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

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1. Introduction:

Lynch syndrome screening should be performed on all colorectal cancers (CRC) at diagnosis, in line with NICE diagnostics guidance (https://www.nice.org.uk/guidance/dg27). The primary screening test can either be immunohistochemistry (IHC) for the four mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, MSH6) or microsatellite instability (MSI) testing, to be followed by BRAF testing +/- MLH1 promoter methylation testing in cases showing MLH1 loss or MSI-high.

MMR IHC has some advantages over MSI testing in that it can be performed on tiny amounts of tumour (e.g. as seen in biopsies), it determines the likely genes affected (to correlate with the germline), it can identify clonal loss, and is easy to deliver in most histopathology departments with a significantly faster turnaround time. However, MSI testing is centrally commissioned and may be easier to implement in centres with funding or staffing constraints.

2. Which specimen and block to test:

Staining only needs to be performed once per tumour (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. This also avoids possible changes in protein expression due to neoadjuvant therapy. If the biopsy contains high grade dysplasia only, it is acceptable to stain this if no other specimen exists, but a caveat should be inserted into the report (see template reports below). 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 mucosa (to act as an internal control), where possible.

In patients with more than one cancer, ensure that staining has been performed independently on all tumours. Synchronous right and left sided cancers can show a different MMR status.

3. Recognised reporting pitfalls:

Normal staining should consist of clean nuclear staining within the tumour cells. Background stromal/inflammatory cells and normal mucosal epithelial cells at the crypt base provide a positive internal control. Loss of nuclear staining within the tumour, reduced intensity staining (when compared to adjacent stromal/normal epithelial 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.

One well recognised pitfall is loss of MSH6 protein expression after neoadjuvant therapy. If neoadjuvant therapy has been used and testing was performed on a resection block

showing isolated MSH6 loss, staining should be repeated on the pre-treatment biopsy if possible. The loss of MSH6 in this context is often patchy rather than complete.

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. Rarer patterns of loss include combined loss of PMS2/MSH6, MLH1/PMS2/MSH6 and loss of all four proteins (sometimes referred to as "null phenotype").

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 either BRAF mutation or 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.

Very rare patterns of loss include loss of staining in the tumour and adjacent stromal cells in constitutional mismatch repair deficiency (usually isolated PMS2 loss in children with multiple early onset cancers, due to the loss of both alleles in the germline) and mixed positive/negative cells in somatic mosaicism (usually due to reversion of an inherited mutation in a variable percentage of cells).

5. What to do if MLH1 is abnormal:

According to the NICE diagnostics guidance, cases with abnormal MLH1 should subsequently undergo BRAF codon 600 mutational analysis. BRAF testing should also be requested if MLH1/PMS2 are abnormal in association with abnormal expression of MSH6 +/- MSH2.

Some centres are sending cases showing MLH1 loss directly for MLH1 methylation testing and missing out the BRAF step. The advantage of this pathway is that it harmonises with endometrial cancer testing and reduces the number of tests required in a proportion of patients, although the turnaround times are likely to be longer and the failure rate higher when compared to BRAF testing. Other centres are missing out methylation testing and sending patients with no evidence of a BRAF mutation directly for germline testing, which reflects a recent change in the National Genomic Test Directory for Rare Disease testing where BRAF or methylation testing is now required prior to germline testing.

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

6. Requesting BRAF testing

BRAF mutational testing in CRC showing MLH1 protein loss appears on the National Genomic Test Directory for Cancer (test code M1.1), and is therefore available through your

local Genomic Laboratory Hub. The test will usually be performed as part of a multigene panel including analysis of the KRAS and NRAS genes. 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!).

If no BRAF mutation is detected, MLH1 promoter methylation testing should be performed if the NICE diagnostics guidance pathway is being followed in full. This may be automatically undertaken by the GLH on the residual DNA or it may require pathology to request it as a separate test, depending on agreed local pathways. Additional sections may be required for repeat DNA extraction. MLH1 promoter methylation testing also appears on the National Genomic Test Directory for Cancer (test code M1.5).

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 endometrial carcinoma.

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.

If the block contains high-grade dysplasia only and staining is normal:

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

Clinicopathological Correlation:
There is no evidence of mismatch repair deficiency in this specimen containing high-grade dysplasia. However, no adenocarcinoma is present in the material, therefore it is possible that the pattern of staining observed is not representative of any associated invasive component. If a subsequent sample containing invasive adenocarcinoma materialises, this should be sent for repeat testing. If no such sample is expected and there is any clinical suspicion of a hereditary bowel cancer syndrome, a Clinical Genetics referral should be made for consideration of genetic counselling and germline testing, if clinically appropriate.

SUMMARY RESULT:
NO EVIDENCE OF MISMATCH REPAIR DEFICIENCY (HIGH-GRADE DYSPLASIA ONLY TESTED).

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, BRAF codon 600 mutational 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), BRAF 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, BRAF codon 600 mutational 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 a BRAF mutation or 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), BRAF 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, BRAF codon 600 mutational 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 a BRAF mutation or 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), BRAF 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.

If there are extensive areas of loss that are most likely due to poor fixation but no alternative material is available to test and the status is not entirely conclusive, MSI testing should be requested for confirmation and the correlation and results paragraphs modified as below:

Clinicopathological Correlation:
There is no convincing evidence of mismatch repair deficiency, however areas of poor fixation preclude a definitive assessment. MSI testing will be undertaken for confirmation of the MMR/MSI status. A supplementary report will be issued when these results become available.

SUMMARY RESULT:
MISMATCH REPAIR STATUS NOT CONCLUSIVE DUE TO POOR FIXATION, MSI TESTING REQUESTED.