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Effective Identification of Lynch Syndrome in Gastroenterology Practice

Charles Muller, MD¹, Lindsay Matthews, MD², Sonia S. Kupfer, MD¹, Jennifer M. Weiss, MD, MS^{2,3,*}

¹Section of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Chicago, Chicago, IL, USA

²Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

³Division of Gastroenterology and Hepatology, Department of Medicine, University of Wisconsin School of Medicine and Public Health, 1685 Highland Ave, Room 4200, Madison, WI, 53705, USA

Abstract

Purpose of review: Identification of Lynch syndrome is important from an individual patient and public health standpoint. As paradigms for Lynch syndrome diagnosis have shifted in recent years, this review will discuss rationale and limitations for current strategies as well as provide an overview of future directions in the field.

Recent findings: In recent years, the use of clinical criteria and risk scores for identification of Lynch syndrome have been augmented by universal testing of all newly diagnosed colorectal cancers with molecular methods to screen for mismatch repair deficiency with high sensitivity and specificity. Studies of implementation and outcomes of universal testing in clinical practice have demonstrated significant heterogeneity that results in suboptimal uptake and contributes to disparities in diagnosis. Emerging technologies, such as next-generation sequencing, hold significant promise as a screening strategy for Lynch syndrome.

Summary: Universal testing for Lynch syndrome is being performed with increasing frequency, although real-world outcomes have demonstrated room for improvement. Future directions in Lynch syndrome diagnosis will involve optimization of universal testing workflow and application of new genetics technologies.

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*Correspondence jmw@medicine.wisc.edu.

Conflict of Interest

Charles Muller and Lindsay Matthews declares no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Keywords

Lynch syndrome; Amsterdam Criteria; Bethesda Guidelines; PREMM Model; Universal Tumor Screening

Introduction

Lynch syndrome is the most common cause of hereditary colorectal cancer (CRC), accounting for between 3–5% of all CRC cases [1]. Lynch syndrome results from a germline mutation in one of the four mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, or *PMS2*) or in the *EPCAM* gene that is located upstream of *MSH2*. Such deleterious mutations lead to accumulation of replication errors (insertions and deletions) within repetitive DNA sequences, known as microsatellite instability (MSI), and a predisposition to development of malignancy [2, 3].

In addition to having high rates of CRC with earlier age of onset, patients with Lynch syndrome are also at increased risk for endometrial, ovarian, gastric, small bowel, urothelial, pancreaticobiliary, brain, and sebaceous skin cancers [4, 5]. Identification of individuals with Lynch syndrome is crucial to enable implementation of life-saving cancer screening and risk-modification strategies in both affected individuals and their at-risk family members [6–10]. Endoscopic surveillance of Lynch syndrome patients, for example, has been shown to reduce CRC-related mortality by up to 71% [11]. Underscoring the importance of Lynch syndrome diagnosis, the US Department of Health and Human Services included improving identification of individuals with Lynch Syndrome as one of its two genomics goals for the Healthy People 2020 initiative [12].

In this review, we will provide an overview of traditional clinical methods of Lynch syndrome diagnosis and their performance characteristics. We will also discuss the rationale for universal tumor testing as a cost-effective strategy with improved sensitivity and specificity compared to clinical methods. We will review the successes and limitations to implementation of universal testing in clinical practice and its associated outcomes and provide a brief overview of novel genetic tools, including next-generation sequencing, and their role in Lynch syndrome diagnosis.

Clinical criteria for diagnosing Lynch syndrome: Amsterdam Criteria and Bethesda Guidelines

The original Amsterdam Criteria were published in 1991 by the International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (HNPCC) [13]. The purpose was to develop a minimum set of objective clinical criteria to aid in research on Lynch syndrome and were later adopted in clinical practice. All of these criteria, commonly referred to as the “3–2–1 rule”, must be present for the diagnosis: (1) at least *three* relatives should have histologically verified CRC; one should be a first-degree relative to the other two, (2) spanning at least *two* successive generations, and (3) *one* of the CRCs should be diagnosed before 50 years of age [13]. In addition, a diagnosis of Familial Adenomatous Polyposis must be excluded. This initial set of criteria were criticized for focusing only on CRC and

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were expanded in 1999, known as the Amsterdam II Criteria, to include extra-colonic malignancies associated with Lynch syndrome (endometrial, small bowel, ureter and renal pelvis) [14]. The purpose of the expanded criteria was to identify Lynch syndrome families that do not present with the colon-only Amsterdam I Criteria, such as families that include predominantly endometrial cancers due to an *MSH6* mutation. Originally developed for research studies with a focus on specificity over sensitivity, performance of the Amsterdam II Criteria for clinical diagnosis is suboptimal with a sensitivity of 22% (range 13–67%) and a specificity of 98% (range 97–100%) [8•, 15–19]. In the clinic, high specificity of Amsterdam criteria means that a patient who meets these criteria is likely to have Lynch syndrome and should be referred for genetic testing. However, the low sensitivity of these criteria means that many individuals with Lynch syndrome will be missed and that these criteria should not be used as a screening test. Despite these limitations in performance characteristics, in our experience, insurance companies continue to use Amsterdam II Criteria as guidelines to approve germline genetic testing and therefore might deny coverage to individuals with Lynch syndrome who would benefit from testing.

As a means to improve sensitivity for identification of Lynch syndrome, the Bethesda Guidelines (1996) [20] and Revised Bethesda Guidelines (2004) [21] were developed which combine clinical and pathologic information to help identify individuals who should have further tumor testing for microsatellite instability (MSI), a hallmark of Lynch syndrome (Table 1) [8•, 22, 23]. Approximately 90% of Lynch syndrome-associated CRCs are MSI-high, supporting use of the Bethesda Guidelines as a potentially effective screening tool to identify individuals who should be referred for germline genetic testing [8•, 17, 24–26]. In comparison to the Amsterdam Criteria, the Bethesda Guidelines have a higher sensitivity in multiple studies ranging from 94–96%, but less specificity (25%–27%) [21, 27, 28]. Additionally, in the study by Syngal *et al.* [28] although individuals who met the Bethesda Guidelines were more likely to be referred for genetic testing, the majority did not complete testing indicating that family history is still underused in clinical practice [21]. At this point, the Bethesda guidelines are becoming largely irrelevant due to the implementation of universal tumor testing which is discussed later in the review.

Family history screening tools

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Family history is a critical component of cancer risk assessment and is incorporated into the Amsterdam Criteria and Bethesda Guidelines; however, both of these clinical criteria are inconvenient for daily practice and have suboptimal test characteristics. In addition, completeness and accuracy of physician-collected family histories are often lacking when compared to self-administered family cancer history questionnaires [29], and some patients lack knowledge of family history [30]. In order to improve test characteristics compared to older criteria, multiple family history screening tools have been developed for ease of use in routine clinical practice, however, they still require patient knowledge of family history. Kastrinos *et al.* [31], developed a simple, risk assessment tool that includes the following three questions: (1) Do you have a first-degree relative (mother, father, brother, sister or child) with any of the following conditions diagnosed before age 50? Colon or rectal cancer, cancer of the uterus, ovary, stomach, small intestine, urinary tract (kidney, ureter, bladder),

bile ducts, pancreas, or brain; (2) Have you had any of the following conditions diagnosed before age 50? Colon or rectal cancer, colon or rectal polyps; (3) Do you have three or more relatives with a history of colon or rectal cancer (this includes parents, brothers, sisters, children, grandparents, aunts, uncles, and cousins)? Individuals who answer yes to any question should be referred for additional assessment or genetic evaluation. This three-question tool successfully identified 77% of high-risk individuals and 95% of mutation carriers and was easily incorporated into an open-access colonoscopy program [31]. Gunaratnam *et al.* [32] integrated the three-question CRC risk assessment tool [31] into their electronic template for scheduling outpatient colonoscopy procedures. Answering “yes” to at least one of the three questions resulted in an immediate electronic alert, as well as a printed alert for the colonoscopist to encourage discussion and possible referral to genetics clinic. They were able to demonstrate the feasibility of integrating this simple cancer risk assessment tool in a busy community-based, open-access colonoscopy practice. Unfortunately, only a small percent of individuals who screened positive (9%, $N=77/848$ patients) were actually referred for genetic counseling [32], suggesting that a more systematic process is necessary to ensure that individuals potentially at-risk for Lynch syndrome complete the work-up with a genetic counseling referral and germline genetic testing, if indicated.

In an attempt to increase the specificity of the previously described three-question tool for identifying patients at highest risk for genetic syndromes, Guivatchian *et al.* [33] increased the age at diagnosis of Lynch syndrome-associated cancers in first-degree relatives from 50 to 60 and expanded the tool to include the following: (1) Do you have a first-degree relative diagnosed with colon polyps before the age of 60? (2) Have you had any of the following diagnosed before age 50? Cancer of the uterus, ovary, stomach, small intestine, urinary tract (kidney, ureter, bladder), bile ducts, pancreas, or brain; (3) Have you had a total of 10 or more colon polyps removed in your lifetime? More than 98% of the 700 patients recruited for the study successfully completed the expanded five-question tool, providing a CRC risk assessment that was immediately relevant to patient care in an outpatient colonoscopy setting [33].

Prediction models

Multiple computerized prediction models have been developed that offer a quantitative systematic approach to identify an individual’s risk for carrying a germline mutation in a DNA mismatch repair gene. These models incorporate both clinical features, as well as family history and have comparative performance with the previously described clinical criteria [15, 18, 34, 35]. The first three models, MMRpredict [15], MMRpro [36], and PREMM_{1,2} [37], were developed in 2006 with more recent iterations of the PREMM model in 2011 and 2017 (Table 2) [38, 39••]. MMRpredict [15] was developed in a cohort of 870 patients with CRC under the age of 55 who underwent germline genetic testing for mutations in *MLH1*, *MSH2*, and *MSH6*. The two-stage multivariable logistic regression model included only clinical variables in the first stage and incorporated tumor test results for immunohistochemical (IHC) staining and MSI in the second stage. MMRpro [36] involves the application of Bayes rule and mendelian laws and includes more extensive family history for both first- and second-degree relatives. While performance is comparable

to MMRpredict (Table 2), MMRpro requires knowledge of more extensive family history, and does not include information about tumor location or other Lynch syndrome-associated cancers besides endometrial cancer. The PREMM_{1,2} model [37] incorporates other Lynch syndrome-associated cancers, but was only developed to predict risk of germline mutations in *MLH1* and *MSH2*. In 2011, PREMM_{1,2} was expanded to the PREMM_{1,2,6} model [38] to include predictions for mutations in *MSH6*. A cutoff score of 5% is used for all of these models to recommend further workup with genetic counseling referral and consideration for germline genetic testing. These 3 models have comparable sensitivity and specificity with variable ease of use [40]. However, it is important to note that all of these models were primarily developed focusing on CRC. Mercado *et al.* [41] examined the performance of MMRpredict, MMRpro, and PREMM_{1,2,6} in detecting Lynch syndrome among individuals with endometrial cancer and found much lower discrimination using the 5% cutoff (AUC 0.64, 0.54, and 0.67, respectively).

The newest iteration of the PREMM models, PREMM₅, incorporates quantification of an individual's risk of carrying a pathogenic germline mutation in all five Lynch syndrome genes (including *PMS2* and *EPCAM*) to provide a more comprehensive risk assessment [39••]. Kastrinos *et al.* [39••] used clinical and germline data from over 18,000 individuals with germline genetic testing for all five genes to develop this model and compared its performance to PREMM_{1,2,6}. At scores $\geq 2.5\%$, the performance characteristics of PREMM₅ surpassed PREMM_{1,2,6} even for asymptomatic individuals and those with a *PMS2* mutation [39••]. In their paper, Kastrinos *et al.* [39••] mention two major advantages to using PREMM₅. One is the performance in individuals unaffected by cancer. Previous versions of the predictive models were developed and validated in cohorts where a majority of individuals had cancer [15, 34, 36, 40–43], while 46% of the development cohort for the PREMM₅ model had no personal history of cancer but had a family history of Lynch syndrome-associated cancers. The second advantage is that PREMM₅ does not require information about molecular tumor testing to make a prediction of an individual's risk for having Lynch syndrome. PREMM₅ is simple, publicly available without the need to download any software (<https://premm.dfci.harvard.edu/>) and easy to use at point-of-care to quickly identify individuals who might benefit from germline genetic testing for Lynch syndrome.

Universal testing

Universal laboratory-based tumor testing for Lynch Syndrome.—Clinical history-based tools such as Amsterdam II and revised Bethesda criteria have been used for years to identify patients with Lynch syndrome and guide decisions regarding genetic testing. However, these tools have been shown to miss up to 28% of Lynch syndrome cases even when used correctly [44]. Moreover, screening modalities based on family history have potential to contribute to racial and ethnic disparities in Lynch syndrome diagnosis, as minority patients are less likely to be asked about family history by providers [45] or to be able to provide extensive family history information when asked [30]. Acknowledging these limitations, laboratory-based methods to detect mismatch repair deficiency in CRC tumors using IHC of MMR proteins or molecular testing for MSI have gained wider acceptance as

an adjunctive method to identify patients with cancer and their family members at risk for Lynch syndrome (Figure 1).

Universal tumor screening refers to the use of these molecular methods in all newly diagnosed CRCs to identify individuals at risk for Lynch syndrome. These strategies are aimed at identifying the approximately 15% of colorectal tumors with MMR deficiency [2]. MMR deficient tumors can arise as a result of a germline mutation in one of the MMR genes, as in Lynch syndrome, or from sporadic epigenetic silencing of *MLH1* through promoter hypermethylation, which is present in approximately 70% of cases and is often associated with BRAF mutations [3, 46, 47]. A more recently described cause of abnormal IHC or MSI testing is double (or biallelic) MMR somatic mutations that appear to be almost as common as Lynch syndrome. Double somatic mutations acquire 2 somatic alterations (either mutations or loss of heterozygosity) leading to MSI-H cancers [48]. In our practice, paired germline and somatic testing is done in cases of abnormal tumor testing as it facilitates testing for all known causes of MSI-H tumors (germline mutation, *MLH1* promoter hypermethylation and double somatic mutations). Identification of sporadic microsatellite unstable tumors is important and can have management implications, as these tumors have improved response to immune therapies such as PD-1 inhibitors [49].

Tumor screening algorithms consist first of either polymerase chain reaction (PCR) techniques targeting a well-described set of microsatellites to detect MSI [22, 47] or IHC of tumors to detect loss of MMR proteins [50]. If initial tumor testing demonstrates MSI-H (defined as 3 or more microsatellite loci demonstrating altered length) [22] or loss of *MLH1*, further analysis is performed to detect sporadic *MLH1* silencing due to *MLH1* promoter hypermethylation or somatic BRAF mutation [51, 52]. If *MLH1* promoter methylation testing and BRAF mutation analysis are negative or IHC demonstrates loss of *MSH2* or *MSH6*, then further assessment with germline testing for Lynch syndrome is warranted [8, 51]. One advantage of IHC over MSI testing, in addition to its decreased cost, is that the former method requires less tumor tissue. Up to 14% of tumor specimens provide insufficient or poor-quality DNA for completion of PCR-based MSI testing [53, 54]. IHC has also been shown to reliably detect MMR deficiency in colorectal biopsy specimens [55, 56], allowing for diagnosis of Lynch syndrome to help inform treatment decisions before tumor resection. IHC has the added benefit of identifying the likely gene target affected, which can facilitate downstream germline testing. Both laboratory-based screening tests have been shown to significantly outperform clinical assessment tools at identification of patients with Lynch syndrome. The sensitivity of MSI testing and IHC are 77–91% and 83%, respectively, while the specificity for each method is approximately 90% [57].

Cost-effectiveness of universal tumor testing.—A key step in the adoption of universal tumor testing for Lynch syndrome in newly diagnosed CRCs is demonstration that it is cost-effective. Some “universal” screening strategies that have been studied employ age cutoff criteria, wherein molecular screening methods are only used in cases under a certain age, enabling lower costs with a potentially small decrease in sensitivity [58]. Strategies employing age restrictions of 50 and 70 can miss up to 50% and 15% of Lynch syndrome patients, respectively [59, 60]. As a result, most analyses have shown that truly universal testing strategies are cost-effective when compared to either no screening or to strategies

limiting screening to those younger than 50 [61]. Although few studies have compared universal tumor testing to selective testing with an age cutoff of 70, one study did demonstrate universal testing to have an acceptable incremental cost effectiveness ratio (ICER) [61]. Given comparable performance characteristics and lower cost, multiple analyses have shown that IHC is the more cost-effective of the laboratory-based strategies when compared to MSI testing [62, 63••]. By allowing identification and surveillance of additional mutation carriers, cascade testing of second-degree or higher order relatives has been demonstrated to be crucial to cost-effectiveness of universal testing [61]. Indeed, cost-effectiveness of universal testing is improved as more relatives are tested, with most studies demonstrating that acceptable ICER is reached when 2–3 relatives undergo cascade testing [63••]. Selective CRC screening strategies using either clinical criteria or risk prediction models as screening prior to tumor or germline testing have not been shown to be as cost-effective as universal approaches [61, 64].

Implementation of universal tumor testing.—The improved performance characteristics of molecular-based methods to detect patients with Lynch syndrome as well as cost-effectiveness has led several societies to advocate for universal testing of all newly diagnosed CRCs for MMR deficiency with either IHC or PCR-based MSI testing [8, 57, 65, 66•]. Universal testing was first proposed by Hampel *et al.* in 2008 [44], but has been more widely adopted in national guidelines as of 2014 [8•, 66•]. Despite these endorsements, widespread adoption of universal tumor screening to date has been slow, with tumor testing rates as low as 21–28% [67, 68]. Large academic centers and National Cancer Institute designated Comprehensive Cancer Centers have led the way with early implementation of successful universal screening programs [50, 69], while performance among community practices has lagged, but has improved over time [67]. Population-based studies have demonstrated that universal tumor testing is performed less often in underserved and minority patients, suggesting that these groups are disproportionately affected by geographic and practice-based variation in performance of tumor testing [67, 68, 70]. Frequently cited barriers to implementation of universal tumor testing include unfamiliarity with guidelines, concerns about cost, lack of laboratory or genetics services, inadequate stakeholder involvement, and absence of a universal testing “champion” or a designated department that claims responsibility for the universal testing program [71–73]. With these challenges in mind, concerted multi-disciplinary public health efforts are needed to overcome these barriers and optimize implementation of universal testing on a large scale [59].

For racial and ethnic minority groups, in whom Lynch syndrome is underdiagnosed [74–76], universal tumor testing holds promise when implemented successfully to level the playing field and remove traditional barriers to diagnosis including access to specialists and reliance on family history [30, 45, 68, 76]. However, studies of universal testing outcomes in “real-world” practice have shown that even when undergoing tumor screening at an equal rate with comparable rates of abnormal testing results, minority patients are still less likely than their non-Hispanic white counterparts to receive genetics referrals or undergo germline testing [69]. Although other studies have demonstrated heterogeneity and inadequacy of follow-up and downstream testing after abnormal tumor testing results in clinical practice [77, 78], minority patients are particularly vulnerable to these deficiencies [69]. Proposed

strategies to improve operations downstream of universal tumor testing include creation of a “champion” to follow-up testing results, automatic genetic counseling at post-operative visits to prevent loss to follow-up, and creation of centralized pathology cores to standardize testing procedures and reporting [59, 69, 77–79].

Next-generation tumor and germline sequencing.—With recent advances of next-generation sequencing technology and increasing use in clinical oncology, efforts have been made to use targeted next-generation sequencing to identify MMR deficiency in CRC [80–82]. Potential benefits include the ability to quantify tumor mutational burden as a surrogate for MSI status and to simultaneously identify other actionable mutations for which targeted mutation testing is already recommended by guidelines, such as *RAS* oncogene mutations, both of which can impact therapeutic decisions [81, 83, 84]. Sensitivities over 90% have been reported for MSI testing by next-generation sequencing [80–82, 85]. Universal tumor sequencing has also been demonstrated to outperform traditional multi-step universal tumor testing strategies in identifying Lynch syndrome patients specifically, with a sensitivity of 100% in one cohort [54]. Development of targeted next-generation sequencing for microsatellite instability is not without limitations. The panel used by Nowak *et al.* [81], for example produced false positive detections in tumors with *POLE* mutations, which also harbor a hypermutated phenotype. Specificity of this approach for identification of MMR deficiency was still 98%, and discrimination of *POLE*-mutated tumors was achieved through sequencing of *POLE* genes, highlighting the powerful potential of sequencing approaches.

Such tumor sequencing methods of screening for Lynch syndrome may create ethical questions regarding informed consent as a genetic test. Whereas current methods for universal tumor testing do not directly identify pathogenic mutations and therefore do not require rigorous informed patient consent [71, 86], next generation sequencing is being promoted in part because of its ability to guide downstream germline testing through identification of pathogenic mutations in MMR genes or even in other cancer-susceptibility genes unrelated to Lynch syndrome [54]. Patients with pathogenic MMR mutations identified from tumor sequencing would still require germline testing, but less costly single-mutation confirmatory testing could be used instead of full gene sequencing or multi-gene panel testing [81].

Conclusion

Identification of patients with Lynch syndrome has significant implications for individual patients, their families, and our healthcare system as a whole. Given the profound impact of effective and timely diagnosis on an individual and population level, strategies to improve diagnosis in a cost-effective and equitable manner are a public health priority. Newer clinical risk assessment tools have improved test characteristics over traditional criteria and can be used in busy practices especially for unaffected individuals with family history of Lynch syndrome-associated cancers. Universal tumor testing represents an improved strategy for Lynch syndrome identification among cancer patients and is endorsed by major societies. Although implementation of universal tumor testing in clinical practice is improving over time, there is heterogeneity across practices in adherence to recommendations and follow up.

Next generation sequencing represents the next frontier in identification of Lynch syndrome patients and also has implications for treatment and prognosis.

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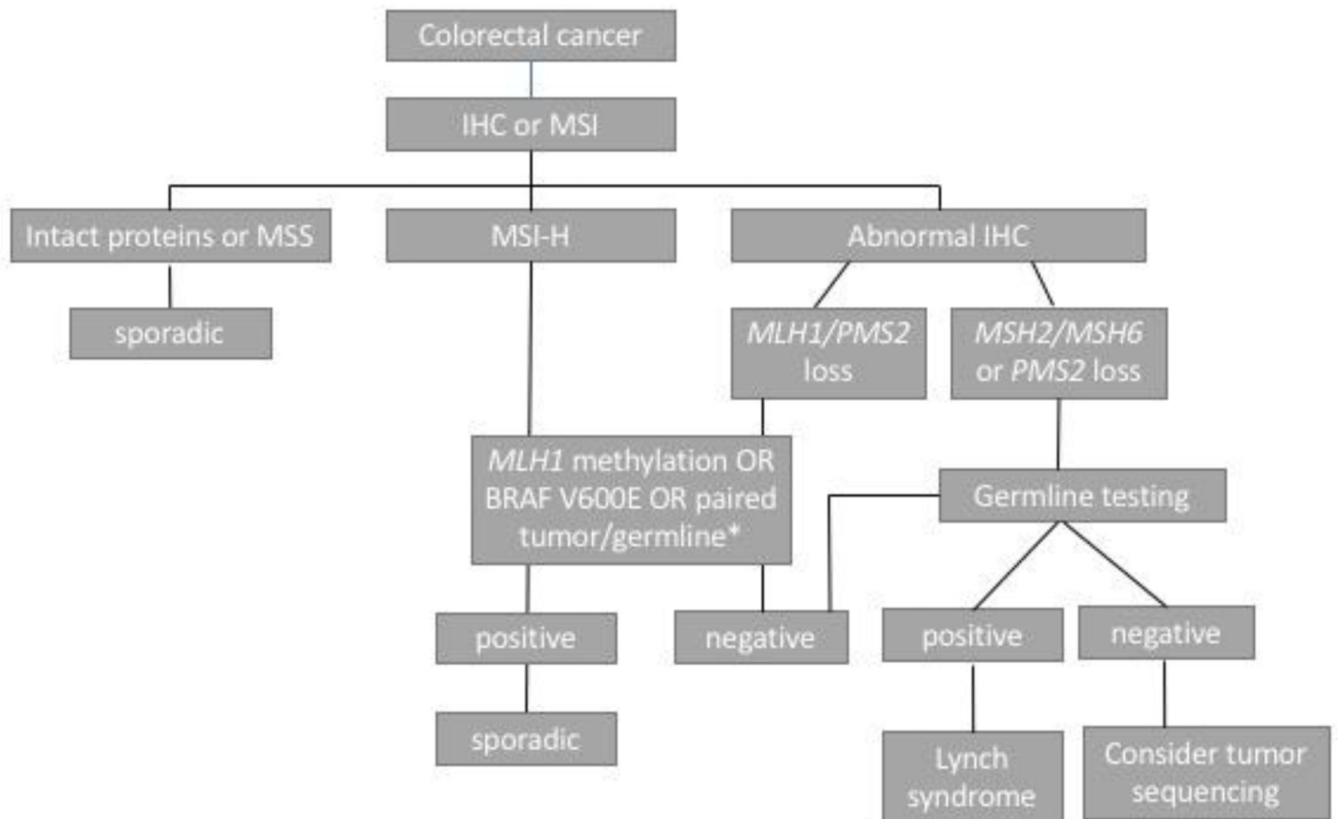


Figure 1. Algorithm for colorectal tumor testing for Lynch syndrome.

Testing can be done with immunohistochemistry (IHC) for mismatch repair proteins or microsatellite instability (MSI) testing by polymerase chain reaction. If testing reveals intact staining of proteins or microsatellite stability (MSS), this likely represents sporadic cancer in the absence of family history consistent with Lynch syndrome. If testing reveals microsatellite instability-high (MSI-H) or abnormal IHC, additional testing is warranted. In the case of MSI-H or loss of *MLH1/PMS2*, a number of potential strategies can be followed such as testing for *MLH1* hypermethylation or *BRAFV600E*. If hypermethylation or a *BRAF* mutation are found, sporadic colorectal cancer is likely. If this testing is negative, germline testing is warranted. An alternative strategy is to perform paired tumor and germline testing (highlighted with *) as this evaluates *MLH1* hypermethylation, double somatic mutations and germline testing in a single test. In the case of loss of *MSH2* and/or *MSH6* or *PMS2* only, germline testing is the next step. If germline testing confirms a mutation, Lynch syndrome is diagnosed. If germline testing is negative, then somatic tumor testing can be considered to evaluate for double somatic mutations as an explanation for abnormal tumor testing.

Table 1.

Revised Bethesda Guidelines [21]

<p>Tumors from individuals should be tested for microsatellite instability (MSI) if they meet any of the following criteria:</p> <ol style="list-style-type: none">(1) Colorectal cancer diagnosed in an individual <50 years of age(2) Presence of synchronous/metachronous colorectal cancer, or other Lynch syndrome-associated cancers (endometrial, stomach, ovarian, pancreas, small bowel, ureter and renal pelvis, biliary tract, brain, sebaceous glands, and keratoacanthomas) regardless of age(3) Colorectal cancer with MSI-high histology (tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern) diagnosed in an individual <60 years of age(4) Colorectal cancer in one or more first-degree relatives with a Lynch syndrome-associated tumor, with one of the cancers diagnosed <50 years or age(5) Colorectal cancer in two or more first- or second-degree relatives with Lynch syndrome-associated cancers, regardless of age

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Table 2.

Comparison of Predictive Models *

Model	MMRpredict [15]	MMRpro [36]	PREMM ₅ [39••]
Year	2006	2006	2017
Genes	<i>MLH1, MSH2, MSH6</i>	<i>MLH1, MSH2, MSH6</i>	<i>MLH1, MSH2, MSH6, PMS2, EPCAM</i>
Variables	For patient: - Age at CRC diagnosis - Sex - Tumor location (proximal/distal) - Synchronous and/or metachronous tumors For FDRs: - CRC (yes/no) - Youngest age of CRC (<50/>50) - Endometrial cancer (yes/no)	For patient, FDRs, SDRs: - Relation to patient - CRC (yes/no); age at diagnosis - Endometrial cancer (yes/no); age at diagnosis - Current age if unaffected - Results of MSI/IHC - Results of previous germline testing	For patient: - Age - Sex - CRC (yes/no) - Other LS-associated cancer (yes/no) From affected side of family: - Number of FDRs and SDRs with CRC - Number of FDRs and SDRs with endometrial cancer - Any relatives with other LS-associated cancer (yes/no) *Prior versions include PREMM _{1,2} [37] and PREMM _{1,2,6} [38]
Performance	<i>MLH1/MSH2/MSH6</i> (AUC, 0.85; 0.77–0.93)	<i>MLH1/MSH2/MSH6</i> (AUC, 0.79; 0.74–0.84)	<i>MLH1</i> (AUC, 0.89; 0.87–0.91) <i>MSH2/EPCAM</i> (AUC, 0.84; 0.82–0.86) <i>MSH6</i> (AUC, 0.76; 0.73–0.79) <i>PMS2</i> (AUC, 0.64; 0.60–0.68)
Source	Website [87]	Website; software [88]	Website [89]

CRC = colorectal cancer, FDR = first degree relative, SDR = second degree relative, LS = Lynch syndrome

* Adapted from “Criteria and prediction models for mismatch repair gene mutations: a review” by Win *et al*, 2013, J Med Genet, 50:785–93 [34]