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Title

Molecular assessment of colorectal cancer through Lynch syndrome screening

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Unstructured abstract (150 words)

Since 2017, the National Institute for Health and Care Excellence (NICE) has recommended molecular testing of all patients with newly diagnosed colorectal cancer (CRC) to identify those with suspected Lynch syndrome who should be referred to clinical genetics for germline testing. The pathway involves firstly determining the mismatch repair (MMR) expression status by immunohistochemistry (IHC) or performing microsatellite instability testing. This may be followed by BRAF V600E mutation testing and then MLH1 promotor hypermethylation analysis depending on the result. This approach identifies patients that are most likely to have underlying germline mutations in the MMR genes as opposed to somatic causes of deficient MMR. Here we demonstrate a case with loss of MLH1 protein expression and discuss the subsequent testing strategy according to NICE guidance.

Keywords

Mismatch repair, Lynch syndrome, colorectal cancer,

Main text

Case report

A 54 year old woman presented to her GP with fatigue and altered bowel habit. She was referred for an urgent colonoscopy which revealed an exophytic caecal tumour from which biopsies were taken. Histological examination showed fragments of partially ulcerated large intestinal mucosa with high grade adenomatous dysplasia. Focally there was evidence of neoplastic glands in the submucosa with surrounding desmoplasia, in keeping with invasive moderately differentiated adenocarcinoma.

As per NICE recommendations, the biopsy underwent subsequent molecular testing. MMR protein expression was initially assessed by IHC. This showed a diffuse loss of nuclear expression of MLH1 and PMS2 within the tumour cells. Background stromal cells and normal mucosa showed retained expression of these two markers. Normal nuclear expression of MSH2 and MSH6 was noted throughout the tumour, stromal cells and normal mucosa (Figure 1). As the IHC showed loss of MLH1, BRAF codon 600 mutation status was ascertained by pyrosequencing. Macrodissection was performed prior to DNA extraction with plenty of tumour cells in the extracted area (estimated tumour percentage = 50%). Pyrosequencing showed no evidence of a BRAF mutation (Figure 2). Finally, due to the wild type BRAF result, MLH1 promotor methylation testing was performed by pyrosequencing, which showed the presence of hypermethylation of the MLH1 promoter region

(Figure 3). This result is in keeping with somatic MLH1 gene silencing due to an epigenetic event rather than a germline mutation. The IHC, BRAF and methylation results were summarised in a supplementary report to the original biopsy with a recommendation that a clinical genetics referral was not required in this case.

Discussion

Approximately 15% of colorectal cancers develop in association with MMR deficiency (dMMR), approximately 80% of which is caused by somatic changes, usually hypermethylation of the MLH1 promoter region [1]. This occurs more commonly in right sided, elderly female patients. However, around 3% of CRC is due to underlying germline mutations in the MMR genes known as Lynch syndrome [2]. In order to determine which patients should be referred for germline testing, an initial multistep testing approach on the tumour tissue is recommended by NICE. This should allow more patients with Lynch syndrome to be diagnosed and managed accordingly, and for family members to be screened.

There are two options for the first line of tumour testing: microsatellite instability (MSI) testing or IHC for the four MMR proteins; MLH1, MSH2, MSH6, PMS2. MMR proteins function as dimers of MLH1/PMS2 and MSH2/MSH6. Within these dimers, MLH1 and MSH2 are the dominant protein, and are required to be functional for normal PMS2 and MSH6 expression. However, MLH1 and MSH2 expression is retained with an underlying defect in PMS2 or MSH6 respectively. For patients with somatic loss of MMR protein tumour expression, the vast majority show MLH1 loss, with losses of the other MMR proteins being less common. As a result, the NICE guidance recommends a direct referral to clinical genetics if the MSH2, MSH6 or PMS2 IHC result is abnormal. Note that in this context, PMS2 loss requires isolated loss of PMS2 with retained MLH1 expression.

If there is either evidence of MSI or MLH1 protein loss, this is significantly more likely to be caused by somatic changes rather than Lynch syndrome. BRAF V600E mutations are strongly associated with somatic MLH1 loss but are almost never seen in patients with Lynch syndrome. For this reason BRAF mutational analysis is performed next in these patients to identify those with likely somatic causes [3].

Finally for those patients with MSI or MLH1 loss and wild type BRAF status, the NICE guidance recommends methylation analysis of the MLH1 promotor region. The remaining patients with MSI/MLH1 loss and no evidence of a BRAF mutation or MLH1 promoter hypermethylation are also recommended for germline testing to exclude Lynch syndrome.

Beyond the identification of Lynch syndrome, there is significant value in MMR testing as a prognostic and predictive biomarker. Patients with dMMR tumours have a more favourable stage-adjusted prognosis and are less likely to metastasize [4]. Some studies have shown that dMMR tumours respond less well to 5-fluorouracil based chemotherapy [5]. However, there is currently much interest in the role of immune blockade for these patients as early studies show they receive significant clinical benefit with immunotherapy [6].

Conclusion

Routine molecular testing of all colorectal cancers is recommended by NICE to identify individuals who require referral to clinical genetics to test for Lynch syndrome. The tumour testing pathway is a

multistep process to rule out those that are more likely due to somatic loss of MMR gene function. MMR status also provides useful prognostic/predictive information and may act as a future biomarker to select patients for immune checkpoint blockade.

Practice points

- 15% of CRC show MMR protein deficiency, of which 12% are somatic and 3% are due to underlying germline mutations known as Lynch syndrome
- A multi-step tumour testing approach is recommended by NICE to determine patients who are most likely to be germline carriers
- Initial testing includes either MSI or MMR IHC, followed by BRAF mutation analysis and MLH1 promotor methylation testing in patients with MLH1 loss.

Self Assessment questions

PMS2 forms a dimer with which of the other following mismatch repair proteins:

A. MSH2
B. MLH1
C. MSH6
D. MLH3

Answer: B MLH1

Which of the following is the NICE recommended first line test for molecular assessment of colorectal cancers for Lynch screening?

A. BRAF protein expression or BRAF mutation status
B. MLH1 promoter hypermethylation
C. KRAS mutation testing
D. Microsatellite instability testing or mismatch repair protein IHC
E. PMS2 gene mutation testing

Answer: D Microsatellite instability testing or mismatch repair protein IHC

Sporadic mismatch repair loss is associated with which of the following
A. Right sided colorectal tumours
B. Left sided colorectal tumours
C. Rectal tumours
D. Anal tumours

Answer: A Right sided colorectal tumours

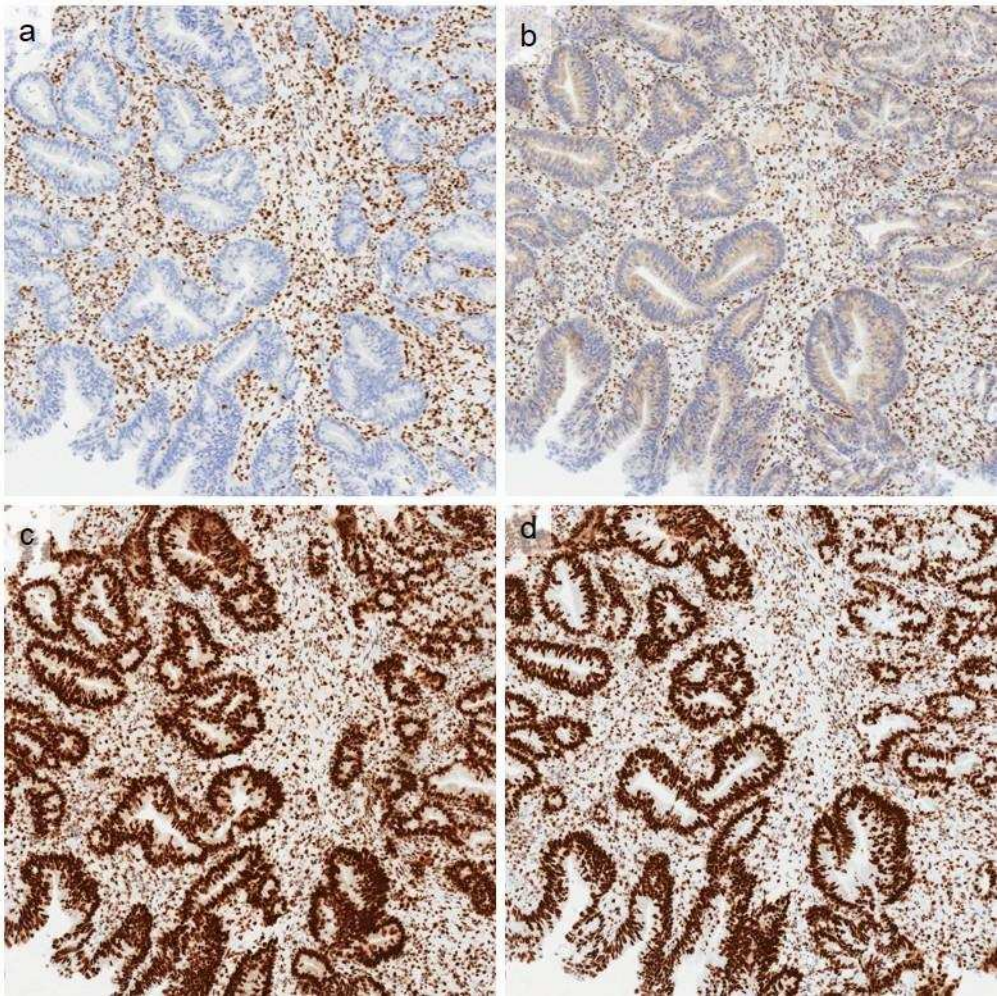

Figure 1. Four-panel IHC for the MMR proteins (a) loss of MLH1 expression in the tumour cells. **(b)** loss of PMS2 expression in the tumour cells. **(c)** normal positive MSH2 expression. **(d)** normal positive MSH6 expression

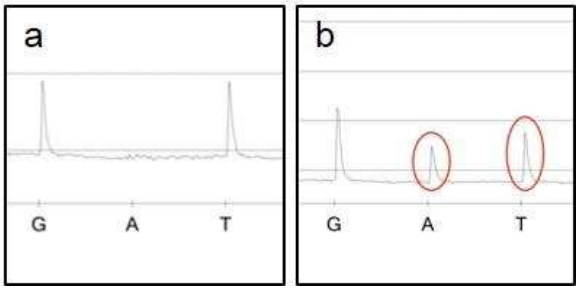

Figure 2. Pyrogram for BRAF codon 600 mutation status. (a) Result from this case showing a wild-type result with no "A" peak present and a normal sized "T" peak as expected at this site; wild-type sequence guanine (G) followed by thymine (T) . **(b)** Example of a mutant result with a reduced height of the "T" peak and an extra A peak present showing that a significant proportion of the DNA tested contain the V600E mutation; mutated sequence guanine (G) followed by adenine (A).

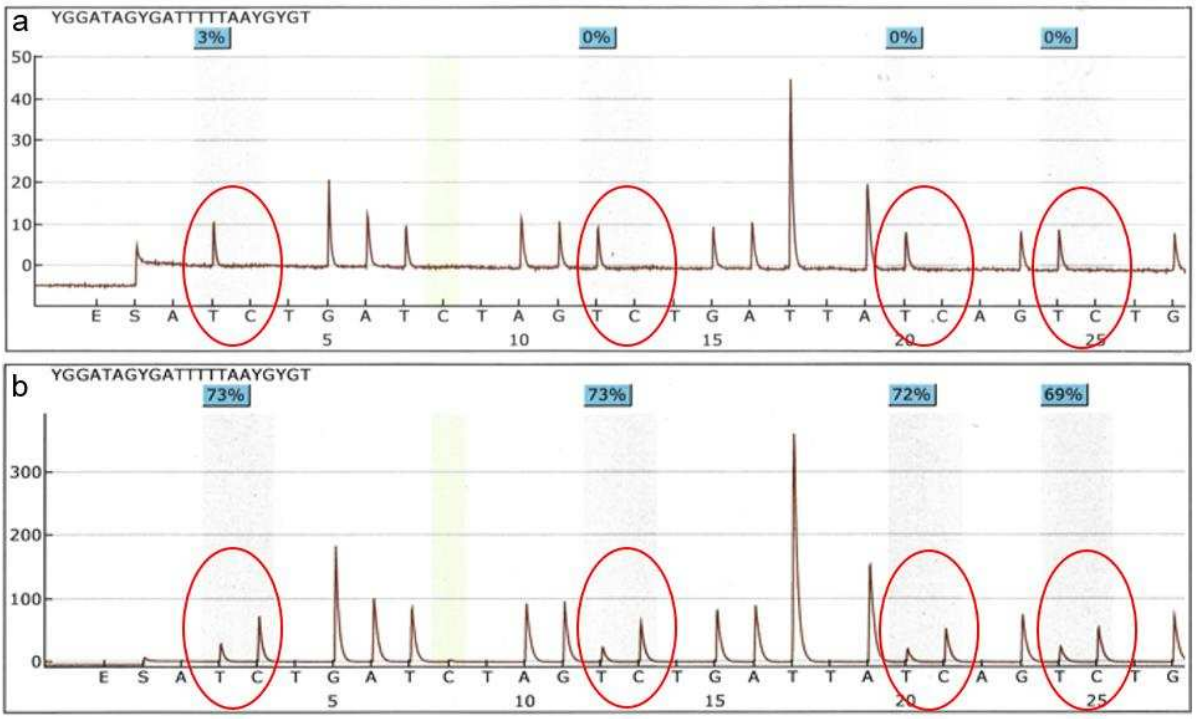

Figure 3. Pyrogram for Methylation Assay for MLH1 promoter region. (a) Example of an unmethylated result. **(b)** Result from this case showing the presences of hypermethylation. At the positions highlighted, the proportion of the "C" peak height compared to the preceding "T" peak height indicates if hypermethylation is present.

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