue to ascertain the result.

For cohort 1, MMR IHC was carried out using four antibodies directed against MLH1 (M1 Ventana clone ready for use and Optiview kit revelation, Tucson, AZ), MSH2 (clone G219-1129 Ventana ready to use; Optiview kit revelation), MSH6 (44 BD Biosciences clone and ultraView kit revelation, Meylan, France), and PMS2 (clone EPR3947 Ventana ready for use; Optiview kit revelation with amplification) proteins on the BenchMark XT device (Ventana Medical Systems). For cohort 2, MLH1 and MSH2 expression was evaluated using an antibody against MLH1 (clone G168-728; PharMingen, San Diego, CA) and an antibody against MSH2 (clone FE11; Calbiochem, Cambridge, MA) and the analysis carried out using the Bond max platform (Leica Microsystem, Nanterre, France).

MMR protein loss was defined by the absence of IHC staining in the nucleus of tumor cells while normal cells remained stained, ensuring the technical validity of the experiment. Loss of nuclear expression of at least one protein was sufficient to establish dMMR status; otherwise, the tumor was considered pMMR. The four staining patterns observed were combined MLH1/PMS2 loss, combined MSH2/MSH6 loss, isolated PMS2 loss, and isolated MSH6 loss. It is worth noting that the physicians carrying out the initial testing were not aware of the results of the test carried out by the other technique. Both expert centers carried out MSI and MMR IHC tests, whenever possible, on a sample prior to any chemotherapy or radiotherapy.

For all cases with discordant results between MSI and MMR IHC tests, re-examination of the molecular MSI profile and the MMR IHC staining was carried out by experts. When only two MMR proteins had been tested (cohort 2), a new experiment was carried out with two additional MMR

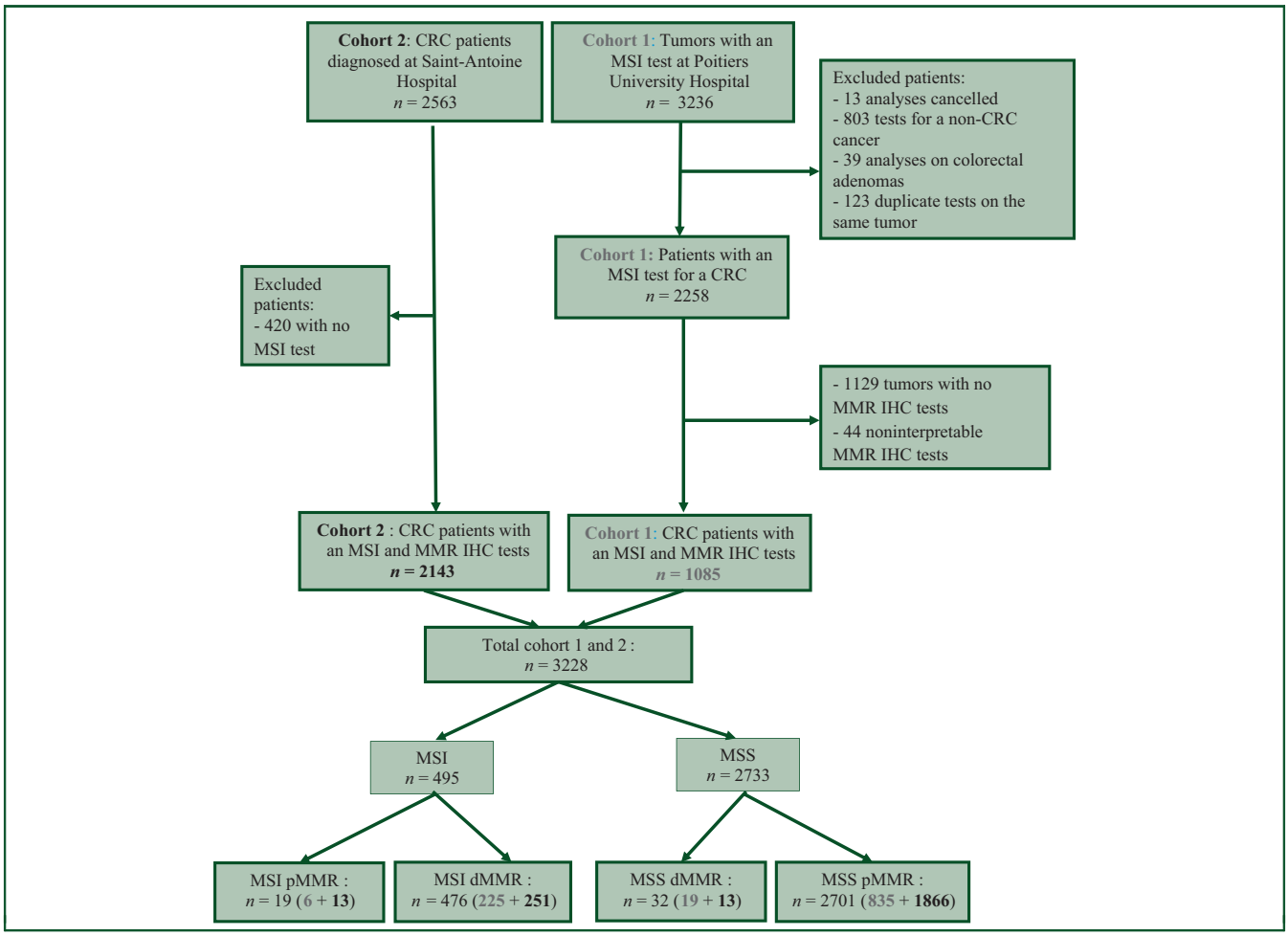

Figure 1. Flowchart.
Bold and Grey refers to the cohort 2 and 1 respectively.
CRC, colorectal cancer; dMMR, DNA mismatch repair system deficient; IHC, immunohistochemistry, MSI, microsatellite instability, MSS, microsatellite stable, pMMR, DNA mismatch repair system.

proteins MSH6 (clone 44; Becton Dickinson, Lexington, NC) and PMS2 (clone A16-4; BD PharMingen, Le Pont de Claix, France); then, if the two tests remained discordant, new MSI and MMR IHC tests were carried out on different tumor areas (provided that tumor material was sufficient).

Patient and tumor characteristics

Patient and tumor characteristics, as well as *MLH1* promoter hypermethylation, were collected. Determination of sporadic dMMR/MSI versus suspected germline (LS) cases was based on MMR protein expression, family history (Amsterdam II criteria[30]), *BRAF* status (exclusion of LS if *BRAF* mutated), and *MLH1* promoter methylation status (exclusion of LS if *MLH1* promoter hypermethylation). When available, information on germline exploration to confirm LS status was collected.

Statistical methods

Continuous variables were described with mean, median, minimum, maximum, and standard deviation. Qualitative variables were described with frequency and percentages. Comparison was carried out with the Mann−Whitney test for continuous variables and the chi-square test or Fisher's exact test for qualitative variables. A P value <0.05 was considered statistically significant. All statistical tests were two sided. All analyses were carried out using StatView software (SAS Institute, Cary, NC).

RESULTS

Patient and tumor characteristics

Considering cohort 1, 3236 patients had an MSI test on their tumor tissue at the Poitiers University Hospital. Among these, 2258 had a confirmed diagnosis of CRC (Figure 1). After excluding CRCs with no MMR IHC, 1085 were finally included in cohort 1. Mean age at diagnosis was 64.2 ± 13.1 years and there were 44.1% of women (Table 1).

Considering cohort 2, 2563 CRCs diagnosed between January 2006 and December 2014 at the Saint-Antoine Hospital were routinely screened for MMR IHC. Because of missing MSI testing ($n = 420$), 2143 were finally included in cohort 2. Mean age at diagnosis was 66.0 ± 13.8 years and there were 43.9% of women. Patients were significantly older in cohort 2 with more frequent rectal tumors (33.7% versus 18.2%).

Table 1. Patient and tumor characteristics

Characteristics	Cohort 1 ($N = 1085$)	Cohort 2 ($N = 2143$)	*P* value
Age (years), mean ± SD	64.2 ± 13.1	66.0 ± 13.8	<0.001
Sex, *n* (%)			0.92
Female	479 (44.1)	941 (43.9)	
Male	606 (55.9)	1202 (56.1)	
Tumor site, *n* (%)			<0.001
Right colon	430 (39.6)	585 (27.3)	
Left colon	411 (37.9)	697 (33.7)	
Rectum	198 (18.3)	697 (33.7)	
Transverse colon	25 (2.3)	88 (4.2)	
Colon of unknown site	21 (1.9)	2 (0.1)	
Missing data	0	74	
Tumor stage, *n* (%)			<0.001
0	9 (1.8)	23 (1.1)	
I	38 (7.6)	392 (18.6)	
II	144 (29.0)	547 (26.0)	
III	134 (27.0)	492 (23.4)	
IV	172 (34.6)	651 (30.9)	
Missing data	588	38	
MSI status, *n* (%)			<0.001
MSI	231 (21.3)	264 (12.3)	
MSS	854 (78.7)	1879 (87.7)	
MMR IHC status, *n* (%)			<0.001
pMMR	841 (77.5)	1879 (87.7)	
dMMR	244 (22.5)	264 (12.3)	
Lynch status in dMMR/MSI CRCs ($N = 476$), *n* (%)			<0.001
Confirmed Lynch syndrome	7 (3.1)	80 (33.7)	
Suspected Lynch syndrome	43 (19.1)	4 (1.7)	
Sporadic cases	144 (64.0)	153 (64.6)	
Missing data	31	14	

CRC, colorectal cancer; dMMR, DNA mismatch repair system deficient; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite stable; pMMR, DNA mismatch repair system proficient; SD, standard deviation.

Initial results of microsatellite instability and immunohistochemistry of mismatch repair proteins

Most CRCs presented MSS status ($n = 2733$, 84.7%). MSI was more frequently detected in cohort 1 (21.3%) than in cohort 2 (12.3%) ($P < 0.001$). IHC of the four MMR proteins (cohort 1) identified 22.5% dMMR CRCs, whereas only 12.3% were detected by IHC of only two MMR proteins in cohort 2 ($P < 0.001$; Table 1). Among the dMMR/MSI CRCs, there were 3.1% of confirmed LS and 19.1% of suspected LS in cohort 1 as compared with 33.7% and 1.7% in cohort 2.

In the overall cohort, among the MSI CRCs ($n = 495$), 19 tumors were pMMR (6 in cohort 1, 13 in cohort 2; Figure 1 and Supplementary Tables S1 and S2, available at https://doi.org/10.1016/j.esmoop.2021.100120), scoring at a 0.6% MSI/pMMR rate of discordance ($n = 19/3228$). Among the MSS CRCs ($n = 2733$), 32 tumors were dMMR (19 in cohort 1, 13 in cohort 2), which represents a discordance rate of 1.0% dMMR/MSS ($n = 32/3228$). Therefore, the initial overall discordance rate reached 1.6% ($n = 51/3228$), 2.3% ($n = 25$) in cohort 1 and 1.2% ($n = 26$) in cohort 2 (Figure 1).

Control immunohistochemistry of mismatch repair proteins tests

In cohort 1, the review of all initial MMR IHC tests and, if necessary, new MMR IHC tests by expert pathologists of the 25 discordant cases enabled reclassification of 16 cases (64.0%; Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2021.100120). The main misclassifications were related to a pathologist's misinterpretation (case numbers 6, 7, 8, 9, 14, and 16) and poor antibody-binding quality (case numbers 1, 2, 10, 12, 15, and 21). Two pMMR/MSI cases with a first MMR IHC test carried out on biopsy were corrected by the MMR IHC test on the surgical specimen (dMMR; case numbers 20 and 22). Two cases presented tumor heterogeneity with two distinct pMMR and dMMR populations (case numbers 5 and 18).

In cohort 2, all 13 pMMR/MSI cases, using only MLH1 and MSH2 MMR IHC tests, were subsequently tested for PMS2 and MSH6 expression by IHC. Nine (69.2%) were reclassified dMMR/MSI as they presented PMS2 ($n = 2$) or MSH6 ($n = 7$) isolated loss (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2021.100120; case numbers 39-47). In addition, human errors explained four other misclassified cases related to multiple simultaneous tumors (case numbers 27, 32, 33, and 34). All the remaining discordant cases in this cohort, dMMR/MSS and pMMR/MSI ($n = 13$), were reviewed by expert pathologists and, if necessary, new MMR IHC tests were carried out. No other error of MMR IHC tests was identified.

Overall, control MMR IHC tests successfully identified 29 misclassifications (56.9%; 16 in cohort 1 and 13 in cohort 2) among the 51 initially discordant cases.

Control of microsatellite instability molecular tests

In cohort 1, review of all initial MSI tests and, if necessary, new MSI tests ($n = 11$) by expert biologists of the 25 discordant cases enabled reclassification of 6 cases (24.0%) (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2021.100120). Indeed, three initial dMMR/MSS cases were reclassified as dMMR/MSI on testing of new tumor areas (case numbers 4, 11, and 13). Two cases presented tumor heterogeneity with two distinct populations, one dMMR/MSI and one pMMR/MSS (case numbers 5 and 18). Heterogeneity was suspected for one case but could not be ascertained due to exhaustion of the tumor material (case number 25).

In cohort 2, review of all initial MSI tests and, if necessary, new MSI tests ($n = 8$) by expert biologists of the 26 discordant cases enabled reclassification of 6 cases (23.1%) (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2021.100120). Indeed, five cases initially classified as dMMR/MSS due to sampling difficulties for the molecular biology test (tumoral cells inferior to 10%) were reclassified as dMMR/MSI on testing of new tumor areas (case numbers 26, 29, 30, 35, and 36) and one pMMR/MSI case was reclassified as pMMR/MSS (case number 49).

Overall, control of MSI tests identified 12 misclassifications (23.5%; 6 in cohort 1 and 6 in cohort 2) among the initially 51 discordant cases and 10 misclassifications among the 22 remaining discordant cases after the control of MMR IHC tests. Therefore, 12 cases (23.5%) remained discordant after reviewing (5 in cohort 1 and 7 in cohort 2).

Table 2. Characteristics of discordant cases

Characteristics	Overall cohort of discordant cases ($n = 12$)	Cohort 1 (Poitiers)			Cohort 2 (Saint Antoine)		
		Nondiscordant cases ($n = 1080$)	Discordant cases ($n = 5$)	*P*	Nondiscordant cases ($n = 2136$)	Discordant cases ($n = 7$)	*P*
Age (years), mean ± SD	59.82 ± 17.49	64.15 ± 13.09	65.46 ± 13.64	0.99	66.07 ± 13.76	55.79 ± 19.78	0.10
Sex, *n* (%)				0.39			0.10
Female	2 (16.7)	478 (44.3)	1 (20.0)		940 (44.0)	1 (14.3)	
Male	10 (83.3)	602 (55.7)	4 (80.0)		1196 (56.0)	6 (85.7)	
Location, *n* (%)				0.70			0.46
Right colon	6 (50.0)	428 (39.6)	2 (40.0)		581 (28.2)	4 (57.1)	
Left colon	4 (33.3)	408 (37.8)	3 (60.0)		696 (33.7)	1 (14.3)	
Rectum	2 (16.7)	198 (18.3)	0 (0)		695 (33.7)	2 (28.6)	
Transverse colon	0 (0)	25 (2.3)	0 (0)		88 (4.3)	0 (0)	
Colon of unknown site	0 (0)	21 (2.0)	0 (0)		2 (0.1)	0 (0)	
Missing data	0	0	0		74	0	
BRAF^{V600E} status, *n* (%)				0.58			0.38
Mutated	0 (0)	164 (22.3)	0 (0)		218 (13.2)	0 (0)	
Wild type	9 (100)	570 (77.7)	4 (100)		1434 (86.8)	5 (100)	
Missing data	3	346	1		484	2	
KRAS status, *n* (%)				0.99			0.49
Mutated	2 (22.2)	294 (38.4)	1 (25.0)		729 (34.6)	1 (20.0)	
Wild type	7 (77.8)	472 (61.6)	3 (75.0)		1380 (65.4)	4 (80.0)	
Missing data	3	314	1		27	2	
NRAS status, *n* (%)				0.99			—
Mutated	0 (0)	21 (4.2)	0 (0)		—	0 (0)	
Wild type	6 (100)	476 (95.8)	2 (100)		—	4 (100)	
Missing data	6	583	3			3	
Hypermethylation of *MLH1* gene promoter, *n* (%)		$N = 225$		0.09	$N = 251$		0.08
Presence	1 (16.7)	80 (71.4)	0 (0)		153 (70.5)	1 (25.0)	
Absence	5 (83.3)	32 (28.6)	2 (100)		64 (29.5)	3 (75.0)	
Missing data	6	113	3		34	3	
Tumor stage, *n* (%)				0.08			0.77
0	0 (0)	9 (1.8)	0 (0)		23 (1.1)	0 (0)	
I	2 (18.2)	38 (7.7)	0 (0)		390 (18.6)	2 (28.6)	
II	6 (54.5)	140 (28.4)	4 (100)		545 (26.0)	2 (28.6)	
III	2 (18.2)	134 (27.2)	0 (0)		490 (23.3)	2 (28.6)	
IV	1 (9.1)	172 (34.9)	0 (0)		650 (31.0)	1 (14.2)	
Missing data	1	587	1		38	0	
Lynch syndrome, *n* (%)		$N = 225$		0.01	$N = 251$		0.27
Confirmed LS	5 (55.6)	7 (3.6)	1 (33.3)		80 (33.7)	4 (66.7)	
Suspected LS	2 (22.2)	43 (22.2)	2 (66.7)		4 (1.7)	0 (0)	
Sporadic unstable CRCs	2 (22.2)	144 (74.2)	0 (0)		153 (64.6)	2 (33.3)	
Missing data	3	31	2		14	1	

CRC, colorectal cancer; LS, Lynch syndrome; SD, standard deviation.

Remaining discordant cases

The final overall discordance rate after control tests was 0.4% ($n = 12/3228$), with five pMMR/MSI and seven dMMR/MSS cases. Among the dMMR/MSS CRCs, two had an isolated loss of MSH6, two a loss of MSH2 ± MSH6, two a loss of MLH1 ± PMS2, and one an isolated loss of PMS2.

There were 10 men (83.3%) and 2 women (16.7%) among discordant cases (Table 2), and their mean age was 59.82 ± 17.49 years. The tumors were mainly located in the right colon ($n = 6$, 50.0%). No tumor harbored a *BRAF*^{V600E} mutation and one had a hypermethylation of the *MLH1* promoter. Seven patients had suspected or confirmed LS, including two patients with *PMS*2 germline mutation and one patient with *MSH2* germline mutation. No case had prior chemotherapy or radiotherapy on the samples used for MSI and MMR IHC tests.

MMR immunohistochemistry test with two versus four proteins

In cohort 1, 22 MSI cases and 12 MSS cases had an isolated loss of PMS2 or MSH6. If only two MMR proteins (MHL1 and MSH2) had been initially tested for IHC determination, 12 dMMR/MSS discordant cases would have been missed and classified wrongly as pMMR/MSS. Moreover, 22 cases would have been classified wrongly as pMMR/MSI discordant cases. Overall, if MMR IHC with MLH1 and MSH2 antibodies was carried out alone, 3.1% dMMR cases would have not been identified ($n = 34/1085$).

MMR IHC testing with only MSH6 and PMS2 proteins could have been another strategy for MMR exploration.[31] If only two MMR proteins (PMS2 and MSH6) had been initially tested in cohort 1, four cases with isolated loss of MLH1 and

seven cases with isolated loss of MSH2 would have been missed and wrongly classified as pMMR (1.0%).

Comparison of MMR immunohistochemistry versus microsatellite instability testing

Focusing on whether to carry out one or two tests, MMR IHC and/or MSI tests, we determined the rate of misclassified cases, as well as the sensitivity and specificity of each test. In the overall cohort, if only MSI testing had been carried out, 495 MSI CRCs would have been identified but 32 dMMR/MSS CRCs (1.0%) would have been ignored (Figure 1). By contrast, if only MMR IHC had been carried out, 508 dMMR CRCs would have been identified but 19 pMMR/MSI CRCs would have been ignored (0.6%).

In cohort 1, after excluding discordant cases and cases with tumor heterogeneity, sensitivity and specificity of MSI testing were 98.7% (n = 228/231) and 99.9% (n = 848/849), respectively (Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2021.100120). Sensitivity and specificity of MMR IHC testing were 98.7% (n = 228/231) and 98.7% (n = 838/849), respectively. In cohort 2, after excluding discordant cases, sensitivity and specificity of MSI testing were 96.6% (n = 260/269) and 99.9% (n = 1866/1867), respectively. Sensitivity and specificity of MMR IHC test were 96.6% (n = 260/269) and 100% (n = 1867/1867), respectively.

DISCUSSION

Our study combines two large cohorts from expert centers in an attempt to evaluate the discordance between IHC of MMR proteins and MSI molecular testing by Pentaplex, the objective being to correctly identify dMMR/MSI CRC. The rate of discordant cases of 1.6% is relatively low but has major consequences at the individual level (i.e. missed LS or eligibility for ICI). Our results showed that dual screening with both MSI testing and MMR IHC with a four-antibody panel should be carried out to avoid having CRC patients with undetected MSI and/or dMMR status. Indeed, when double testing is carried out, discordances can be revealed and rechecked, enabling significant reduction of wrongly classified cases. After re-reading, 76.5% of discordant cases in our study were reclassified as nondiscordant. Finally, only 0.4% of cases remained discordant. This strategy aimed at identifying dMMR/MSI CRC had previously been applied only in relatively old series with a low number of patients. In addition, our results provide significant new data that can help to identify true dMMR/MSI mCRC cases for ICI treatment.

Discordance rate in our study is slightly lower than that in other series, which analyzed fewer patients with older MSI testing techniques. In a cohort of 1144 CRCs, while Lindor et al.[19] reported a discordance rate of 2.36%, MSI testing was carried out using multiple techniques/panels and IHC explored only two MMR proteins, MLH1 and MSH2. Analyzing 1003 CRCs, Watson et al.[20] found a low 1% discordance rate, which was rather surprising insofar as they did not use recommended panels (Bethesda or Pentaplex) for MSI testing but BAT-26 microsatellite alone. Besides, IHC of the four MMR proteins was carried out only on MSI CRCs and not on the whole cohort, leading to probable underestimation of the number of discordant cases. Chen et al.[21] studied fewer CRCs (n = 569) and found a higher rate of discrepancy (8.08%). They compared IHC of the four MMR proteins with MSI testing using the Bethesda panel. Their limitation was due to the fact that the Bethesda panel is less sensitive and specific than the Pentaplex panel.[17,18,32] Cohen et al.[22] carried out a study using IHC of the four MMR proteins and Pentaplex tests on a low number of patients (n = 92). They found a higher rate of discrepancy (9.8%) but did not provide any explanations, other than that the tests were re-done in a more experienced reference center. Finally, Jaffrelot et al.[24] reported a 1.1% discordance rate in a large cohort of 2528 different cancers, using IHC of the four MMR proteins and Pentaplex tests. The rate of discordance was equivalent to our study even though molecular MSI testing is known to have lower performances in malignancies other than CRC.[33,34] Finally, although the rate of discordance is low in the literature, it varies depending on the panel used for MSI testing and on whether two or four MMR proteins are studied by IHC.

In our study, reanalysis and expert reviews disclosed the presence of 39 'false' discordant cases. In the literature, multiple factors explaining discordant cases have been identified, and they were confirmed by our study. These factors include tumor heterogeneity with a dual tumor contingent (pMMR/MSS and dMMR/MSI),[35,36] the expertise of pathologists and molecular biologists,[37,38] the quality of the tissue sampling used and the quality of tissue fixation,[23] polymorphisms of BAT-25 and BAT-26 in African patients,[39] low rates of tumor cells (<30%),[40] inactive mutant proteins which remain detected by IHC,[41] and inflammatory tumors with lymphocyte-rich stroma that can interfere with IHC interpretation.[42,43] In our study, the most frequent causes of 'false' discordance cases were attributed to the following: tumor sampling bias, experience of the pathologists, antibody-binding quality, and IHC using only two MMR proteins in the Saint-Antoine cohort. Heterogenous MMR protein staining has previously been thoroughly described, and is often due to lack of tissue fixation.[44-46] To diagnose dMMR CRC, full disappearance of immunostaining in the nucleus of the tumor cells should be observed with sustained labeling of normal tissue (stroma and normal mucosa). In our series, while we did not quantify cases with heterogeneous MMR protein staining, all of them were analyzed by expert pathologists aware of these patterns. It is worth noting that prior chemotherapy or radiotherapy on samples used for MMR IHC or MSI tests did not explain the discordant cases in our cohort.

After identifying these misclassified cases, 0.4% (12 cases) remained discordant between MSI and MMR IHC tests without any established explanations. Similar profiles have been described in the literature, especially isolated loss of PMS2 or MSH6 and MSS status. In our study, two dMMR/MSS cases presented isolated loss of MSH6, which could be explained by partial redundancy of MSH6 and

MSH3 protein function. When the MSH6 protein is mutated, the MSH2/MSH3 heterodimer still operates and DNA mismatch errors are partially corrected.[47] By analogy with isolated loss of MSH6, isolated loss of PMS2 ($n = 1$) can eventually be explained by a functional redundancy of the proteins PMS2 and PMS1. In addition, *MLH1*-mutated LS is known to have a more aggressive phenotype than *MSH2*/*MSH6* mutated LS, confirming the weaker impact of MSH2/MSH6 impairment on MMR function.[48] As in our study, rare MSI cases with the presence of all four proteins in IHC have been described in the literature.[20,21,24] A recent work showed that ~6% of MSI cases retained mismatch repair protein expression and that these would be missed by IHC-based testing, thereby hindering patient access to immunotherapy.[49] The majority of these cases harbor germline or somatic mismatch repair gene missense mutations; consequently inactive mutant proteins remain detected by IHC.[41]

Most discordant cases were dMMR/MSS with loss of expression of PMS2, MSH6, or MSH2, but no loss of MLH1 expression. It has been suggested that hypermethylation of the *MLH1* promoter completely inhibits *MLH1* transcription and causes complete loss of MLH1 protein expression and unambiguous MSI status.[50] Isolated loss of one MMR protein (PMS2, MSH2, or MSH6) with a weaker impact of impairment on MMR function (dMMR/MSS status), in addition to germline missense mutations (pMMR/MSI status), may explain the high percentage of discordance cases observed in LS.[51] Consequently, most discordant cases were confirmed or suspected LS (7 cases). Double somatic inactivation of one MMR gene could be the mechanism underlying MMR inactivation in suspected LS with no germline MMR mutation, as has been reported for *MSH6*.[52]

Some discordant cases may arise from the MSI test that is used. Indeed, the Pentaplex panel is largely used worldwide as it has higher sensitivity than the Bethesda panel (95% versus 85%-89%).[32] Future advances could determine MSI status using the next-generation sequencing (NGS) method with a large panel of several microsatellites to avoid false-positive and false-negative results.[53,54] Indeed, NGS permits parallel high-throughput sequencing of a high number of microsatellites and genes, and it may consequently identify MSI, tumor mutation burden as well as other targetable molecular alterations. ESMO guidelines present NGS as an alternative molecular test to assess MSI.[12] However, NGS is more expensive than PCR or IHC approaches, many hospitals have limited access to NGS, and some challenges need to be considered. First, the microsatellite markers used should be chosen carefully to obtain a pan-cancer panel with high sensitivity and specificity for MSI detection. Second, the method used should present a low limit of detection for possible application in samples with few tumor cells.[55]

Another source of discordance highlighted in our study was the use of two MMR proteins for IHC instead of four. Shia et al.[31] showed that MMR IHC testing with MSH6 and PMS2 proteins yielded similar results as compared with four MMR proteins. Nevertheless, interpretation of MSH6 and PMS2 staining is sometimes complex and rare cases with isolated loss of MSH2 expression are not detected.[13] As in our study, IHC with only the two MLH1 and MSH2 proteins is less sensitive (80%-90%) than IHC with the four MMR proteins tested (85%-95%).[30,33,56]

One current issue that remains is the use of only one of the two techniques (IHC or molecular MSI test). In our study, aside from the cost and the time required to carry out these tests, the completion of only one test would have ignored ~1% of dMMR/MSI CRCs, which could have led to a risk of underdiagnosis of LS and ineffective treatment with ICI. In stage IV CRC, it has been shown that 30%-60% of tumors with primary resistance to ICI were in fact discordant.[57]

The key strengths of our study are its multicentric nature and, to our knowledge, the largest series with both MMR IHC and MSI testing up until now. Our methodology enabled us to evaluate not only the impact of MMR IHC test with two or four MMR proteins, but also the impact of the re-reading of MMR IHC and MSI tests. It is worth noting that LS is more frequent in cohort 2 because it is a tertiary center where some young patients with a dMMR/MSI CRC (i.e. LS cases and not elderly sporadic cases) are referred to be included in trials with ICI, and it is an expert center in Paris for CRC management. Nevertheless, discordant rates in both cohorts are similar before and after re-reading. In addition, MMR IHC and MSI tests have been carried out in expert centers with possibly different results in centers with less experience. According to ESMO guidelines, both MSI and MMR IHC tests should be carried out for all mCRC, if possible.[12] MSI and MMR IHC tests should be avoided on small endoscopic biopsies except if it is mandatory for patient management. One alternative is to carry out first MMR IHC and then PCR. The gold standard remains both MMR IHC and Pentaplex, awaiting validation for MSI tests using NGS.[53,54]

To conclude, our study shows a low rate of discordance between MSI and MMR IHC tests when Pentaplex and IHC of four MMR proteins are used. An IHC panel of four antibodies should be preferred to two-antibody panels to avoid missing out dMMR cases, and both the MSI and the MMR IHC tests should be carried out. Discordant cases should be re-read to detect 'common' errors, for example, due to poor quality of tissue sampling and/or poor quality of tissue fixation and staining. If discordance remains unexplained, the tests should be repeated and analyzed by an expert team. Discordance is a major issue in the selection of patients for ICI treatment. The limits of MMR IHC and MSI tests must be known by physicians to avoid misinterpretation, which may lead to clinical management errors, especially use of ICI.

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DISCLOSURE

The authors have declared no conflicts of interest.

REFERENCES

1. Lefol C, Sohier E, Baudet C, et al. Acquired somatic MMR deficiency is a major cause of MSI tumor in patients suspected for "Lynch-like syndrome" including young patients. *Eur J Hum Genet*. 2021;29(3):482-488.
2. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology*. 2010;138(6):2073-2087.e3.
3. Levine AJ, Phipps AI, Baron JA, et al. Clinicopathologic risk factor distributions for MLH1 promoter region methylation in CIMP-positive tumors. *Cancer Epidemiol Biomarkers Prev*. 2016;25(1):68-75.
4. Bapat B, Lindor NM, Baron J, et al. The association of tumor microsatellite instability phenotype with family history of colorectal cancer. *Cancer Epidemiol Biomarkers Prev*. 2009;18(3):967-975.
5. Tougeron D, Mouillet G, Trouilloud I, et al. Efficacy of adjuvant chemotherapy in colon cancer with microsatellite instability: a large multicenter AGEO Study. *J Natl Cancer Inst*. 2016;108(7):djv438.
6. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol*. 2010;28(20):3219-3226.
7. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509-2520.
8. Overman MJ, Lonardi S, Wong KYM, et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J Clin Oncol*. 2018;36(8):773-779.
9. Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol*. 2017;18(9):1182-1191.
10. Snowsill T, Coelho H, Huxley N, et al. Molecular testing for Lynch syndrome in people with colorectal cancer: systematic reviews and economic evaluation. *Health Technol Assess*. 2017;21(51):1-238.
11. Zhang X, Li J. Era of universal testing of microsatellite instability in colorectal cancer. *World J Gastrointest Oncol*. 2013;5(2):12-19.
12. Luchini C, Bibeau F, Ligtenberg MJL, et al. ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach. *Ann Oncol*. 2019;30(8):1232-1243.
13. Pearlman R, Markow M, Knight D, et al. Two-stain immunohistochemical screening for Lynch syndrome in colorectal cancer may fail to detect mismatch repair deficiency. *Mod Pathol*. 2018;31(12):1891.
14. Suraweera N, Duval A, Reperant M, et al. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. *Gastroenterology*. 2002;123(6):1804-1811.
15. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for Cancer Detection and Familial Predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res*. 1998;58(22):5248-5257.
16. Coelho H, Jones-Hughes T, Snowsill T, et al. A systematic review of test accuracy studies evaluating molecular micro-satellite instability testing for the detection of individuals with lynch syndrome. *BMC Cancer*. 2017;17(1):836.
17. Goel A, Nagasaka T, Hamelin R, Boland CR. An optimized pentaplex PCR for detecting DNA mismatch repair-deficient colorectal cancers. *PLoS One*. 2010;5(2):e9393.
18. Xicola RM, Llor X, Pons E, et al. Performance of different microsatellite marker panels for detection of mismatch repair-deficient colorectal tumors. *J Natl Cancer Inst*. 2007;99(3):244-252.
19. Lindor NM, Burgart LJ, Leontovich O, et al. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. *J Clin Oncol*. 2002;20(4):1043-1048.
20. Watson N, Grieu F, Morris M, et al. Heterogeneous staining for mismatch repair proteins during population-based prescreening for hereditary nonpolyposis colorectal cancer. *J Mol Diagn*. 2007;9(4):472-478.
21. Chen M-L, Chen J-Y, Hu J, et al. Comparison of microsatellite status detection methods in colorectal carcinoma. *Int J Clin Exp Pathol*. 2018;11(3):1431-1438.
22. Cohen R, Hain E, Buhard O, et al. Assessment of local clinical practice for testing of mismatch repair deficiency in metastatic colorectal cancer: the need for new diagnostic guidelines prior to immunotherapy. *Ann Oncol*. 2018;29:179-180.
23. Fazzalari L. Étude Des Discordances Entre Les Analyses d'immunohistochimie et de Biologie Moléculaire à La Recherche d'une Instabilité Microsatellitaire Avec Les Analyses de Génétique Constitutionnelle Dans Le Cadre Du Syndrome de Lynch. Médecine Humaine et Pathologie. 2017. Available at: https://dumas.ccsd.cnrs.fr/dumas-01547059. Accessed April 12, 2021.
24. Jaffrelot M, Selves J, Fares N, et al. Characterization of atypical dMMR (deficient MisMatch Repair) tumors: a study from a large cohort of 4948 cases. *Ann Oncol*. 2019;30:870.
25. André T, Shiu K-K, Kim TW, et al. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer. *N Engl J Med*. 2020;383(23):2207-2218.
26. Le DT, Kim TW, Van Cutsem E, et al. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. *J Clin Oncol*. 2020;38(1):11-19.
27. Cohen R, Hain E, Buhard O, et al. Association of primary resistance to immune checkpoint inhibitors in metastatic colorectal cancer with misdiagnosis of microsatellite instability or mismatch repair deficiency status. *JAMA Oncol*. 2019;5(4):551-555.
28. Kim JH, Kim SY, Baek JY, et al. A phase II study of avelumab monotherapy in patients with mismatch repair-deficient/microsatellite instability-high or POLE-mutated metastatic or unresectable colorectal cancer. *Cancer Res Treat*. 2020;52(4):1135-1144.
29. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst*. 2004;96(4):261-268.
30. Genevay M, Benusiglio PR, Hutter P, Chappuis PO. Lynch syndrome: when pathologist and clinician have the opportunity to reduce the risk of developing cancer. *Rev Med Suisse*. 2011;7(303):1502-1506.
31. Shia J, Tang LH, Vakiani E, et al. Immunohistochemistry as first-line screening for detecting colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome: a 2-antibody panel may be as predictive as a 4-antibody panel. *Am J Surg Pathol*. 2009;33(11):1639-1645.
32. Buhard O, Suraweera N, Lectard A, et al. Quasimonomorphic mononucleotide repeats for high-level microsatellite instability analysis. *Dis Markers*. 2004;20:251-257.
33. Hampel H, Frankel W, Panescu J, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. *Cancer Res*. 2006;66(15):7810-7817.

34. Faulkner RD, Seedhouse CH, Das-Gupta EP, et al. BAT-25 and BAT-26, two mononucleotide microsatellites, are not sensitive markers of microsatellite instability in acute myeloid leukaemia. *Br J Haematol*. 2004;124(2):160-165.
35. Tachon G, Frouin E, Karayan-Tapon L, et al. Heterogeneity of mismatch repair defect in colorectal cancer and its implications in clinical practice. *Eur J Cancer*. 2018;95:112-116.
36. Chapusot C, Martin L, Bouvier AM, et al. Microsatellite instability and intratumoural heterogeneity in 100 right-sided sporadic colon carcinomas. *Br J Cancer*. 2002;87(4):400.
37. Tests somatiques recherchant une déficience du système MMR au sein des tumeurs du spectre du syndrome de Lynch. INCa 2016. Available at: https://www.e-cancer.fr/Expertises-et-publications/Catalogue-des-publications/Tests-somatiques-recherchant-une-deficience-du-systeme-MMR-au-sein-des-tumeurs-du-spectre-du-syndrome-de-Lynch. Accessed April 12, 2020.
38. Overbeek LIH, Ligtenberg MJL, Willems RW, et al. Interpretation of immunohistochemistry for mismatch repair proteins is only reliable in a specialized setting. *Am J Surg Pathol*. 2008;32(8):1246-1251.
39. Pyatt R, Chadwick RB, Johnson CK, et al. Polymorphic variation at the BAT-25 and BAT-26 loci in individuals of African origin. Implications for microsatellite instability testing. *Am J Pathol*. 1999;155(2):349-353.
40. Wang Y, Shi C, Eisenberg R, Vnencak-Jones CL. Differences in microsatellite instability profiles between endometrioid and colorectal cancers. *J Mol Diagn*. 2017;19(1):57-64.
41. Peltomäki P, Vasen H. Mutations associated with HNPCC predisposition — update of ICG-HNPCC/INSiGHT mutation database. *Dis Markers*. 2004;20(4-5):269-276.
42. Kim H, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *Am J Pathol*. 1994;145(1):148-156.
43. Tougeron D, Fauquembergue E, Rouquette A, et al. Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. *Mod Pathol*. 2009;22(9):1186-1195.
44. 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(4):293-300.
45. Chen W, Frankel WL. A practical guide to biomarkers for the evaluation of colorectal cancer. *Mod Pathol*. 2019;32(1):1-15.
46. Klarskov L, Ladelund S, Holck S, et al. Interobserver variability in the evaluation of mismatch repair protein immunostaining. *Hum Pathol*. 2010;41(10):1387-1396.
47. 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(4):301-307.
48. Dominguez-Valentin M, Sampson JR, Seppälä TT, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. *Genet Med*. 2020;22(1):15-25.
49. Hechtman JF, Rana S, Middha S, et al. Retained mismatch repair protein expression occurs in approximately 6% of microsatellite instability-high cancers and is associated with missense mutations in mismatch repair genes. *Mod Pathol*. 2020;33(5):871-879.
50. Veigl ML, Kasturi L, Olechnowicz J, et al. Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. *Proc Natl Acad Sci U S A*. 1998;95(15):8698-8702.
51. Liu W, Zhang D, Tan SA, Liu X, et al. Sigmoid colon adenocarcinoma with isolated loss of PMS2 presenting in a patient with synchronous prostate cancer with intact MMR: diagnosis and analysis of the family pedigree. *Anticancer Res*. 2018;38(8):4847-4852.
52. 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(4):301-307.
53. Salipante SJ, Scroggins SM, Hampel HL, et al. Microsatellite instability detection by next generation sequencing. *Clin Chem*. 2014;60(9):1192-1199.
54. Hempelmann JA, Scroggins SM, Pritchard CC, et al. MSIplus for integrated colorectal cancer molecular testing by next-generation sequencing. *J Mol Diagn*. 2015;17(6):705-714.
55. Baudrin LG, Deleuze J-F, How-Kit A. Molecular and computational methods for the detection of microsatellite instability in cancer. *Front Oncol*. 2018;8:621.
56. Svrcek M, Lascols O, Cohen R, et al. MSI/MMR-deficient tumor diagnosis: which standard for screening and for diagnosis? Diagnostic modalities for the colon and other sites: differences between tumors. *Bull Cancer*. 2019;106(2):119-128.
57. Loupakis F, Depetris I, Biason P, et al. Prediction of benefit from checkpoint inhibitors in mismatch repair deficient metastatic colorectal cancer: role of tumor infiltrating lymphocytes. *Oncologist*. 2020;25(6):481.