Protocols 5-8

Background information for Lynch Syndrome evaluation and investigation

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Q: What is the sensitivity of IHC?
Overall IHC is 95-100% sensitive (Shia, 2008, Caldes et al., 2004). MLH1 can give false positive staining, which typically appears “patchy” (van Riel et al., 2010, Shia, 2008, Mangold et al., 2005). Hence, any abnormality in staining of MLH1 should be investigated as per absence of MLH1, starting with BRAF mutation testing (Shia, 2008).

Q: What is the likely explanation if the IHC is abnormal and the germline mutational testing is normal?
Current sequencing and MLPA technologies are highly sensitive for detecting Lynch syndrome. A MMR tumour phenotype, resulting in abnormality in IHC and/or MSI, commonly arises from somatic tumour events, including somatic MLH1 promoter hypermethylation and biallelic large scale deletions/point mutations (Sourrouille et al., 2012) (Cunningham et al., 2001). Mechanisms of pathogenic mutation aside from those detectable by sequencing of the germline and MLPA (including the EPCAM region) have been reported very infrequently. It is therefore not appropriate to manage these individuals/families as Lynch syndrome on the basis of the abnormal IHC alone. If there is a significant family history, IHC should be sought in a second family member (see FAQs) and if there is concordance of the IHC abnormality, additional studies should be initiated. Otherwise, these individuals/families should be managed as negative for Lynch and advised colonoscopic surveillance on the basis of their family history of cancer.

Q: What is the role of BRAF mutation testing?
Overall, ~ 15% of all colorectal cancers tumours show a MMR tumour phenotype (ie abnormality on IHC/microsatellite instability/). About 70-80% these (and ~90% of MMR phenotype with loss of MLH1) will be sporadic and due to somatic MLH1 promoter hypermethylation. The BRAF mutation is present in ~70% of these cases and is a useful test by which to filter out these sporadic tumours (Cunningham et al., 2001). BRAF V600E is extremely rare in Lynch syndrome tumours (Bouzourene et al., 2010).

Q: Does somatic MLH1 promoter hypermethylation cause MMR phenotype in other cancers?
Yes. Overall ~20-30% of all endometrial cancers show a MMR tumour phenotype (ie microsatellite instability/abnormality on IHC). Of these ~10% are due to germline mutations and 80-90% are due to MLH1 promoter hypermethylation. However, the BRAF V600E mutation is rare in endometrial tumours, so can not be used to filter out this sporadic group (Peterson et al., 2012).
Q: Does somatic promoter hypermethylation of other genes cause a MMR phenotype?
No. It seems that only MLH1 is commonly mutated somatically via somatic promoter hypermethylation to give a MMR-phenotype (Nagasaka et al., 2010). However (see above) other biallelic somatic events in other genes can likewise cause a MMR phenotype and abnormality on IHC.

Q: What is the relative proportion of sequence mutations versus other mechanisms of mutation?
~5-10% of germline MLH1 mutations, ~20-50% of germline MSH2 mutations and ~30% of mutations in PMS2 are due to large deletions/insertions. Large deletions/rearrangements of MSH6 are rare (Vaughn et al., 2010). MLPA should be performed as an adjunct to testing of any of the 4 genes. Constitutional epimutations of MLH1 arise infrequently. These will be detectable on paired MLH1 promoter methylation studies of blood and tumour: abnormalities of promoter methylation will be detected in the germline as well as the tumor. There is a recurrent deletion upstream of MSH2 in the EpCam gene (formerly known as TACSTD1) which alters MSH2 methylation, silencing gene expression and accounts for ~5% of MSH2-related Lynch syndrome (Rumilla et al., 2011). This deletion detectable via MLPA of MSH2 using the current MRC Holland kit. There is little evidence for more widespread causes of heritable MSH2 hypermethylation beyond that caused by the EpCAM deletion (Rumilla et al., 2011). There are two cases in the literature in which there is loss of MSH2, unexplained by germline mutation, abnormal MSH2 promoter methylation and no EpCAM deletion (Nagasaka et al., 2010) (Rumilla et al., 2011). We do not currently recommend MSH2 promoter hypermethylation analysis.

Q: Are there other mechanisms by which the IHC protein loss can be entirely accounted for by tumour-specific mutational events?
Yes. Sourreille et al studied tumours showing loss of proteins using HRM and MLPA and reported detecting double somatic hits (not present in the germline) in 3/10 MSH2 deficient CRC tumour samples and 1/7 MLH1 deficient tumour. A single somatic hit was detected for 4/10 and 3/7 (second hit could be tumour or germline) (Sourrouille et al., 2012). Somatic tumour events are reported in a number of series and are likely to account for the majority of cases in which there is loss of staining on IHC with no germline mutation detected (Borresen et al., 1995, Jeong et al., 2003). It is therefore not appropriate to manage such individuals/families as Lynch syndrome but they should be managed on the basis of their family history of cancer and advised screening accordingly (eg colonoscopy on the basis of family history of CRC).

Q: What patterns of IHC loss of staining are typical?
Typically:
- Germline mutation (or promoter hypermethylation) of MLH1 will result in concurrent loss of MLH1/PMS2 or, less commonly, isolated loss of MLH1
- Mutation of MSH2 will result in loss of MSH2/MSH6 or, less commonly, isolated loss of MSH2
- Mutation of PSM2 will result in isolated loss of PMS2
- Mutation of MSH6 will result in isolated loss of MSH6
Occasionally:
- Mutation of MLH1 will result in seemingly isolated loss PMS2 due to false positive staining of MLH1 (although typically this staining is ‘patchy’)
- Mutation in MSH2 will result in isolated loss of MSH6

Q: Does the presence of a K-RAS tumour mutation guide investigation for Lynch Syndrome?
No. KRAS mutations are common (~40%) in Lynch CRCs (Oliviera 2004). This frequency of KRAS mutations is comparable to MSS sporadic CRC. There is a significantly lower but appreciable frequency of KRAS mutations (~22%) in sporadic MS-H (MLH1-promoter hypermethylation) group. KRAS and BRAF mutations can co-occur (Stepanius 2011).

Q: Should MLH1 germline mutational testing be undertaken if there is isolated loss of PMS2?
Yes. If germline mutational testing of PMS2 is normal. This is because there can be false positive staining of MLH1. Series have detected a frequency of pathogenic germline mutations in MLH1 in individuals with apparently isolated loss of PMS2 in the tumour (Halvarsson et al., 2006)

Q: Should PMS2 germline mutational testing be undertaken if there is loss of MLH1+PMS2 and no germline mutation in MLH1 is detected?
No. Series have detected no frequency of pathogenic germline mutations in PMS2 in individuals with loss of MLH1+PMS2 (Clendenning et al., 2013).

Lynch Syndrome: Background

Q: How common is Lynch syndrome?
Population prevalence: 1:440 (Chen et al., 2006); accounts for ~ 1-3% of CRCs (Cunningham et al., 2001, Hampel et al., 2005)

Q: What is the relative proportion of mutations four Lynch genes in Lynch families
From an unselected series of 1066 colorectal cancers, the relative proportions were: 5 MLH1, 13 MSH2, 3 MSH6, 2 PMS2 (Hampel et al., 2005)
Mutations in PMS2 are generally only reported to account for only ~2-5% of Lynch overall (Hampel et al., 2005, Talseth-Palmer et al., 2010). However, historically, in some reported series, there may be under-ascertainment of MSH6 and PMS2 as the genes have been identified more recently and the cancer risks are lower. PMS2 is difficult to sequence on account of pseudogenes and requires more complex sequencing approaches eg long-range techniques.

Q: What is the difference between the different Lynch clinical criteria
**Amsterdam I** criteria just include colorectal cancer and stipulate: three cases, one case <50y, cases over 2 generations, one individual must be FDR of other two, cancers must be confirmed, polyposis (ie FAP) has been excluded (Vasen et al., 1991).

**Amsterdam II** is an extension of Amsterdam I to include other Lynch-related tumours (colorectal, endometrial, ovarian, gastric, small intestinal, hepatobiliary, renal pelvic and transitional cell ureteric carcinomas (Vasen et al., 1999)

**Original Bethesda** (1997) criteria were developed to be more inclusive than Amsterdam criteria included two cases (one<50) and single case<45 (Rodriguez-Bigas et al., 1997). The list of Lynch-related tumours was the same as Amsterdam II.

**Updated Bethesda** (2004) criteria are more inclusive than the original Bethesda criteria and also includes CRC diagnosed in 3 or more FDRs or SDRs of any age (FDR or SDR of proband), a single case of CRC<50, multiple CRCs (synchronous/metchronous) or other Lynch-related tumours in the same individual at any age and adenomas in an individual<45. The spectrum of Lynch-related cancers was extended to include pancreatic, brain (glioblastoma) and skin tumours. These guidelines were published in two journals with some inconsistencies and there is contention regarding the conclusions of the meeting, in particular with regard to the inclusion of individuals with adenomas age <45 and the relaxation of three cases who can be FDRs or SDRs (Umar et al., 2004a, Umar et al., 2004b)

Q: **What is the germline mutation pick-up rate the different Lynch clinical criteria?**
Amsterdam II: pick-up rate: ~50%
Bethesda: pick-up rate: ~20% (Hampel et al., 2005, Syngal et al., 2000))

Q: **Which tumours should be considered part of Lynch syndrome?**
The Amsterdam criteria include: colorectal, endometrial, ovarian, gastric, small intestinal, hepatobiliary, renal pelvic and transitional cell ureteric carcinomas (Vasen). The revised Bethesda criteria also includes: brain, pancreas and skin. The inclusion of pancreatic cancer is contentious: the frequency of pancreatic cancer has been reported in some series to be elevated in Lynch; HR 8.6 (4.7-15.7) (Kastrinos et al., 2009). The associations of Lynch syndrome with brain tumours (most commonly Glioblastoma) is referred to as Turcot syndrome. The brain tumours typically exhibit abnormality on IHC/MSI. The co-occurrence of an internal Lynch-related tumour with sebaceous neoplasms (typically sebaceous adenomas, sebaceous epitheliomas, sebaceous carcinomas, and keratoacanthomas) is referred to as Muir Torre syndrome. The skin lesions typically exhibit MSI and are most strongly associated with mutations in MSH2 and MSH6.

Q: **Are the cancer risks in Lynch consistent across the genes?**
No. There is good evidence that mutations in MSH6 confer a lower risk of CRC (possibly particularly to women) and a higher risk of endometrial cancer (Ramsoekh et al., 2009, Baglietto et al., 2010, Bonadona et al., 2011, Barrow et al., 2013, Hendriks et al., 2004, Plaschke et al., 2004). The cancer risks associated with mutations in PMS2 are lower in reported series but less accurately defined.
MUTYH Polyposis syndrome: background

Q: How common is MUTYH-related CRC?
In a large meta-analysis, in all population-based CRC, the frequency was of individuals with biallelic mutations was 101/25231 (0.4%) (Theodoratou et al., 2010).

Q: What are the cancer risks associated with biallelic mutations in MUTYH?
~80% by age 80 (95% CI 65%-85%); penetrance by age 60 ~43% (30-58%) (Lubbe et al., 2009)

Q: What is the age of onset of CRC associated with MUTYH?
The mean age of CRC was 49.5 (Y179C homozygotes); 57.9 (G392D homozygotes) and 52.5 y (compound heterozygotes) (Lubbe et al., 2009). In this series 0/257 CRC diagnosed at <40 years carried biallelic mutations in MYH.

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Q: What is the mutational spectrum of MUTYH?
Y179C and G396D (previously designated Y165C and G382D) account for > 90% of all pathogenic mutations in MUTYH in Western European populations (ratio ~3 G396D :2 Y179C). The combined carrier frequency in the population is ~1% (Lubbe et al., 2009, Theodoratou et al., 2010). Other mutations in MUTYH can be detected on sequencing but are individually rare (Dallosso et al., 2008). Other founders include:
- Northern European origin c.1147delC (p.Ala385ProfsX23)
- Dutch c.1214C>T (p.Pro405Leu)
- Italian c.1437_1439del (p.Glu480del)
- British Indian c.1438G>T (p.Glu480X)
- Pakistani p.Tyr104X
- Spanish, Portuguese
- Tunisian c.1227_1228dup (p.Glu410GlyfsX43)
- Japanese, Korean p.A359V. However, these account for lower proportions of disease in these populations.

Q: Do MUTYH-related CRCs typically demonstrate microsatellite instability?
No. In a series of 27 individuals with CRC and biallelic mutations in MUTYH, 9/9 for whom tumour tissue was available demonstrated microsatellite stability (Lubbe et al., 2009)

Q: Are polyps always present in those with MUTYH-related CRC?
No. In a series of 27 individuals with CRC and biallelic mutations in MUTYH, in those in whom there was reliable polyp information, 30% had no synchronous polyps and 30% had <3 synchronous polyps and ~40% had >3 polyps(Lubbe et al., 2009)

Q: Do monoallelic mutations confer a risk of CRC?
In a large meta-analysis, it seems that a marginally significant mono-allelic effect was demonstrated for the Y179C variant alone. This confers a small, non-clinically relevant risk of CRC (OR 1.34 (95% CI: 1.00–1.80))

See: http://www.icr.ac.uk/protocols
Polyps and National Bowel screening

Q: How common are adenomatous polyps in the general population?
The prevalence of adenomas at autopsy and via colonoscopic studies in the general population is 15% (age 50-59) and 33% (age 70+)(DiSario et al., 1991, Loeve et al., 2004)

Q: What types of polyps are more likely to progress into adenocarcinomas?
Adenomatous polyps have greater malignant potential than hyperplastic polyps or hamartomatous polyps. The likelihood for malignant progression in adenomatous polyps is greater for Villous>Tubulovillous>Tubular and for sessile (overall ~36% malignancy rate) >pedunculated (overall <10% malignancy rate).
The likelihood of progression relates to adenomatous polyp size: <1cm (<3% malignancy rate), 1-2cm (20% malignancy rate), >2cm (58% malignancy rate)

Q: How rapidly do adenomatous polyps grow and become cancerous?
Studies suggest that the transformation from an adenoma into a carcinoma typically takes > 5 years and may be as long as 20 years (Morson, 1976). This process may be more rapid for MMR-pathway than CIN-pathway CRCs, and if there is germline predisposition.

Q: What program of screening is offered within the National Bowel Cancer Screening Programme
FOB kits are sent out biennially to individuals age≥ 60. Individuals with abnormal results are offered colonoscopy.
See (http://www.cancerscreening.nhs.uk/bowel).

Q: What is the cancer detection rate of the NBCSP
In the pilots, the return rate of FOB kits was 55-60% (Professor David et al., 2006). The FOB test is positive in 1-2% of individuals. In these ~1/10 have CRC and 4/10 have adenomatous polyps.

Q: Are any advances planned in the NBCSP
One-off flexible sigmoidoscopy at 55 is due to be introduced in soon as part of the NBCSP.
References


R., NIELSEN, M., PARRY, S., TYLER, E., MOSKVINA, V., CHEADLE, J. P. & SAMPSON, J. R. 2008. Inherited predisposition to colorectal adenomas caused by multiple rare alleles of MUTYH but not OGG1, NUDT1, NTH1 or NEIL 1, 2 or 3. Gut, 57, 1252-5.


PLASCHKE, J., ENGEL, C., KRUGER, S., HOLINSKI-FEDER, E., PAGENSTECHER, C., MANGOLD, E., MOESLEIN, G., SCHULMANN, K., GEBERT, J., VON KNEBEL


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