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Point/Counterpoint

Immunohistochemistry versus Microsatellite Instability Testing For Screening Colorectal Cancer Patients at Risk For Hereditary Nonpolyposis Colorectal Cancer Syndrome

Part I. The Utility of Immunohistochemistry

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The utility of immunohistochemical detection of DNA mismatch repair (MMR) protein in screening colorectal tumors for hereditary nonpolyposis colorectal cancer (HNPCC) syndrome has been the focus of much intensive research over the last 10 years. Particular attention has been given to the relative usefulness of immunohistochemistry (IHC) versus testing of tumor microsatellite instability (MSI). Earlier work that focused on mutL homolog 1 (MLH1) and mutS homolog 2 (MSH2) has created a false impression that IHC has a lower sensitivity than MSI testing in predicting germline mutation. More recent studies that included postmeiotic segregation increased 2 (PMS2) and MSH6, on the other hand, have demonstrated an IHC predictive value that is virtually equivalent to that of MSI testing. Such added value of PMS2 and MSH6 can be explained by the biological and biochemical properties of the MMR proteins. On the premise that IHC with PMS2 and MSH6 is as sensitive as MSI testing, given that IHC is easily available and generally inexpensive and, importantly, identifies the affected gene, it is reasonable to regard IHC as a more optimal first-line screening tool than MSI testing for identifying HNPCC. MSI testing can provide a fallback position in equivocal situations, while remaining an important research tool. However, for IHC to be used as a first-line screening test requires that both pathologists and clinicians be aware that IHC results may be construed as "genetic information," and that appropriate procedures should be established to ensure patient understanding and consent. ***(J Mol Diagn 2008, 10:293–300; DOI: 10.2353/jmoldx.2008.080031)***

Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome—a condition originally characterized as familial clustering of colorectal and other types of cancer,[1,2] is now molecularly defined as a cancer-predisposing syndrome secondary to a deleterious germline mutation in one of a set of DNA mismatch repair (MMR) genes, namely, mutL homolog 1 (*MLH1*), mutS homologs 2 and 6 (*MSH2*, *MSH6*), and postmeiotic segregation increased 2 (*PMS2*).[3] In this condition, affected persons carry one mutated copy of the gene in all their tissues, and a somatic mutation or loss of the second normal allele in colorectal or other epithelium inactivates the gene and impairs mismatch repair function, resulting in neoplasia. As such, detection of the deleterious germline mutation has evolved into the ultimate diagnostic criterion for HNPCC.

Screening for mutation, however, is both time-consuming and expensive. This is largely because of the heterogeneity of the mutation spectrum of the MMR genes. Such difficulties make preselection of high-risk patients necessary. Consequently, there have been tremendous research efforts over the last 10 years that aim at designing the most efficient workup algorithm.[3–5] Among the commonly studied screening tools, ie, family history, tumor pathological characteristics, tumor DNA microsatellite instability (MSI), and tumor MMR protein detection by immunohistochemistry (IHC), the relative usefulness of

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Table 1. Literature Data on Sensitivity of MLH1- and MSH2-IHC versus MSI Testing in Predicting Germline Mutation in *MLH1* and *MSH2* in Colorectal Carcinoma

	Abnormal IHC or MSI-H/pathogenic mutation					
	IHC			MSI		
References	Overall	*MLH1*	*MSH2*	Overall	*MLH1*	*MSH2*
Marcus et al 1999[17]	16/16	5/5	11/11	16/16	5/5	11/11
Debniak et al 2000[18]	4/6	2/4	2/2	5/6	3/4	2/2
Dieumegard et al 2000[19]	5/7	3/4	2/3	8/8	5/5	3/3
Cunningham et al 2001[20]	5/5	2/2	3/3	5/5	2/2	3/3
Salahshor et al 2001[12]	22/28	14/20	8/8	27/30	19/22	8/8
Stone et al 2001[21]	4/4	4/4	—	4/4	4/4	—
Terdiman et al 2001[22]	11/13	6/7	5/6	13/13	7/7	6/6
Furukawa et al 2002[23]	8/8	3/3	5/5	—	—	—
Wahlberg et al 2002[24]	4/9	1/5	3/4	14/14	8/8	6/6
Hendriks et al 2003[25]	29/33	18/21	11/12	19/21	13/15	6/6
Hoedema et al 2003[26]	4/4	—	4/4	3/4	—	3/4
Mangold et al 2005[27]	67/82	29/44	38/38	—	—	—
Pinol et al 2005[28]	11/11*	3/4	6/7	10/11	4/4	6/7
Shia et al 2005[29]	25/32	4/9	21/23	27/28	6/7	21/21
Barnetson et al 2006[30]	21/23	12/12	9/11	19/24	10/12	9/12
Niessen et al 2006[31]	15/16	7/8	8/8	17/17	8/8	9/9
Total	251/297 (85%)	113/152 (74%)	136/145 (94%)	187/201 (93%)	94/103 (91%)	93/98 (95%)

*One case: MLH1 present/MSH2 lost but with mutation on MLH1; one case: MLH1 lost/MSH2 present but with mutation on MSH2.

MSI testing versus IHC has been the topic of much intensive investigation.

Historically, DNA MSI, a molecular manifestation of tumors bearing DNA MMR deficiency, was the phenotype that led to the discovery of human MMR genes and their causative role in HNPCC.[6–9] Such a breakthrough occurred in 1993; testing of MSI has since remained a prominent methodology in HNPCC-related research and clinical work.

Around 1996, monoclonal antibodies against MMR proteins started to become available; first came antibodies to MSH2[10,11] and then to others. Such antibodies rendered IHC detection of MMR protein possible, providing an alterative methodology for detecting MMR deficiency.

Paralleling the recognition of the greater impact of germline mutations in *MLH1* and *MSH2* in HNPCC, much research work on the utility of IHC focused on the products of these two genes. Such work, unfortunately, has accentuated the limitations of MLH1 IHC in predicting *MLH1* mutation,[12] therefore creating a false impression[13] that IHC was inferior to MSI testing. Other, mostly more recent work realized a significant compensatory effect of PMS2 antibody on the detection of *MLH1* mutation,[14] and as such, demonstrated an apparent improvement of the performance of IHC by adding PMS2 to the antibody panel.[4,15,16] In this article, we summarize both the early and more recent literature data on the use of IHC, discuss the biological and biochemical basis of how IHC works in MMR-deficient tumors, and outline the advantages and limitations of this methodology. Our analysis indicates that IHC is in fact a more optimal first-line screening tool than MSI testing for identifying HNPCC.

Literature Review

In this review, pertinent literature reports that analyzed the effectiveness of IHC versus MSI in predicting germline mutation in colorectal tumors are divided into two groups according to the IHC antibodies used. The first group includes those that primarily assessed MLH1 and MSH2 (with or without MSH6),[12,17–31] and the second group, those that assessed all four proteins (MLH1, MSH2, MSH6, and PMS2).[4,14,15]

Results from the most pertinent studies belonging to the first group are summarized in Table 1. After weighing for sample size, IHC with MLH1/MSH2 antibodies had a sensitivity of 85% in predicting germline mutation in *MLH1/MSH2*. This was apparently lower than the 93% sensitivity of MSI testing. It became clear on further analysis that this low sensitivity of IHC was largely caused by a low rate of *MLH1* mutation detection by MLH1 IHC (74%).

Results from the most pertinent studies belonging to the second group are summarized in Table 2. As shown here, with the use of all four antibodies, the sensitivity of IHC in predicting *MLH1* mutation increased to 92%, which was equivalent to that of MSI testing. Such an improvement was largely the result of the ability of PMS2 IHC to predict *MLH1* mutation (mechanisms to be explained below). This is further supported by a study by de Jong et al[14] (not included in Table 2 because of lack of correlative data on other genes) in which PMS2 IHC detected 23% (8/35) of *MLH1* mutated tumors that were missed by MLH1 IHC. Table 2 also shows that MSH6 IHC predicted *MSH6* mutation in 8 of 8 tumors; in contrast, high frequency MSI was seen in only 2 of the 8 cases.

In summary, literature review suggests that IHC with MLH1/MSH2 has a lower sensitivity than MSI testing in predicting gene mutation; however, inclusion of PMS2 and MSH6 significantly increases the sensitivity of IHC, resulting in a predictive value that is virtually equivalent to that of MSI testing.

With regard to the specificity of IHC in predicting germline mutation, data are more limited as the analysis re-

Table 2. Literature Data on Sensitivity of MLH1-, MSH2-, MSH6-, and PMS2-IHC versus MSI Testing in Predicting Germline Mutation in *MLH1*, *MSH2, MSH6,* and *PMS2* in Colorectal Carcinoma

	Abnormal IHC or MSI-H/pathogenic mutation									
	IHC					MSI				
Reference	Overall	*MLH1*	*MSH2*	*MSH6*	*PMS2*	Overall	*MLH1*	*MSH2*	*MSH6*	*PMS2*
Hampel et al 2005[4]	21/23	4/5	12/13	3/3	2/2	21/23	5/5	12/13	2/3	2/2
Southey et al 2005[15]	18/18	9/9	4/4	4/4	1/1	13/18	9/9	4/4	0/4*	0/1
Lagerstedt Robinson et al 2007[16]	23/25	10/11	12/13	1/1	0	21/25	9/11	12/13	0/1	0
Total	62/66 (94%)	23/25 (92%)	28/30 (93%)	8/8 (100%)	3/3 (100%)	55/66 (83%)	23/25 (92%)	28/30 (93%)	2/8 (25%)	2/3 (67%)

*All 4 are MSI-L (Ten microsatellite markers assessed: 3 dinucleotide repeats - D5S346, D17S250, and 2S123, and 7 mononucleotide repeats - BAT-25, BAT-26, BAT-40, MYB, TGFßRII, IGFIIR, and BAX. In this study, the degree of instability was scored as stable, low, and high when 0 to 1, 2 to 5, and 6 to 10 markers were identified as unstable, respectively).

quires that large numbers of cases (including clinically unsuspected cases) be tested for mutation so as to achieve a meaningful group of mutation-negative cases. Many studies reported IHC data in mutation-positive cases only. In general, however, the specificity is believed to be high; one study[29] reported 95% specificity for both MLH1 and MSH2 IHC.

It is to be noted that whereas both MSI and the four-antibody IHC have similar value in predicting germline mutation, the concordance rate between these two tests is less than perfect; the sensitivity of IHC in predicting MSI is about 92%.[32] This is because both tests may miss cases that are detectable by the other. Specifically, MSI may detect cases that have abnormalities in MMR genes that are not covered by the IHC antibody panel and therefore not detectable by IHC; on the other hand, as shown in Table 2, IHC can detect *MSH6* mutation cases that may not show high frequency MSI and therefore can be missed by MSI testing.

The Biological and Biochemical Basis of How IHC Works in MMR-Deficient Tumors

Not All Pathogenic Mutations Result in Loss of Protein by IHC

In general, IHC is reliable in screening for mutations that result in truncation or degradation of the protein. IHC, however, cannot distinguish between mutant proteins commonly resulting from missense mutations and wild-type polypeptides.

Most mutations in *MSH2* are protein truncating; consequently, most *MSH2*-mutant colorectal tumors are expected to show absent MSH2 expression by IHC.[27] However, more than one-third of the mutations in *MLH1* are missense mutations that may result in mutant proteins that are catalytically inactive but antigenically intact.[12,33] Thus, on IHC, these mutant proteins may result in a false-normal staining pattern. Moreover, false-normal staining for MLH1 can occur even with protein-truncating mutations and large in-frame deletions in *MLH1*,[12,24,27,34] the mechanism for which is unclear.

Yet another element that could potentially result in a false-normal staining relates to the second hit that inactivates the second normal allele. This second hit might result in a nonfunctional but antibody-binding MLH1 protein detectable by IHC. Such a possibility exists as various individuals with an identical germline mutation in MLH1 have been shown to exhibit different MLH1 IHC staining patterns.[27]

Mutation of MLH1 or MSH2 Will Result in Concurrent Loss of MLH1/PMS2 *or* MSH2/MSH6*, Respectively, by IHC, Whereas Mutation of PSM2 or MSH6 Will Result in Isolated Loss of* PMS2 *or* MSH6 *Only*

In their functional state, the MMR proteins form heterodimers.[35–38] MSH2 dimerizes with MSH6, forming the functional complex, MutSα[38]; and MLH1 dimerizes with PMS2, forming MutLα.[37,39] It has been shown that the MSH2 and MLH1 proteins are the obligatory partner of their respective heterodimer.[14,40–42] Their abnormalities can result in proteolytic degradation of their dimer and consequent loss of both the obligatory and secondary partner proteins (exceptions include some *MLH1* mutations: when the mutation results in an antigenically active mutant MLH1 protein as discussed above, there may be loss of PMS2 only). The reverse, however, is not true. When mutation occurs in genes of the secondary proteins, ie, *MSH6* and *PMS2*, there may not be concurrent loss of the obligatory proteins, MSH2 and MLH1. This is because the function of the secondary proteins may be compensated by other proteins, such as MSH3, MLH3, and PMS1. As a result, mutations of *MLH1* or *MSH2* often cause concurrent loss of MLH1/PMS2 or MSH2/MSH6, respectively, by IHC, whereas mutations of *PSM2* or *MSH6* often cause isolated loss of PMS2 or MSH6 only.

A curious phenomenon worth noting here is that mononucleotide repeats of 7 or more elements (ie, A7, C8, etc) exist in the coding sequence of some MMR genes, namely *MSH2*, *MSH6*, and *PMS2*.[43,44] Theoretically, such encoded microsatellites can undergo secondary mutation in HNPCC and result in a pattern of protein loss that deviates from the common situations described above. For example, concurrent loss of MLH1 and MSH6 may occur because of secondary loss of MSH6 in an *MLH1*

mutation case. Such aberrant patterns (with complete loss of a second, "unexpected" protein), however, are extremely rare.[45] This is so, probably because secondary mutation does not occur during carcinogenesis, but rather, only in subclones of an established MSI cancer. It follows that such a mechanism may explain partial loss of IHC staining in some tumors (see section on IHC staining pattern below).

Summary

In summary, IHC with antibodies to only MLH1/MSH2 is not able to detect all *MLH1* or *MSH2* abnormalities, as certain pathogenic mutations may be associated with retained protein expression. However, PMS2 and MSH6 antibodies have the capability of detecting most abnormalities in *MLH1* and *MSH2*, in addition to detecting mutations in the genes that encode themselves, ie, *PSM2* and *MSH6* (notably, mutations in *MSH6* have been reported to account for up to 13% of families with MMR gene mutations[46]). Thus, it becomes easy to understand why the studies that used MLH1/MSH2 IHC yielded a lower predictive value than those that included PMS2 and MSH6 (as shown above, under Literature Review). Parenthetically, the secondary nature of MSH6 protein in MutS complexes, ie, the functional redundancy between MSH6 and MSH3, may explain, at least in part, why some *MSH6* mutations do not result in high frequency MSI in the tumor.

Advantages of IHC

IHC Is Easily Available; Testing at the Time of Colectomy Is Efficient

As IHC is available as part of the routine service in the general pathology laboratories, it constitutes a convenient technique to general pathologists. This is important because pathologists are at the forefront in recognizing HNPCC. The lack of a premorbid clinical phenotype (hence the difficulty to identify it clinically), coupled with the presence of specific histological patterns in its cancers, renders HNPCC a disease that is often first recognized by the pathologists. Indeed, the revised Bethesda Guidelines have defined an important role for pathologists.[3] For colorectal cancer patients age 50 to 60 years, the Guidelines recommend that further testing be done when the tumor morphology is suggestive as determined by the pathologist. Currently, the Guidelines are defined to select tumors for "MSI testing." With today's knowledge about IHC, it is foreseeable that the next version will state that the Guidelines are to select tumors for "either IHC or MSI testing." In that scenario (IHC as the standard first-line screening tool), an at-risk colorectal cancer patient will be able to obtain the information about the MMR proteins from the routine pathology report at the time of colectomy. When the patient reaches the Clinical Genetics Service, the next test would be germline mutation on the gene indicated by IHC. There is no longer any need to retrospectively retrieve the paraffin tumor material. Such an approach should be applicable to the majority of at-risk patients, restricting the need for MSI testing to only the rare cases in which IHC is not informative and yet there is a clinical suspicion.

IHC Is in General Regarded As an Inexpensive Technique

The cost of IHC and MSI testing may vary among different countries and health care systems. Earlier analysis indicated that IHC was about threefold less expensive than MSI testing.[18] Such estimation, however, may no longer be accurate today as newer techniques are being used. Indeed, the cost of MSI can be reduced by new methods such as fluorescent multiplex PCR-capillary electrophoresis. The cost of IHC may potentially be further reduced as well. We have recently proposed a new two-antibody panel (composed of PMS2 and MSH6) to replace the current four-antibody panel for IHC use as a first-line screening tool.[45] Such a proposal is based on the biochemical properties of MMR proteins as described above, and data from the literature and our own work indicating that PMS2 and MSH6 have the ability to predict mutations on all four MMR genes. According to this proposal, in cases in which abnormality is detected by the two-antibody panel, a second-step IHC using additional antibodies (MLH1 in the case of PMS2 abnormality and MSH2 in the case of MSH6 abnormality) may be performed to pinpoint further the gene for mutation testing. (It is expected that the majority of cases picked up by PMS2 will more likely have a mutation in *MLH1* than in *PMS2*, and the majority of cases picked up by MSH6 will more likely have a mutation in *MSH2*.) In cases where no abnormality is detected by PMS2 or MSH6, no further IHC testing would be necessary. This implies that the majority of colorectal cancer patients who fulfill the revised Bethesda Guidelines but do not harbor MMR deficiency (estimated to be about 70%)[28] could have been spared half the IHC work and yet still have the ability to achieve the same predictive value from this test.

IHC Helps Identify the Mutated Gene

IHC reveals which particular MMR gene may be defective, and as such it enables efficient mutation analysis on the target gene. Such ability is not possessed by MSI testing. The ability of IHC to identify the mutated gene also encourages the use of alternative procedures in cases in which standard methods fail. For example, by applying the multiplex ligation-dependent probe amplification method to tumors that showed abnormal PMS2 IHC, Halvarsson et al[47] identified a deletion of exons 14 to 19 of *MLH1* that was missed by conventional sequencing.

IHC May Detect MMR-Deficient Cases That Can Potentially Be Missed by MSI Testing

As noted by our literature review (Table 2), mutations in *MSH6* tend to result in weaker or no MSI in the tumors, a

phenomenon also well demonstrated both by cell line studies[48,49] and by studies with *MSH6*-mutant mice.[50] Such *MSH6* cases may be missed by MSI testing but can be detectable by MSH6 IHC.

Limitations of IHC

IHC with MLH1 *Antibody Alone Has a Low Sensitivity in Detecting Mutation of MLH1*

Early IHC work noted a low sensitivity of MLH1 IHC, and this was regarded as a major drawback of IHC. However, as demonstrated in the more recent studies and discussed above, such a drawback is largely overcome by the addition of PMS2 to the IHC antibody panel.

IHC Staining Pattern May Vary, Resulting in Uncertainty in Interpretation

A limitation that has drawn much attention is the IHC staining quality.[32] Three major patterns have caused much confusion: i) focal staining (with or without weakened intensity); ii) lack of positive internal control, mostly in negatively stained tumors; and iii) cytoplasmic staining. These patterns are also referred to as "weak staining," "heterogeneous staining," or "clonal pattern" in the literature.

Focal staining, often with weak intensity, with or without positive internal control in the negatively stained regions, is a pattern most commonly seen with MLH1. This pattern has been suggested to reflect certain types of gene mutation.[32] Careful analyses, however, fail to support such an association, as its occurrence spans the mutation spectrum[27] and can be seen in mutation-negative cases as well.[29] Thus, alternative mechanisms have been suggested and include tumor microenvironment and tissue preservation. It is interesting to note here that tissue hypoxia and oxidative stress have been demonstrated to impair MMR function in genetically MMR-proficient tissues.[51,52] As such, regional hypoxia in a tumor could potentially be a reason for regional staining to be lost or weak. Better tissue preservation could potentially reduce such effects of tissue microenvironment and improve the performance of IHC.

Focal staining with unimpaired intensity is a pattern that seems to be more frequently seen with MSH6. In such cases, the areas of tumor cell staining can be very focal, sometimes, constituting only $<10\%$ of the tumor. Positive internal control is often present throughout the tumor. The mechanism for this phenomenon is unclear. Anecdotally, we have encountered such extremely focal staining for MSH6 in MLH1-deficient tumors (cases initially misinterpreted as concurrent loss of MLH1/MSH6). Such experience prompted us to speculate that secondary mutation in *MSH6* in subclonal populations of an MSI tumor might be a potential explanation (as discussed above, under The Biological and Biochemical Basis of How IHC Works in MMR-Deficient Tumors).

The second staining pattern of concern, lack of convincing positive internal control in an otherwise IHC-negative (protein lost) case, is again not well understood from a mechanistic point of view. Such cases had been regarded as "staining un-interpretable" and therefore excluded from analysis in some early IHC studies.[29,32] It has been our experience that, on careful examination or repeated staining in such cases, at least some positive staining can be revealed in the background benign cells, whereas the tumor cells remain negative. Therefore, these cases are probably best regarded as IHC-abnormal before more definitive data become available. Recently, we have encountered a case of an endometrial carcinoma in which the stain for MSH6 demonstrated this very staining variation and the patient was found to have a pathogenic germline mutation on *MSH6*. The MSI testing in this case did not show instability in any of the five standardized markers.

The third staining variation, cytoplasmic staining, bears no known significance. There are no data as yet to indicate that its presence is reflective of protein deficiency.

Although these various staining patterns exist, with experience, an accurate interpretation of IHC staining is still easily achievable, especially in colorectal tumor specimens. In general, the presence of nuclear staining in the tumor cells, even when it is focal and weak, is good evidence of retained MMR protein. In the rare situation where there is lack of positive internal control in an otherwise IHC-negatively stained tumor, we recommend repeating the stain in search for positive stromal cells. If such repeated attempts still fail to reveal positive stromal cells (a scenario rare in colorectal cancer cases in our experience), as long as the tumor cells remain negative, this tumor is best regarded as IHC abnormal or inconclusive.

IHC May Not Be Reliable in Small Biopsy Samples

Given that IHC staining for MMR protein can be focal, its performance may be affected by tissue sampling. Specifically, lack of staining in a small biopsy sample may not be reliably interpreted as loss of protein in the entire tumor, thus limiting the utility of IHC in biopsy specimens. Specific data to demonstrate such an effect of sampling in biopsy specimens, however, are scare in the literature. Work in this area is ongoing at our institution.

IHC Results May Be Interpreted As "Genetic Information," Which Can Complicate the Use of IHC as a First-Line Screening Tool

Although loss of MLH1 is a frequent occurrence in sporadic MSI colorectal cancers, loss of the other MMR proteins most often indicates an underlying germline defect. Therefore, although no germline testing has been performed, genetic information can be gleaned from IHC testing (that is not available from MSI testing). As it is the

general perception that reporting of genetic information requires patient consent, IHC testing can therefore be construed as medicolegally relevant if it is to be initiated by pathologists who do not have the ability to directly obtain patient consent. Such an issue deserves our attention, and may be resolved by good communication between the pathologists and surgeons or clinicians. An agreement needs to be reached whereby the surgeons and clinicians will discuss IHC tests with their patients who fall in a defined high-risk category for which IHC will be routinely performed and obtain patient consent before the pathology material reaches the pathology department. Under such an agreement, the pathologists can then automatically perform the IHC in predefined patient populations.

The Sensitivity of IHC Is Dependent on Its Antibody Panel

Although the genes covered by the four IHC antibodies are believed to account for the majority of hereditary MMR-deficient cases, other putative MMR genes exist that could potentially be pathogenic as well. Unless new antibodies become available, IHC will not be able to detect such other genes. In contrast, MSI testing has the potential to predict pathogenic abnormalities in any MMR gene (although exceptions are already known to exist, such as the *MSH6* mutation cases as discussed above). Vasen et al[53] noted that, as long as the role of other putative MMR genes has not been elucidated, IHC analysis cannot completely replace MSI. This is true particularly from a research and investigational point of view.

Summary

In summary, limitations to IHC do exist. Most limitations, however, can be remedied by an appropriate IHC antibody panel, optimized laboratory procedures, and knowledge and experience in MMR IHC interpretation. The inability of IHC to reliably detect MMR abnormality in biopsy specimens, should it be proven by further data, however, would represent a true drawback, as diagnosis at the time of biopsy could significantly enhance the management decision-making (eg, segmental resection versus subtotal colectomy). (In this scenario, however, MSI testing is not superior to IHC; the presence of MSI in a small biopsy would be equally, or even more, unreliable.) In addition, it is to be recognized that on the one hand, IHC has the ability to pinpoint the specific gene and therefore it may be construed as a "genetic test" that carries medicolegal implications; on the other hand, IHC's ability is dependent on its antibody panel, and it may miss cases in which the fundamental defect resides in other untested genes.

Conclusion And Perspectives

Nearly 100 years after a pathologist, Warthin, first recognized the clinical condition of what is now known as HNPCC,[2] we are finally able to pinpoint the specific germline mutation in at least a significant proportion of these patients and families. Of the two techniques—MSI testing and IHC (with antibodies including PMS2 and MSH6)—that are commonly evaluated as screening tools for identification of HNPCC, our literature review demonstrates a virtually equivalent informative value in predicting germline mutation (IHC may even be superior in the case of *MSH6* mutation). Under such a premise, given that IHC is more easily available and in general inexpensive, that it also detects the mutated gene, and that its limitations can be largely overcome by the pathologists' experience, it is our view that IHC should be placed as the first choice for first-line screening to identify patients for genetic testing. However, such a strategy requires that both the pathologists and clinicians are cognizant of the fact that genetic information can be gleaned from IHC testing, and that appropriate procedures need to be established to ensure patients' understanding and consent. Meanwhile, MSI testing is not to be abandoned by any means, as it can provide a fallback position in situations where IHC is normal and yet clinical suspicion exists, or IHC is inconclusive. MSI testing will also remain a significant research tool for discovering new MMR gene abnormalities.

Notably, amid all constructive debate on the utility of IHC versus MSI testing, there is a constant improvement of laboratory techniques and emergence of new knowledge and new methods. For example, tests on MLH1 promoter methylation and B-raf mutation have been shown to be useful in distinguishing sporadic MSI cases from HNPCC. It is hopeful that in the future, tests such as IHC and MSI (or even mutation testing) will become equally easily accessible and affordable; and detection of HNPCC cancers and mutation carriers will be as simple as diagnosing any other routine pathological entity.

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