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ALGORITHMIC LABORATORY TESTING FOR DIAGNOSIS OF LYNCH SYNDROME, IMPLEMENTED AT MARSHFIELD LABS

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Marshfield Labs has implemented a Lynch syndrome testing algorithm.

BACKGROUND

Colorectal carcinoma is the third most frequently diagnosed malignancy in the United States. Approximately 2.5% of cases are associated with Lynch syndrome, representing the most common form of inherited nonpolyposis colorectal cancer syndrome and characterized by deficient DNA mismatch repair (MMR) and high-level microsatellite instability (MSI-H). Diagnosis of Lynch syndrome facilitates appropriate surveillance of the patient and relatives who also carry the mutation, allowing for early detection and treatment of colorectal carcinomas and other syndromic tumors (e.g., carcinomas of the endometrium, small intestine, stomach, ovary, pancreatobiliary tract, and ureter/renal pelvis). Surveillance modalities include periodic endometrial biopsy, transvaginal ultrasound, measurement of serum CA-125 level, urinalysis and urine cytologic examination, annual skin surveillance, and upper endoscopic surveillance.[1-4] Among patients beyond childbearing age, prophylactic hysterectomy and salpingo-oophorectomy are considered.[5]

The Revised Bethesda Guidelines (based on a combination of clinical and histologic considerations) are sensitive in screening for patients whose tumor tissue should undergo testing in the further evaluation for Lynch syndrome (Table 1).[6] Some studies have shown an improved Lynch syndrome detection rate by testing all colorectal tumors. Attesting to the recognized importance of Lynch syndrome proband identification,

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many cancer centers in the United States reflexively test all colorectal carcinomas for deficient DNA MMR by immunohistochemistry (IHC). Universal testing of colorectal carcinomas for Lynch syndrome may be evolving into standard practice.

Lynch syndrome results from inherited (germline) mutations that predispose the patient to deficient DNA MMR function, leading to genomic instability and tumor development. Lynch syndrome testing of tumor tissue is predicated on the principle that either aberrant nonexpression of an MMR protein or MSI-H connotes an MMR defect. Since approximately 11% of patients with Lynch syndrome exhibit preserved MMR protein expression but are MSI-H, a normal IHC panel should prompt molecular testing for MSI-H.[7] Mismatch repair protein malexpression and MSI-H are also demonstrated by a subset of noninherited colorectal carcinomas, the latter identified by the presence of a BRAF gene mutation. As such, sequencing for specific MMR gene mutations is performed only in the absence of a BRAF gene mutation.

TESTING AT MARSHFIELD LABS

Implementation of Marshfield Labs' Lynch Syndrome Testing Algorithm for Colorectal Carcinoma (Figure 1) is recommended for patients suspected of manifesting this inherited disorder. Tumors that show either MMR protein malexpression (by IHC) or MSI-H (by polymerase chain reaction) are reflexively tested for a BRAF V600E gene mutation; in the absence of a BRAF mutation, peripheral blood should be tested to confirm the presence of a deleterious germline MMR gene mutation, thereby establishing the diagnosis of Lynch syndrome. Tumor tissue IHC testing is not only instrumental in selecting patients for germline mutation analysis, but also targets the particular MMR gene to evaluate. Tumors that are MMR protein deficient/MSI-H and exhibit a BRAF V600E mutation are considered to manifest acquired (i.e., nonheritable) MSI-H as a result of somatic hypermethylation of the MLH1 gene promoter. Patients showing preserved MMR protein expression with microsatellite stability or only low-level microsatellite instability are highly unlikely to have Lynch syndrome.

The Lynch Syndrome Testing Algorithm for Colorectal Carcinoma may be initiated by either the clinician or pathologist who suspects the patient to manifest Lynch syndrome. The MMR protein IHC panel (comprised of antibodies to MLH1, MSH2, MSH6, and PMS2) is performed on formalin-fixed, paraffin-embedded colorectal carcinoma tissue. If a carcinoma from the patient or a first-degree relative is not available, testing may be performed on an adenomatous polyp. For Marshfield Clinic patients, reflexive molecular testing is performed only on those cases for which insurance carrier benefits have been reviewed and authorizations approved.

As an alternative to the complete testing algorithm, IHC alone or in conjunction with MSI testing may be requested. Performing MSI testing without previous IHC assessment of MMR protein expression is not recommended. In the evaluation for Lynch syndrome, BRAF mutation testing should be ordered in isolation only if deficient MMR protein expression or MSI-H already has been documented. Of note, among colorectal carcinoma patients in general, there is a role for determining MSI status in guiding the choice of adjuvant systemic therapy for deeply invasive colorectal carcinomas without lymph nodal or distant metastasis; MSI-H is linked to resistance to certain chemotherapeutic agents (most notably, 5-fluorouracil), but may be associated with sensitivity to irinotecan.[8-10]

Lynch syndrome is associated not only with colorectal carcinoma, but also with endometrial, gastric, ovarian, pancreatic, ureteral/renal pelvic (i.e., urothelial carcinoma), biliary tract, brain (i.e., glioblastoma), cutaneous (i.e., sebaceous neoplasm and keratoacanthoma), and small intestinal tumors. It is not uncommon for a patient with Lynch syndrome to present initially with a noncolorectal tumor. However, testing of

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a colorectal carcinoma from a first-degree relative or colorectal adenomatous polyp from the patient is preferred to that of a noncolorectal tumor. As an alternative, an endometrial carcinoma may be tested for both MMR protein expression and MSI; there is no role for BRAF gene testing in distinguishing between a sporadic and Lynch syndrome-associated endometrial carcinoma. Although the data do not support a role for screening all cutaneous sebaceous neoplasms for MMR protein malexpression, among patients suspected of Lynch/Muir-Torre syndrome, IHC may be of utility in guiding germline gene mutation testing. The role of testing tumors of other anatomic sites has not been well established. In some cases, proceeding directly to germline MMR gene mutation testing of peripheral blood is appropriate. Performance of the complete Lynch Syndrome Testing Algorithm should be reserved for colorectal tumors.

The MMR protein IHC and BRAF V600E gene mutation molecular testing are offered at Marshfield Labs, and assessment for MSI is performed at a reference facility.

Test results are communicated as a report with a series of addenda. The final addition to the report will summarize the interpretation of the entire test panel. Patients fulfilling the Revised Bethesda Guidelines but without laboratory evidence to support a diagnosis of Lynch syndrome may have another familial cancer syndrome; as such, regardless of the Lynch syndrome testing results, it is recommended that a referral be made for formal medical genetics evaluation and counseling.

ORDERING INFORMATION

Marshfield Clinic providers may order the testing algorithm (Figure 1) or an individual test by:

a) contacting the anatomic pathology office (715-221-6100) and requesting the involvement of the pathologist serving on the Lynch syndrome testing service, or
b) faxing an Authorization for Test Requests form to the histology laboratory (715-221-6181).

Non-Clinic outreach clients may submit a written request or a completed histology requisition (specifying either the Lynch Syndrome Testing Algorithm or individual test) to the Department of Anatomic Pathology at Marshfield Labs.

Specimen requirements include one formalin-fixed, paraffin-embedded tissue block and corresponding hematoxylin/eosin-stained slide that contain both the neoplasm and non-neoplastic crypt-containing mucosa.

Questions may be directed to Drs. Resnick or Sun (715-221-6100).

TABLE 1. REVISED BETHESDA GUIDELINES FOR TESTING OF COLORECTAL TUMORS FOR MICROSATELLITE INSTABILITY[6]

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age
2. Presence of synchronous or metachronous colorectal, or other Lynch syndrome-associated tumors, regardless of age
3. Colorectal cancer with the MSI-H histology diagnosed in a patient who is less than 60 years of age
4. Colorectal cancer or Lynch syndrome-associated tumor diagnosed under age 50 years in at least one first-degree relative
5. Colorectal cancer or Lynch syndrome-associated tumor diagnosed at any age in two first- or second-degree relatives

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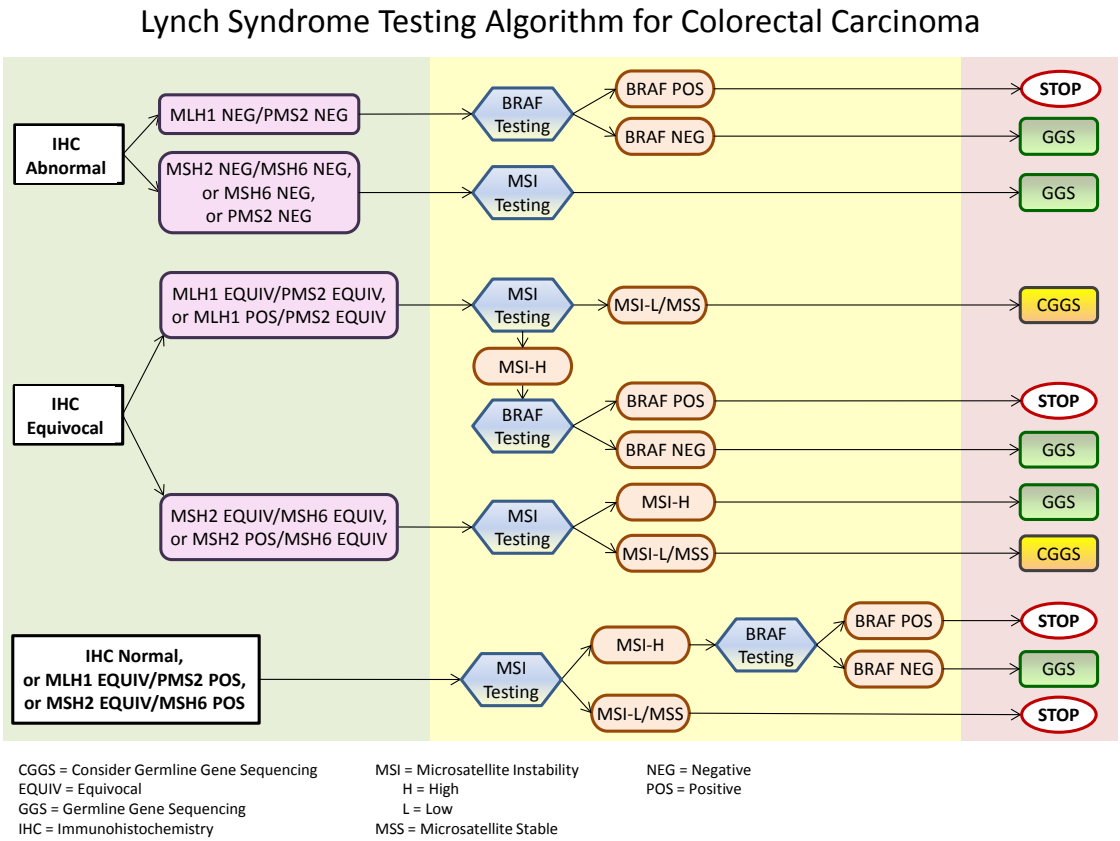

Figure 1. Marshfield Labs' Lynch Syndrome Testing Algorithm for Colorectal Carcinoma.

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