THE PATHCARE NEWS

MISMATCH REPAIR, MICROSATELLITE INSTABILITY AND GERMLINE TESTING IN LYNCH SYNDROME

Lynch syndrome (LS) is the most common cause of hereditary colorectal cancer (CRC), resulting from a germline pathogenic variant in one of the DNA mismatch repair (MMR) genes. The MMR genes associated with LS include *MSH2, MLH1, MSH6* and *PMS2*.

The role of the MMR system is to correct base substitution mismatches and small insertions and deletions that occur during DNA replication. Inactivation of both copies of any of the MMR genes can lead to ineffective mismatch repair which will result in an increased mutation rate in the cell (genomic instability), resulting in carcinogenesis. DNA mismatches commonly occur in regions of repetitive nucleotide sequences called microsatellites. A defective MMR system leads to changes in the number of repeats in these microsatellites, which is termed **microsatellite instability (MSI)**.

MSI is a key characteristic of LS-associated tumours, but it is not limited to only these tumour types. Approximately 15% to 20% of sporadic CRCs also demonstrate MSI (caused by somatic *BRAF* mutations and *MLH1* promoter hypermethylation).

Available tests:

- Testing for **MMR deficiency (dMMR)** is done by **immunohistochemistry (IHC)**, which can detect the loss of MMR protein expression. This test is performed on the tumour tissue.
- Testing for **MSI** is done by **PCR**, which can detect the changes in the numbers of microsatellite repeats. This test is performed on DNA extracted from the tumour tissue and surrounding normal tissue.
- **Lynch syndrome** is confirmed via a **multi-gene panel PCR** test, which can detect germline mutations in the MMR genes. This test is performed on DNA extracted from the patient's blood and saliva.

When is testing for dMMR/MSI relevant?

1. **To determine prognosis**

Several studies have shown that MSI-high tumours have a more favorable prognosis and are less prone to lymph node and systemic metastasis.

2. **To predict response to 5-FU and irinotecan therapy**

Current data suggests that stage II and III MSI-high colorectal tumours do not benefit (and might actually be harmed by) 5-FU therapy and MSI-high tumours may be more responsive to irinotecan than microsatellite stable (MSI-S) tumours.

3. **To predict response to immune checkpoint inhibitors (anti PD-1 therapy):**

dMMR cancers are predicted to have a higher mutational burden, a very large number of mutations associated neoantigens (MANAs), show greater densities of tumour infiltrating lymphocytes and PD-L1 expression. These all increase the chance of response to immune checkpoint inhibitors.

4. **In the detection of Lynch Syndrome**

The role of MSI as a genetic marker of Lynch Syndrome is well established. Both MSI detection and IHC are highly sensitive methods for the identification of a defective MMR system that can be caused by LS. Irrespective of the indication for MSI testing (including for prognostication and treatment planning), current guidelines recommend germline testing for LS in any MSI-high tumour, even in the absence of a personal or family history normally associated with LS. Please refer to the lab update on genetic testing in colorectal and endometrial cancers.

MSI vs IHC in the workup of LS

- In some cases, both MSI and IHC are requested from the onset, and in others it is done sequentially if needed (see algorithm if sequential testing is preferred).
- Both techniques have their own advantages and limitations (see table below).
- If the suspicion is very high for Lynch syndrome (strong family history, young age at onset, synchronous tumours, etc.), then it is more appropriate to start with MMR IHC.
- In the case that the suspicion is lower, then one can start with MSI testing. MSI is better at excluding LS if negative (high negative predictive value).
- If both the MSI and IHC are negative, then one can safely deduce that the tumour is not due to Lynch syndrome.

Details	Advantages	Limitations
Microsatellite instability (MSI)		
PCR test performed on tumour tissue. Involves analysis of 5 microsatellite markers, compared between the tumour tissue and normal tissue. Reported as follows: MSI-high (MSI-H) – if two or more of the five markers show instability MSI-low (MSI-L) – if one of the five markers shows instability MSI-stable (MSI-S) – if none of the markers show instability Current markers used in MSI diagnosis are known to have a reduced sensitivity at detecting MSI in non-CRC tumours (such as endometrial cancers) and in patients with germline *MSH6* pathogenic variants. The sensitivity and specificity of MSI testing for LS are approximately 85 and 90 percent, respectively. About 15-20% of sporadic **colon** cancers show MSI, usually due to loss of MLH1 protein expression. MSI in **rectal** cancers, however, is rare and strongly associated with LS, which gives an excellent positive predictive value (PPV).	• May pick up MMR deficiency even if IHC studies are normal, thus if a protein is present, but non-functional • Requires very little tissue • Highly reproducible	• Requires special skill in microdissection and molecular analysis • MSI cannot be detected in extremely mucinous tumours • A small portion of LS-related tumours will not show evidence of MMR deficiency • Does not identify the exact gene that is likely mutated
MMR immunohistochemistry (IHC)		
Immunohistochemistry test performed on tumour tissue. Loss of staining of a MMR protein can indicate a shortened protein or loss of protein expression. The likelihood of finding a germline pathogenic variant in one of the MMR genes based on IHC results varies depending on the protein that is absent. Abnormality can include patchy expression with loss of nuclear staining, as well as complete loss of expression. Up to 10% of LS tumours that have lost MMR and have MSI do not show any abnormality on IHC. However, when an IHC abnormality is found, it is generally associated with MSI. If it is desired to exclude LS and a tumour shows normal IHC, then it will be necessary to carry out MSI testing. Although testing of tissue from colorectal carcinoma is clearly preferable, testing can be considered on an adenomatous polyp if cancer tissue is not available. Polyps with high-grade dysplasia are more likely to be concordant with germline pathogenic variant status than polyps with low-grade dysplasia.	• Readily available • Can be performed on small biopsies • Is economical • Can identify which of the MMR genes may be causing a MMR-deficient tumour	• Variation in tissue fixation and other technical issues can result in weak or equivocal staining patterns • Some missense germline pathogenic variants will not result in the absence of a detectable protein product • May be less reliable when performed on small tissue samples

When should one suspect a diagnosis of Lynch syndrome in a patient?

- A diagnosis of colorectal cancer (CRC) or endometrial cancer and one or more of the following:
  - Colorectal or endometrial cancer diagnosed before age 50 years
  - Synchronous or metachronous LS-related cancers (e.g. colorectal, endometrial, stomach, small intestinal, hepatobiliary, renal pelvic, ureteral)
  - Colorectal tumour tissue with MSI-high histology (e.g. poor differentiation, tumour-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, medullary growth pattern)
  - Microsatellite instability (MSI) testing showing that tumour tissue (e.g. colon, endometrial) is MSI-high. [see details on MSI in table]
  - Tumour tissue (e.g., colon, endometrial) immunohistochemistry (IHC) demonstrates loss of expression of one or more of the mismatch repair (MMR) gene products: MSH2, MLH1, MSH6, and PMS2. [see details on IHC in table]
  - At least one first-degree relative with any LS-related cancer diagnosed before age 50 years.
  - At least two first-degree relatives with any LS-related cancers regardless of age of cancer diagnosis.
- A family member with colorectal or endometrial cancer who meets one of the above criteria

  Note: Molecular genetic testing ideally begins with a person who has had a LS-related cancer. However, in some families there may be no affected individual who is alive or willing to be tested
- A family member with a confirmed diagnosis of Lynch syndrome

Confirmation of Lynch syndrome diagnosis by means of germline genetic testing

Once MSI testing and/or MMR IHC indicates that the tumour is likely to be caused by Lynch syndrome, then one should proceed with germline molecular genetic testing. A multigene panel test is recommended that includes the MMR genes *(MLH1, MSH2, MSH6* and *PMS2*) and *EPCAM*.

PathCare offers testing for Lynch syndrome as part of our referral service to Invitae (USA). Please see the "Genetic Testing for Colon and Endometrial Cancers" document for more information on how to order this test.

Genetic counselling prior to genetic testing is strongly advised. The genetic counsellor will be able to advise on the appropriate germline testing approach, and facilitate further family studies as needed. For more information on Genetic Counselling services in your area, please call the PathCare Genetic Team on 021 596 3655 or email geneticconsult@pathcare.org

BRAF analysis can also be performed on tumour tissue if needed. Combined *BRAF* mutation and *MLH1* deficiency effectively excludes the possibility of Lynch syndrome. A pooled analysis of COIN, CAIRO, CAIRO2, and FOCUS trials suggested inferior prognosis with MMR deficient colon cancers, in part driven by association with *BRAF* mutations.

References

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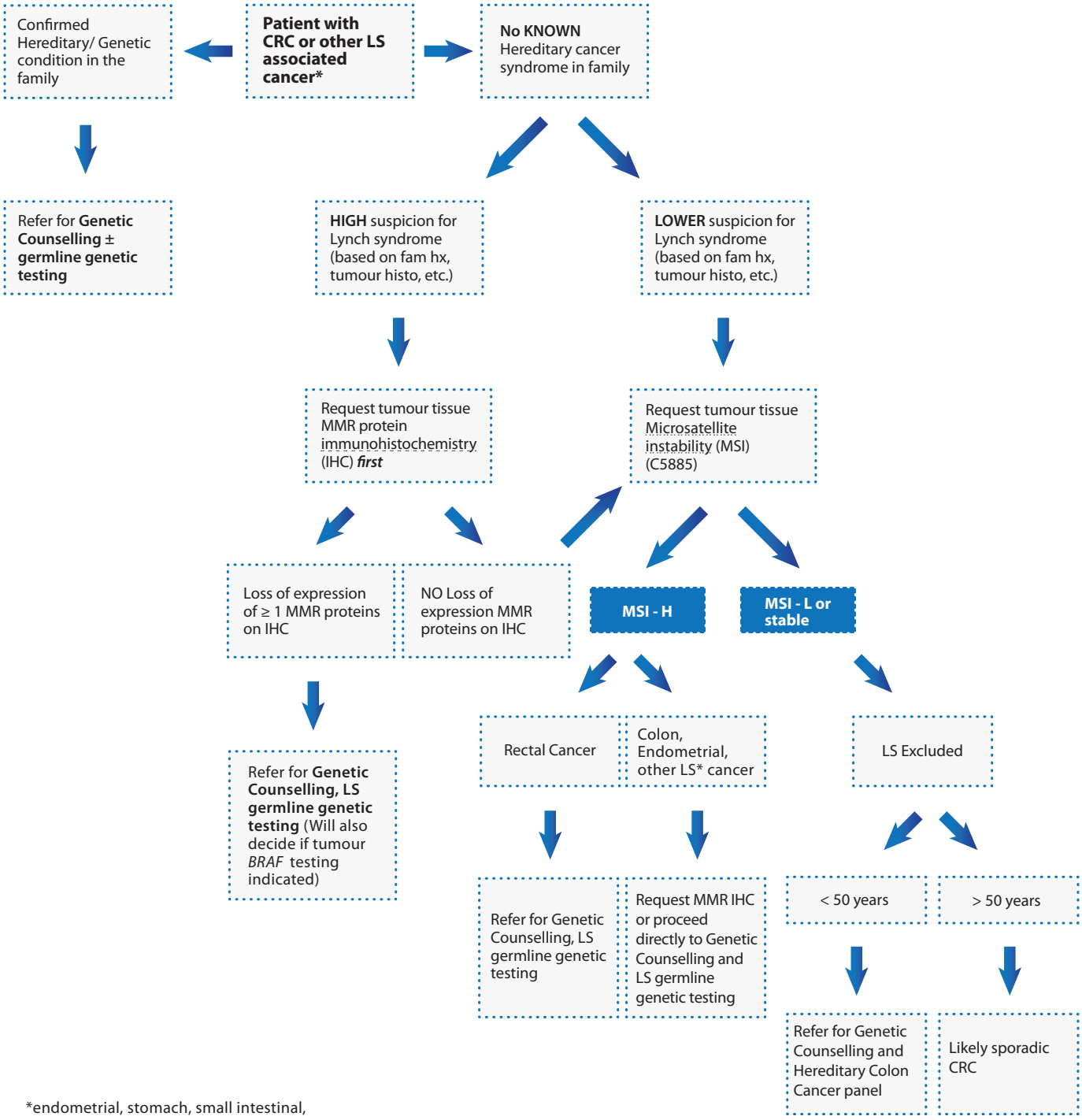
Algorithm for MMR/MSI testing in the context of suspected Lynch syndrome:



*endometrial, stomach, small intestinal, hepatobiliary, renal pelvic, ureteral