Laboratory Assays in Evaluation of Lynch Syndrome in Patients with Endometrial Carcinoma

Bojana Djordjevic, MD[a,*], Russell R. Broaddus, MD, PhD[b]

KEYWORDS

• Lynch syndrome • Endometrial cancer • Molecular diagnostic testing

Key points

- Immunohistochemistry, microsatellite instability analysis, and MLH1 promoter methylation analysis constitute the cornerstone of laboratory molecular testing of endometrial tumors for Lynch syndrome.
- Discrepancies between genetic, immunohistochemistry, and microsatellite instability analyses may arise and can often be explained as a reflection of the underlying tumor biology.
- Although universal endometrial tumor screening is recommended in order to identify the most patients with possible Lynch syndrome, the current practice of screening is highly variable across different centers and countries.
- Lynch syndrome–associated endometrial tumors are associated with several pathologic characteristics that may be used by pathologists to trigger tissue testing.

ABSTRACT

This article reviews the main tissue testing modalities for Lynch Syndrome in the pathology laboratory, such as immunohistochemistry and PCR based analyses, and discusses their routine application, interpretation pitfalls, and troubleshooting of common technical performance issues. Discrepancies between laboratory and genetic testing may arise, and are examined in the context of the complexity of molecular abnormalities associated with Lynch Syndrome. The merits of targeted versus universal screening in a changing healthcare climate are addressed. In the absence of comprehensive screening programs, specific tumor topography and histological features that may prompt pathologist-initiated molecular tumor testing are outlined.

LYNCH SYNDROME

Lynch Syndrome occurs due to a germline mutation in a gene corresponding to a family of DNA mismatch repair (MMR) proteins, Mut L homolog 1 (MLH1), MutS protein homolog 2 (MSH2), MutS homolog 6 (MSH6) and PMS1 Homolog 2, Mismatch Repair System Component (PMS2). The hallmark cancers for Lynch syndrome are colorectal adenocarcinoma and endometrial carcinoma, whereas less common cancer types include ovarian carcinoma, urothelial carcinomas of the ureter/renal pelvis, duodenal adenocarcinoma, and gastric adenocarcinoma. Loss of DNA MMR protein function typically results in high levels of DNA microsatellite instability (MSI). In 15% to 20% of all sporadic endometrial carcinomas, MLH1 immunohistochemical loss and MSI result

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[a] Department of Pathology and Laboratory Medicine, University of Ottawa, The Ottawa Hospital, Eastern Ontario Regional Laboratory, 501 Smyth Road, Ottawa, Ontario K1H 8L6, Canada; [b] Department of Pathology, Unit 85, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA
* Corresponding author.
E-mail addresses: bdjordjevic@toh.on.ca; bojanadjordjevicmd@gmail.com

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from *MLH1* gene promoter methylation with subsequent transcriptional silencing.[1–5]

For most hereditary cancer syndromes, affected individuals are identified from recognition of a constellation of clinical features, such as young age of cancer onset and a strong family history of characteristic cancers, with subsequent germline sequencing of the suspected affected gene. However, tumor tissue testing in the pathology laboratory is a key component of Lynch syndrome diagnosis, involving MMR immunohistochemistry, MSI analysis, and *MLH1* methylation analysis.

TUMOR TESTING AND PITFALLS IN TEST INTERPRETATION

Immunohistochemistry

Immunohistochemistry for MMR proteins is performed using commercially available antibodies that work fairly reliably. Gene mutation of *MMR* genes or methylation of the *MLH1* gene promoter typically results in loss of immunohistochemical expression of the corresponding protein. Complete absence of nuclear expression should be observed in order for a tumor to be considered as having a loss of an MMR marker. Strong nuclear staining in the surrounding endometrial stroma, myometrium, lymphocytes, or normal endometrium should serve as an internal positive control (**Fig. 1**). The MSH2 and MSH6 proteins, and the MLH1 and PMS2 proteins, act as functional pairs.[6] Therefore, when MLH1 protein expression is lost (because of mutation of the *MLH1* gene or methylation of *MLH1* gene promoter), typically there is secondary loss of PMS2 protein expression. Mutation of the PMS2 gene is associated with loss of PMS2 protein but retained MLH1 immunohistochemical expression. Similarly, mutation of the *MSH2* gene typically results in immunohistochemical loss of MSH2 and MSH6 proteins. In contrast, mutation of *MSH6* gene results only in MSH6 protein loss, whereas MSH2 protein expression remains intact.

Regarding MMR immunohistochemistry reporting recommendations, note that, for most cases, the percentage or intensity of staining is not relevant, and the interpretation result should be either positive or negative. Terminology such as “focally positive,” “patchy staining,” “weakly positive,” “positive in x% of cells,” or “equivocal staining” should be avoided. If tumor staining is negative, it should be indicated that internal control stromal cells/normal endometrium are positive.

Several pitfalls in the interpretation of MMR immunohistochemistry exist (**Fig. 2**). Most commonly, false-negative nuclear tumor staining occurs in the setting of an inadequate internal positive control. In contrast, immunohistochemical staining of the tumor cells may be focal or weak. In most cases, this represents genuine nuclear staining. Both of these problems may be resolved by repeating the immunohistochemistry with consideration of prolonging the antigen exposure time or using a different tissue block from the same specimen. Another immunohistochemical issue involves cytoplasmic tumor staining, regardless of the presence or the absence of nuclear staining, especially when the tissue has previously been frozen for the purposes of intraoperative consultation. Cytoplasmic staining should be disregarded in the evaluation of MMR immunohistochemistry. In addition, endometrial stroma or tumor-infiltrating lymphocytes may cause

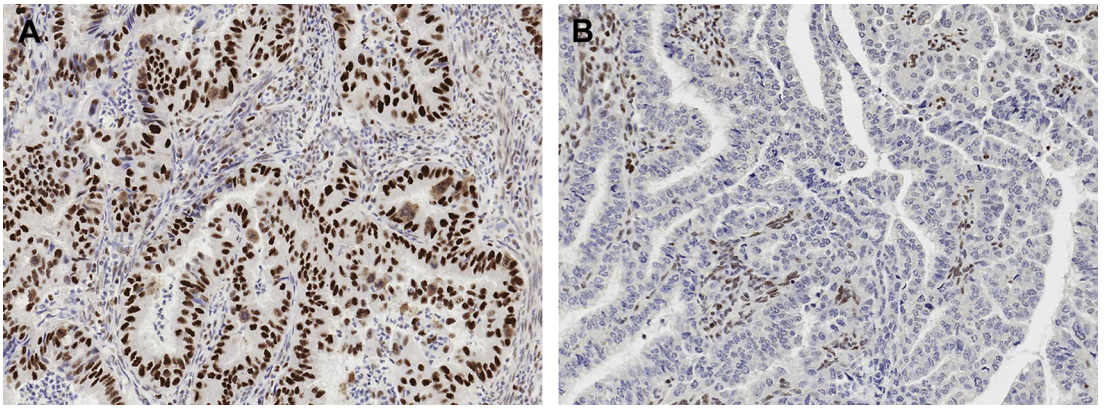

Fig. 1. Application of immunohistochemistry in endometrial carcinoma tissue testing for Lynch syndrome. (*A*) Nuclear expression of mismatch repair proteins (PMS2, 20×). (*B*) Good internal positive control in the surrounding endometrial stroma ensures that the interpretation of mismatch repair protein expression loss is accurate (MSH2, 20×).