

# Molecular testing for Lynch syndrome in people with colorectal cancer: systematic reviews and economic evaluation

Tristan Snowsill,<sup>1\*</sup> Helen Coelho,<sup>1</sup> Nicola Huxley,<sup>1</sup>  
Tracey Jones-Hughes,<sup>1</sup> Simon Briscoe,<sup>1</sup> Ian M Frayling<sup>2</sup>  
and Chris Hyde<sup>1</sup>

<sup>1</sup>Peninsula Technology Assessment Group (PenTAG), University of Exeter, Exeter, UK

<sup>2</sup>Institute of Cancer and Genetics, University Hospital of Wales, Cardiff, UK

\*Corresponding author [t.m.snowsill@exeter.ac.uk](mailto:t.m.snowsill@exeter.ac.uk)

**Declared competing interests of authors:** Ian M Frayling reports personal fees from the UK National External Quality Assessment Scheme for Immunocytochemistry and In Situ Hybridisation (UK NEQAS ICC and ISH) outside the submitted work and is Honorary Medical Adviser to the charity Lynch Syndrome UK.

Published September 2017

DOI: 10.3310/hta21510

## Scientific summary

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Health Technology Assessment 2017; Vol. 21: No. 51

DOI: 10.3310/hta21510

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# Scientific summary

## Background

Lynch syndrome (LS) is the most common form of genetically defined hereditary colorectal cancer (CRC), accounting for 3.3% of colorectal tumours. In the UK, it is estimated that 175,000 people have LS and this leads to > 1100 CRCs per year.

Lynch syndrome is caused by constitutional pathogenic mutations in any one of four deoxyribonucleic acid (DNA) mismatch repair (MMR) genes: *MLH1* (MutL homologue 1), *MSH2* (MutS homologue 2), *MSH6* (MutS homologue 6) and *PMS2* (postmeiotic segregation increased 2). Some constitutional mutations in the *EPCAM* (epithelial cell adhesion molecule) gene also lead to LS through epigenetic silencing of *MSH2*.

For simplicity, the term 'Lynch syndrome' refers to the presence of mutations that cause LS, whether or not an individual has been affected by cancer.

Lynch syndrome is inherited in an autosomal dominant pattern, so if one parent has LS there is a 50% chance of each child inheriting it.

Lynch syndrome increases the risk of CRC, as well as the risks of gynaecological and other cancers. These cancers are frequently observed at younger ages than is typically seen with sporadic cancers.

Frequent colonoscopy with polypectomy in individuals with LS is believed to reduce the incidence of CRC (by allowing the removal of precancerous adenomatous polyps) and improve the survival of CRC (by diagnosing CRC at an earlier stage). Other risk-reducing measures have varying levels of evidence for their effectiveness.

There are usually no signs or symptoms of LS prior to developing cancer, but a blood test can identify constitutional pathogenic mutations. Individuals unaffected by cancer but at high risk of LS can be identified through family history. If a pathogenic mutation is already identified in a relative then a simple predictive genetic test can be performed.

Colorectal cancer patients at risk of LS can also be identified through tests on their tumour cells or DNA. The majority of colorectal tumours from individuals with LS have two distinguishing characteristics: microsatellite instability (MSI) and loss of expression of the MMR proteins.

Deoxyribonucleic acid microsatellites are short repetitive sequences found throughout the human genome. When the MMR system is defective, the lengths of these sequences become variable as errors go uncorrected; this is known as MSI. Molecular MSI testing involves polymerase chain reaction amplification of DNA markers from a microdissected tumour tissue sample and a matched healthy tissue sample from the same patient. Instability in  $\geq 30\%$  of markers is usually denoted as MSI-high (MSI-H), instability in  $< 30\%$  of markers is denoted as MSI-low (MSI-L) and no instability in any markers is denoted as microsatellite stable (MSS).

Loss of expression of MMR proteins can be identified using immunohistochemistry (IHC). Antibodies for the four MMR proteins are used to stain tumour and non-tumour cells. If nuclear staining is abnormal for any MMR protein(s), this suggests that the MMR system is affected.

Some non-LS colorectal tumours display MSI and/or abnormal staining. These are often caused by acquired hypermethylation of the *MLH1* promoter, which can be tested for directly. This is often accompanied by a

somatic V600E mutation in the *BRAF* gene, which is rarely seen in cancers from individuals with LS. *MLH1* promoter methylation and/or *BRAF* V600E testing are used to rule out LS.

If tumour-based tests are suggestive of LS then testing for pathogenic mutations in the MMR and *EPCAM* genes is offered to patients, after genetic counselling.

It is suggested that testing for LS in CRC patients (and then cascading testing to relatives if appropriate) can improve health outcomes and be a cost-effective use of health-care resources.

## Objectives

To investigate whether testing for LS in CRC patients using MSI or IHC (with or without *MLH1* promoter methylation testing and *BRAF* V600E testing) is clinically effective (in terms of identifying LS and improving outcomes for patients) and whether or not it represents a cost-effective use of NHS resources.

## Review of test accuracy studies

### Methods

A systematic review was conducted to assess the diagnostic accuracy of MSI testing and MMR IHC for identifying LS in CRC patients.

Bibliographic databases (including MEDLINE, EMBASE, Web of Science and The Cochrane Library) were searched using terms for LS and terms for MSI or IHC. The search results were limited by date from 2006 and to English-language studies. To identify relevant studies published before 2006 (and any additional studies published after 2006) prespecified and newly identified systematic reviews were screened.

Two reviewers independently assessed titles and abstracts, as well as full-text papers, using prespecified inclusion and exclusion criteria. References were included if they described single-gate or two-gate diagnostic studies recruiting individuals with CRC and investigating the test accuracy of MSI testing and/or MMR IHC, with or without *BRAF* V600E mutation testing and with or without *MLH1* promoter methylation testing. The index test(s) must have been compared with a reference standard and data had to be provided to enable the estimation of sensitivity. Other outcomes were specificity, positive and negative likelihood ratios (LR+ and LR-), positive and negative predictive values (PPV and NPV), concordance with the reference standard, diagnostic yield and test failure rates.

The reference standard was constitutional MMR mutation testing, which had to include DNA sequencing of *MLH1*, *MSH2* and *MSH6* and multiplex ligation-dependant probe amplification (or another appropriate technique for detecting large genomic abnormalities). All participants had to have received both the index test and the reference standard, although for studies recruiting a representative sample of all CRC patients (including studies with age limits) it was acceptable for the reference standard to be applied to all index test positives and to a representative sample of index test negatives.

Data extraction and quality appraisal [using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) tool] was performed by one reviewer and checked by a second. For studies that were not based on high-risk samples, sensitivity, specificity, LR+, LR-, PPV and NPV were independently calculated. For studies based on high-risk samples, only the sensitivity of the index test(s) was calculated. Studies that provided estimates of both sensitivity and specificity had their point estimates plotted in receiver operating characteristic (ROC) space. Because of insufficient data and heterogeneity in methods and results of studies, meta-analysis was not conducted. Narrative synthesis was conducted.

## Results

Ten studies met the diagnostic test accuracy review inclusion criteria. One study had two distinct samples; the results from all 11 samples were considered.

Quality appraisal did not indicate a high risk of bias for any of the studies.

Three of the studies were single-gate studies with population-based samples, with age limits. Four studies were single-gate studies based on high-risk populations. One study had two single-gate samples: a population-based sample (apparently unselected) and a sample based on a high-risk population. The remaining two studies used a variation on the two-gate study design, with only participants with positive reference standard results included. These studies have been termed 'reference standard positive' studies.

Across the studies the reference standard varied in terms of the sequencing methods and genes tested, the techniques used to test for large genomic alterations and deletions, the genes tested for large genomic alterations and deletions and whether unclassified variants were investigated. None of the included studies made a direct comparison between MSI and IHC.

Nine samples provided data on MSI. There was variation between studies in the MSI testing procedures used with regard to microdissection techniques, the panel of markers used and the categories of and thresholds for MSI.

In primary analyses unclassified variants were categorised as negative reference standard results and MSI-L was considered a negative index test result. Sensitivity estimates ranged from 66.7% to 100.0%. The specificity for MSI was calculated from three population-based samples (61.1–92.5%).

When MSI-L was considered to be a positive index test result sensitivity increased and specificity decreased.

Seven samples provided data to assess the accuracy of IHC. Five study samples split IHC data according to the protein assessed (MLH1, MSH2, MSH6 or PMS2), enabling assessment within those studies of the accuracy of IHC for individual proteins (i.e. whether the absence of a particular protein accurately identifies a mutation in that particular gene).

In primary analyses unclassified variants were categorised as negative reference standard results. Sensitivity estimates ranged from 80.8% to 100.0%. For the two population-based studies specificity was estimated as 91.9% and 80.5%.

Three studies provided data for the sensitivity of MLH1 loss of expression (range 50.0–100.0%), three studies provided data for the sensitivity of MSH2 loss of expression (range 22.2–81.8%) and four studies provided data for the sensitivity of MSH6 loss of expression (range 44.4–75.0%). Specificities were also generated for the population-based studies. For MLH1 these ranged from 70.6% to 96.0%, whereas for MSH2 and MSH6 both studies estimated a specificity of > 92%. Only one study provided data for PMS2, providing a sensitivity estimate of 55.6% and a specificity estimate of 87.8%.

## Review of end-to-end studies

### Methods

The diagnostic test accuracy searches were used to identify end-to-end studies of screening for LS in CRC patients, that is, studies that compared patients receiving screening with patients not receiving screening in terms of long-term outcomes such as survival and cancer incidence.

One experienced researcher screened all titles and abstracts for eligibility and a second researcher checked a 10% random sample.

## Results

No eligible studies were identified. Some studies that compared outcomes before and after the introduction of screening were identified but they had been published in only abstract form and could not be included.

## Review of economic evaluations

### Methods

A systematic review was conducted to assess the cost-effectiveness of testing for LS in CRC patients using MSI testing and/or IHC.

Bibliographic databases including MEDLINE, EMBASE, Web of Science, NHS Economic Evaluation Database (NHS EED) and EconLit were searched for economic studies. A date limit of 2013 was used to reflect that this was an update of a previous systematic review of cost-effectiveness.

After two reviewers had completed the screening process, the bibliographies of included papers were scrutinised for further potentially relevant studies. To be eligible for inclusion, studies had to include a population of people with newly diagnosed CRC and had to evaluate MSI and/or IHC.

Studies were quality appraised and their results were tabulated. A narrative synthesis was conducted.

### Results

Nine studies were eligible for inclusion. Seven studies were based in the USA, one study was based in Germany and one in the UK. All studies included strategies to identify LS in people with CRC and their relatives. Modelling was similar across studies, with a diagnostic model to identify LS and a long-term model to estimate the costs and benefits associated with the outcomes of the diagnostic model. Five studies were cost-utility studies.

The studies reported a wide variety of analyses, with varying quality of reporting. No single study answered the decision problem in full, generally because not all of the interventions identified by the National Institute for Health and Care Excellence scope were included or a UK perspective was not used.

Most studies stated that at least one strategy to identify LS could be cost-effective and, when a universal genetic testing strategy was included, strategies that used tumour-based tests to enrich the population appeared to improve cost-effectiveness [reducing incremental cost-effective ratios (ICERs)]. Most studies agreed that the effectiveness of colonoscopy screening, the number of relatives and the prevalence of LS had the biggest effects on cost-effectiveness.

## Independent economic evaluation

### Methods

A model-based economic evaluation was conducted to extrapolate outcomes based on the results of the diagnostic test accuracy review.

The model estimated costs from a NHS and personal social services perspective and quality-adjusted life-years (QALYs). These were discounted at 3.5% per year.

A decision tree was used to estimate how many individuals would be diagnosed with LS (correctly or incorrectly) using 10 possible strategies. One strategy involved no testing for LS, four involved MSI testing, four involved IHC and one involved direct MMR mutation screening.

An individual patient simulation was used to predict long-term outcomes for individuals according to their baseline characteristics and diagnosis. Individuals were simulated until death or age 100 years.

Colorectal cancer and endometrial cancer were modelled. Colonoscopy, gynaecological surveillance, prophylactic gynaecological surgery and aspirin chemoprevention were included. Colonoscopic surveillance was assumed to reduce the incidence of CRC and to affect the stage distribution of CRCs, as estimated from the published literature.

The health-related quality of life for individuals was modelled using an age- and sex-dependent baseline utility and utility decrements for cancer estimated from the published literature.

## Results

In the base-case analysis, four strategies were on the cost-effectiveness frontier. Strategy 1 (no testing) was the cheapest and least effective. Strategy 5 (IHC, *BRAF* V600E and *MLH1* promoter methylation testing) was cost-effective at a conventional cost-effectiveness threshold of £20,000 per QALY. Strategy 3 (IHC and *BRAF* V600E testing) and strategy 2 (testing with IHC) would be cost-effective at higher cost-effectiveness thresholds.

Life expectancy for individuals with LS was improved by 1.2 years for probands and 2.1 years for relatives (strategy 5 vs. strategy 1). Over 300 CRCs were expected to be prevented over the lifetime of each annual cohort.

Subgroup analyses were conducted according to the age of the probands (< 50, < 60, < 70, > 70 years). In all subgroup analyses strategy 5 remained cost-effective compared with strategy 1. When an age limit of 50 years was imposed for probands, strategy 3 was marginally cost-effective, with an ICER of £19,903 per QALY.

A number of scenario and sensitivity analyses were conducted, but strategy 5 remained cost-effective throughout, although cost-effectiveness was marginal when a worst-case assumption (that colonoscopy does not reduce the incidence of CRC) was considered and was also marginal when the CRC risk for individuals with LS was low.

## Conclusions

The review of diagnostic test accuracy studies suggests that MSI testing and IHC are effective, but imperfect, tests to identify LS in CRC patients.

There was no high-quality evidence from end-to-end studies that screening for LS in CRC patients improves long-term outcomes.

Previous economic evaluations suggested that it may be cost-effective to screen for LS in CRC patients using clinical criteria, decision tools and/or tumour-based tests.

The current economic evaluation, which directly addressed the decision problem, suggests that screening for LS is cost-effective at a cost-effectiveness threshold of £20,000–30,000 per QALY.

Two parameters that cannot be easily estimated had a significant impact on cost-effectiveness: the effectiveness of surveillance colonoscopy in reducing the incidence of CRC and the lifetime risk of CRC for individuals with LS. The estimates used for modelling are believed to be the most suitable from the scientific literature, considering the possible sources of bias and the size of the relevant studies.

Research recommendations include:

- evaluate screening for LS in endometrial cancer patients
- evaluate the use of next-generation sequencing panels covering a wide range of cancer predisposition genes in CRC patients.

## Study registration

This study is registered as PROSPERO CRD42016033879.

## Funding

Funding for this study was provided by the Health Technology Assessment programme of the National Institute for Health Research.





ISSN 1366-5278 (Print)

ISSN 2046-4924 (Online)

Impact factor: 4.236

*Health Technology Assessment* is indexed in MEDLINE, CINAHL, EMBASE, The Cochrane Library and the Clarivate Analytics Science Citation Index.

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## This report

The research reported in this issue of the journal was commissioned and funded by the HTA programme on behalf of NICE as project number 15/17/04. The protocol was agreed in January 2016. The assessment report began editorial review in September 2016 and was accepted for publication in March 2017. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The HTA editors and publisher have tried to ensure the accuracy of the authors' report and would like to thank the reviewers for their constructive comments on the draft document. However, they do not accept liability for damages or losses arising from material published in this report.

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