Lynch syndrome screening (endometrial cancer): Guidance for requesting and reporting (v3.0 - 06/04/2022)

Dr Nick West, University of Leeds

1. Introduction:

Lynch syndrome screening should be performed on all endometrial cancers (EC) at diagnosis, in line with NICE diagnostics guidance (https://www.nice.org.uk/guidance/dg42). The primary screening test should be immunohistochemistry (IHC) for the four mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, MSH6), to be followed MLH1 promoter methylation testing in cases showing MLH1 loss.

2. Which specimen and block to test:

Staining only needs to be performed once (if conclusive) on a single tissue block. If there are multiple samples for the patient containing invasive tumour, it is preferable to use the diagnostic biopsy as it is usually more optimally fixed, which reduces the risk of poor quality staining that can be difficult to interpret. If multiple tumour blocks exist for the case, it is preferable to select a block that is well-fixed, contains plentiful invasive tumour, and contains normal background endometrium (to act as an internal control), where possible.

3. Recognised reporting pitfalls:

Normal staining should consist of clean nuclear staining within the tumour cells. Background stromal/inflammatory cells and normal endometrium provide a positive internal control. Loss of nuclear staining within the tumour, reduced intensity staining (when compared to adjacent stromal cells), punctate nuclear staining or cytoplasmic staining are recognised abnormal expression patterns.

Note that good tissue fixation is important to observe clean consistent staining throughout the tumour, hence the recommendation to use biopsy specimens. Resections are prone to areas of reduced/loss of staining in poorly fixed areas leading to difficulties in interpretation. Artefactual loss of staining can be confirmed by the associated loss of adjacent stromal cell staining. Defective mismatch repair (dMMR) is usually present or absent throughout the whole tumour, so positive staining within well-fixed areas of a resection block showing variable staining is reassuring, although it is important to recognise that genuine clonal loss can occasionally occur. Correlation with the adjacent stromal cells is critical to determine whether any loss of staining is genuine.

4. Abnormal staining patterns:

The most common abnormal staining pattern is combined loss of MLH1/PMS2. Most of these cases will have somatic dMMR due to hypermethylation of the MLH1 promoter region leading to loss of gene function. Note that PMS2 requires functional MLH1 to be expressed at the protein level hence the associated loss of PMS2 (despite no abnormality in the PMS2 gene).

Less common patterns of loss, that are more likely associated with Lynch syndrome, include isolated loss of PMS2, loss of MSH2/MSH6 and isolated loss of MSH6. Note that MSH6 requires functional MSH2 to be expressed hence the associated loss with MSH2 defects as for MLH1/PMS2 above.

In cases showing complete MLH1/PMS2 loss, additional complete or clonal loss of MSH6 or MSH2/MSH6 can occur – this is usually due to additional double hit somatic mutations in one or more these genes as a consequence of the high number of mutations seen in dMMR. If complete loss of MSH6 or MSH2/MSH6 is noted on biopsy, it is preferable to stain a resection block (if surgery takes place) to confirm whether loss of staining is clonal or complete. If clonal, this is reassuring if the MLH1 loss is associated with MLH1 promoter

hypermethylation and germline testing is not recommended. If the loss of MSH6 or MSH2/MSH6 staining is complete in a resection block (or in a biopsy if surgery is not being performed), it is recommended that these patients are considered for germline testing as somatic MLH1/PMS2 loss has previously been described in association with germline MSH2/MSH6 mutations.

5. What to do if MLH1 is abnormal:

According to the NICE diagnostics guidance, cases with abnormal MLH1 should subsequently undergo MLH1 methylation testing. Methylation testing should also be requested if MLH1/PMS2 are abnormal in association with abnormal expression of MSH6 +/- MSH2.

Cases showing isolated PMS2 loss or MSH6+/-MSH2 loss without associated MLH1 loss should not undergo methylation testing but be recommended for germline testing.

6. Requesting MLH1 promoter methylation testing

MLH1 promoter methylation testing in EC showing MLH1 protein loss appears on the National Genomic Test Directory for Cancer (test code M215.2), and is therefore available through your local Genomic Laboratory Hub. The test is centrally commissioned so you should not be charged for the test itself, however, the cellular pathology preparation costs need to be met by the local hospital. The GLH cannot usually accept tissue blocks - they require either tissue curls or slide-mounted sections depending on agreed local pathways, with an assessment of tumour %, necrosis % and overall cellularity. If macrodissection is required, they will need an associated H&E slide marked up for the enriched tumour area. It is important that a clean microtome is used when sectioning for DNA extraction, and ideally the pathologist performing the tissue assessment will have completed the Health Education England training and take part in the GenQA Tissue-i scheme (see links below). Further details regarding specific sample requirements should be obtained from your local GLH directly. For centres that are not yet able to perform their own pre-analytical preparation work (sectioning/assessment), other cellular pathology laboratories in your Pathology Alliance may be able to undertake this for you (likely for a fee!).

https://www.england.nhs.uk/publication/national-genomic-test-directories
https://www.england.nhs.uk/genomics/genomic-laboratory-hubs
https://www.genomicseducation.hee.nhs.uk/education/online-courses/tumour-assessment-in-the-genomic-era/
https://genqa.org/tissuei

7. Reporting process:

It is very helpful for the wider MDT if a consistent approach is used for reporting, including the use of standardised templates. The template reports below have been developed through experience of reporting >5,000 MMR cases in Leeds and building on the national recommendations for reporting Lynch screening in EC.

The template reports consist of three sections, the first describes the pattern of staining (including reference to an internal positive control), and the second describes the clinicopathological correlation and recommendation. This is followed by a bottom line summary result. The template reports can be added to the pathology report without modification in most cases, however, rarer patterns do occur and will require some modification.

8. Standard reporting paragraphs for common scenarios.

Normal nuclear expression of all four antibodies:

Immunohistochemistry Result:
Tumour cell nuclei show positive staining for the mismatch repair proteins MLH1, PMS2, MSH2 and MSH6. Associated stromal cells show similar positive nuclear staining.

Clinicopathological Correlation:
There is no evidence of mismatch repair deficiency. According to the NICE guideline for Lynch syndrome screening, this patient does not require referral to Clinical Genetics services, although referral should be considered despite this result in the presence of a strong family/clinical history.

SUMMARY RESULT:
NO EVIDENCE OF MISMATCH REPAIR DEFICIENCY.

MLH1 and PMS2 loss:

Immunohistochemistry Result:
Tumour cell nuclei show loss of expression of the mismatch repair proteins MLH1 and PMS2. Associated stromal cells show positive nuclear staining for both markers. Both MSH2 and MSH6 demonstrate normal nuclear expression within the invasive tumour and associated stromal cells.

Clinicopathological Correlation:
The loss of MLH1 and PMS2 expression by immunohistochemistry indicates mismatch repair deficiency that may be either somatic or due to Lynch or related syndromes. According to the NICE guideline for Lynch syndrome screening, MLH1 promoter methylation analysis will be undertaken to provide further information. A supplementary report will be issued when these results become available.

SUMMARY RESULT:
MISMATCH REPAIR DEFICIENCY DETECTED (MLH1/PMS2 LOSS), MLH1 PROMOTER METHYLATION ANALYSIS REQUESTED.

Isolated PMS2 loss:

Immunohistochemistry Result:
Tumour cell nuclei show loss of expression of the mismatch repair protein PMS2. Associated stromal cells show positive nuclear staining for this marker. MLH1, MSH2 and MSH6 demonstrate normal nuclear expression within the invasive tumour and associated stromal cells.

Clinicopathological Correlation:
The loss of PMS2 expression by immunohistochemistry indicates mismatch repair deficiency that may be associated with Lynch or related syndromes. This patient should be referred to Clinical Genetics services, if clinically appropriate.

SUMMARY RESULT:
MISMATCH REPAIR DEFICIENCY DETECTED (PMS2 LOSS), CLINICAL GENETICS REFERRAL ADVISED.

MSH2 and MSH6 loss:

Immunohistochemistry Result:
Tumour cell nuclei show loss of expression of the mismatch repair proteins

MSH2 and MSH6. Associated stromal cells show positive nuclear staining for both markers. Both MLH1 and PMS2 demonstrate normal nuclear expression within the invasive tumour and associated stromal cells.

Clinicopathological Correlation:
The loss of MSH2 and MSH6 expression by immunohistochemistry indicates mismatch repair deficiency that may be associated with Lynch or related syndromes. This patient should be referred to Clinical Genetics services, if clinically appropriate.

SUMMARY RESULT:
MISMATCH REPAIR DEFICIENCY DETECTED (MSH2/MSH6 LOSS), CLINICAL GENETICS REFERRAL ADVISED.

Isolated MSH6 loss:

Immunohistochemistry Result:
Tumour cell nuclei show loss of expression of the mismatch repair protein MSH6. Associated stromal cells show positive nuclear staining for this marker. MLH1, PMS2 and MSH2 demonstrate normal nuclear expression within the invasive tumour and associated stromal cells.

Clinicopathological Correlation:
The loss of MSH6 expression by immunohistochemistry indicates mismatch repair deficiency that may be associated with Lynch or related syndromes. This patient should be referred to Clinical Genetics services, if clinically appropriate.

SUMMARY RESULT:
MISMATCH REPAIR DEFICIENCY DETECTED (MSH6 LOSS), CLINICAL GENETICS REFERRAL ADVISED.

Complete loss of MLH1/PMS2 with clonal areas or MSH6 and MSH2 loss:

Immunohistochemistry Result:
Tumour cell nuclei show diffuse loss of expression of the mismatch repair proteins MLH1 and PMS2, and clonal areas of loss of MSH2 and MSH6. Associated stromal cells show positive nuclear staining for all four markers throughout.

Clinicopathological Correlation:
The loss of MLH1 and PMS2 expression by immunohistochemistry indicates mismatch repair deficiency that may be either somatic or due to Lynch or related syndromes. According to the NICE guideline for Lynch syndrome screening, MLH1 promoter methylation analysis will be undertaken to provide further information. A supplementary report will be issued when these results become available. If this mismatch repair deficiency is confirmed to be associated with hypermethylation of the MLH1 promoter region, the additional clonal loss of MSH2 and MSH6 is highly likely to be due to somatic double hit mutations in MSH2 due to the hypermutation status of deficient mismatch repair.

SUMMARY RESULT:
MISMATCH REPAIR DEFICIENCY DETECTED (MLH1/PMS2 LOSS WITH CLONAL MSH2/MSH6 LOSS), MLH1 PROMOTER METHYLATION ANALYSIS REQUESTED.

Complete loss of MLH1/PMS2/MSH2/MSH6 on biopsy:

Immunohistochemistry Result:
Tumour cell nuclei show diffuse loss of expression of the mismatch repair proteins MLH1, PMS2, MSH2 and MSH6. Associated stromal cells show positive

nuclear staining for all four markers throughout.

Clinicopathological Correlation:
Loss of all four mismatch repair proteins is rare but is recognised to occur (see J Clin Pathol 2019;72(6):443-447). The loss of MLH1 and PMS2 expression by immunohistochemistry indicates mismatch repair deficiency that may be either somatic or due to Lynch or related syndromes. According to the NICE guideline for Lynch syndrome screening, MLH1 promoter methylation analysis will be undertaken to provide further information. A supplementary report will be issued when these results become available. If this mismatch repair deficiency is confirmed to be associated with hypermethylation of the MLH1 promoter region, the additional loss of MSH2 and MSH6 is likely to be due to somatic double hit mutations in MSH2 +/- MSH6 due to the hypermutation status of deficient mismatch repair.

However, given that there is complete loss of all four proteins in a biopsy specimen, it is recommended that staining is repeated on the resection specimen, if the patient subsequently undergoes surgery, to determine whether the MLH2/MSH6 loss is global or clonal.

SUMMARY RESULT:
MISMATCH REPAIR DEFICIENCY DETECTED (COMPLETE MLH1/PMS2/MSH2/MSH6 LOSS),
MLH1 PROMOTER METHYLATION ANALYSIS REQUESTED.
REPEAT STAINING RECOMMENDED ON THE RESECTION SPECIMEN, IF THE PATIENT UNDERGOES SURGERY.

Additional comment to add to the 'immunohistochemistry result' section if there are artefactual areas of loss due to poor fixation - modify as appropriate if not all antibodies affected:

Some areas of weak/negative staining are observed in both tumour and associated stromal cells for all four antibodies, which are interpreted as being artefactual e.g. due to poor fixation.