Lynch Syndrome
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Histopathology Department
Manchester Royal Infirmary
Bosnian National Congress of Pathology
Sarajevo
November 2016
Lynch Syndrome

- Commonest inherited cause of colorectal cancer, responsible for 3-4% of all cancers
- Estimated 1,100 new diagnoses in UK every year due to Lynch
- Estimated 175,000 people in UK with Lynch Syndrome
- Many unaware of the condition
- Increased risk of cancers at other sites
- Management and surveillance pathways available WHEN people become aware of condition
Lynch Syndrome

- Autosomal dominant disease: previously known as Hereditary Non-Polyposis Colorectal Cancer (HNPCC)
- High risk of cancers of colon, rectum, endometrium, stomach, small intestine, hepatobiliary system, upper ureteric tract, ovary and brain
- Estimated 68–82% lifetime risk of colorectal cancer (CRC)
- HNPCC-related colon cancers present at an early age (mean age 45 years)
- Predominantly located in the proximal colon (60–70%), with approximately 10–30% developing synchronous or metachronous cancers
Lynch Syndrome

Modified Amsterdam Criteria

• There should be at least 3 relatives with an HNPCC associated cancer (colorectal cancer, endometrial, small bowel, ureter or renal pelvis malignancy)

• One affected relative should be a first-degree relative of the other two

• At least two successive generations should be affected

• At least one malignancy should be diagnosed before age 50 years

• FAP should be excluded in the colorectal cancer case(s)

• Tumours should be verified by pathological examination

From Vasen et al 1999
Cancers in Lynch syndrome

- Stomach (1-12%)
- Renal tract transitional cell carcinoma (2-6%)
- Small bowel adenocarcinoma (1-2%)
- Colorectal (right more often than left)
- Ovary (1-6%)
- Endometrial

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<th>Female</th>
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<td>Age 50</td>
<td>45%</td>
<td>20%</td>
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<th>Age 50</th>
<th>Age 70</th>
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<tr>
<td>Ovary</td>
<td>10%</td>
<td>40%</td>
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Fearon and Vogelstein’s model
Fearon and Vogelstein’s model

CRC is not a homogeneous disease
Molecular Pathology of Colorectal Neoplasia

Adenoma-carcinoma sequence

Types of genetic instability

• Chromosomal instability (CIN): majority type occurring in FAP and most sporadic CRCas
• Microsatellite instability (MSI): minority type occurring in Lynch syndrome (loss of DNA mismatch repair proteins) and right sided serrated lesions
• Two largely independent pathways to sporadic CRCa
• Also serrated neoplasia pathway and other rarer routes
The Adenoma - Carcinoma Sequence

Defective DNA Mismatch Repair
Microsatellite Instability (15%)
Mutations in repetitive sequences
Genetic Heterogeneity in Lynch

- Chr 2
  - MSH6
  - MSH2

- Chr 3
  - MLH1

- Chr 7
  - PMS2
Mismatch Repair (MMR)

DNA Mismatch Repair (MMR)

microsatellite

MLH1 ↔ PMS2

MLH1 ↔ PMS1

MLH1 ↔ PMS2

MLH1 ↔ PMS1

MLH1 ↔ PMS2

MLH1 ↔ PMS1

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Molecular Pathology of Colorectal Neoplasia

5 DNA markers or genes with repetitive (microsatellite) sequences (BAT25, BAT26, D2S123, D5S346, D17S250:

- MSI-H (2-5) (15%)
- MSI-L (1) (15%)
- MSS (0) (70%)

Bethesda guidelines

Microsatellite instability

- Change in length of repetitive sequences
  e.g. –CACACA–
- Defects in mismatch repair genes
- Often ‘near-diploid’
Causes of MSI-H CRC

• MSI can be categorized as MSI-H (high), MSI-L (low) or MS-S (stable)
• MSI in itself is not proof of Lynch as 10–20% of sporadic tumours also show some degree of MSI

A. Germline mutation of DNA mismatch repair gene: MLH1, MSH2, MSH6, PMS2 (Lynch syndrome)

B. Somatic methylation of MLH1 (sporadic MSI-H CRC)

C. Hemi-allelic methylation (epimutation) of MLH1 or MSH2 in germline
Lynch Syndrome

• Some tumours have loss of \textit{MSH2} on IHC, but no \textit{MSH2} germline mutations detected.

• \textit{MSH2} hypermethylation detected in tumours; caused by germline deletions in \textit{EPCAM}

• \textit{EPCAM} gene upstream of \textit{MSH2};

• deletion c.859-462\_*1999del abolishes termination of transcription and leads to transcription readthrough of \textit{MSH2}; this leads to methylation of promoter region and inactivation of \textit{MSH2}
Polyp molecular pathogenesis

Conventional pathway
- FAP
  - Germline APC
  - KRAS
  - SMAD4
  - p53
- Chromosomal instability
- CIMP - CIN+
- CIMP - CIN+

Serrated pathway
- Sporadic
  - APC or less commonly β-catenin, axin
  - +/- Aberrant methylation
- Traditional serrated
  - KRAS/BRAF mutation
- Sessile serrated
  - BRAF mutation
  - Aberrant methylation
- Aberrant methylation
- MGMT, p16 methylation
  - CIMP+ MSS
  - CIMP+ MSS
  - CIMP+ MSI-H
  - CIMP+ MSI-H

Microsatellite instability
- Lynch
  - Germline MSH2,6 MLH1,3 PMS1,2
- PPAP
  - Germline POLE POLD1
- Sporadic POLE
- Frameshift mutations e.g TGFRB2, IGFR2

Ultramutated
- Diploid CIMP - MSS
- CIMP - MSI-H
- CIMP - MSI-H
- MSI
- CIMP
- CIN

1-2% ~60% ?1-15% 6-8% 9-12% 2-5% <0.5% ~4%

Polyp molecular pathogenesis
Polyp molecular pathogenesis

Conventional pathway
- FAP
  - Germline APC
- Sporadic
  - APC or less commonly β-catenin, axin

Serrated pathway
- Traditional serrated
  - KRAS/BRAF mutation
- Sessile serrated
  - BRAF mutation
  - Aberrant methylation

Microsatellite instability
- Lynch
  - Germline MSH2,6 MLH1,3 PMS1,2

Ultramutated
- PPAP
  - Germline POLE POLD1
- Sporadic
  - POLE

CIN
- CIMP - CIN+
- ~60%

CIMP
- CIMP - CIMP+/-MSS
- CIMP+ MSS
- ~60%
- ~15%
- 6-8%
- 9-12%
- 2-5%
- <0.5%
- ~4%

MSI
- Diploid CIMP - MSS
MMR IHC in HNPCC

Immunohistochemistry

• 4 antibodies: MLH1, MSH2, MSH6, PMS2
• Internal controls: normal colon, lymphoid tissue
• Comparison between patient’s normal tissue and tumour tissue: if normal colon not available, use internal controls
• Loss of staining in tumour tissue indicates possible mutation
• Functional heterodimers: MLH1 links with PMS2, MSH2 with MSH6
• Often both show loss of staining
MMR IHC in HNPCC

Immunohistochemistry

• Semi-quantitative method

<table>
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<th>Intensity</th>
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<td>0% = 0</td>
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<td>1-10% = 1</td>
<td>1</td>
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<tr>
<td>11-50% = 2</td>
<td>2</td>
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<tr>
<td>51-80% = 3</td>
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<td>&gt;81% = 4</td>
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• Score: % x intensity, range 0-12

• Validity evaluated using known mutations for MLH1 and MSH2

Barrow et al. Histopathology 2010; 56, 331–344
MMR Immunohistochemistry

- MSH2
- Mucinous colon cancer
- MLH1
MLH1 proficient

PMS2 proficient

MSH2 deficient

MSH6 reduced

Endometrial Carcinoma
MLH1 proficient

Ovarian Carcinoma

PMS2 proficient

MSH2 proficient

MSH6 deficient
Skin Sebaceous Adenoma: MMR Immunohistochemistry

Muir-Torre Syndrome: sebaceous tumour & internal malignancy. MSH2 & MSH6 partners
### Amsterdam II Criteria (All of)

1. At least 3 relatives with a Lynch Spectrum cancer (CRC, endometrial, urothelial, small bowel)
2. At least 1 of these is a first degree relative of the other 2
3. At least 2 generations
4. At least 1 of the patients was under age of 50 at the time of their cancer

**Specificity 98%**  
**Sensitivity 42%**

### Bethesda Criteria (one of)

1. Any CRC under the age of 50
2. Synchronous or metachronous CRC or Lynch spectrum cancer (at any age)
3. CRC with typical MSI-H histology under age 60
4. CRC in 1 + first degree relatives with a Lynch spectrum cancer, (1 cancer under age 60)
5. CRC in 2 + first degree relatives, or second degree relatives with a Lynch spectrum cancer regardless of age

**Specificity 38%**  
**Sensitivity 95%**
Investigation

• Moderate risk bowel cancer
• Less clear cut family history (not full Amsterdam)

• When to suspect Lynch syndrome
  – At what point to start to investigate
Is tumour *MLH1* promoter region methylation testing an effective pre-screen for Lynch Syndrome?

**Problem**

Mutation testing time-consuming & expensive

Poor sensitivity/specificity of family history criteria

Unable to differentiate sporadic MLH1 loss CRC from Lynch CRC

**Hypothesis**

“MLH1 promoter region methylation is able to differentiate between sporadic MLH1 loss CRCs and MLH1 Lynch CRCs”

**Katy Newton**

ST3 General Surgery,
Clinical research fellow

General surgery/Genetic Medicine/Histopathology

CMFT
Methods

FFPE tissue
• Sporadic MLH1 loss CRCs (n=71)
• *MLH1* mutation carrier CRCs (n=73)

Novel *MLH1* methylation assay developed

Tumour DNA *MLH1* methylation and *BRAF* mutation testing

Diagnostic test (2 by 2 tables)

Bayesian calculations applied using pre-test risks

Patients with >10% risk to be tested for gene mutation
Pre- and Post-test probabilities of being an *MLH1* mutation heterozygote

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<td>Pre-test risk</td>
<td>&gt;60%</td>
<td>10.5%</td>
<td>4.0%</td>
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<tr>
<td>Post-test normal <em>MLH1</em></td>
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<td>promoter region</td>
<td></td>
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<tr>
<td>True positive</td>
<td>85.5%</td>
<td>31.4%</td>
<td>14.0%</td>
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<tr>
<td>False negative</td>
<td>12.6%</td>
<td>1.2%</td>
<td>0.4%</td>
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<tr>
<td>Post-test wt <em>BRAF</em></td>
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<tr>
<td>True positive</td>
<td>74.9%</td>
<td>19.0%</td>
<td>7.7%</td>
</tr>
<tr>
<td>False negative</td>
<td>17.5%</td>
<td>1.6%</td>
<td>0.6%</td>
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Conclusion

First large scale analysis of performance characteristics of tumour *MLH1* methylation testing

Demonstrated that *MLH1* methylation testing alone is able to define which patients should undergo germline testing

Not useful in Amsterdam Criteria families

Proposed algorithm...
Amsterdam criteria fulfilled

Bethesda criteria fulfilled

? Population based screening - sporadic CRC

Tumour MMR IHC: MLH1, MSH2, MSH6, PMS2

- All present
  - Population risk CRC screening

- MSH2, MSH6, PMS2 loss
  - MLH1 loss
    - MLH1 methylation/BRAF mutation
      - Absent
        - Population risk CRC screening
      - Present

Germline gene mutation testing
ORIGINAL ARTICLE

Tumour MLH1 promoter region methylation testing is an effective prescreen for Lynch Syndrome (HNPCC)

K Newton,1 N M Jorgensen,2 A J Wallace,2 D D Buchanan,3,4,5 F Laloo,6 R F T McMahon,7,8 J Hill,1 D G Evans6

ABSTRACT

Background and aims Lynch syndrome (LS) patients have DNA mismatch repair deficiency and up to 80% lifetime risk of colorectal cancer (CRC). Screening of mutation carriers reduces CRC incidence and mortality. Selection for constitutional mutation testing relies on family history (Amsterdam and Bethesda Guidelines) and tumour-derived biomarkers. Initial biomarker analysis uses mismatch repair protein immunohistochemistry and microsatellite instability. Abnormalities in either identify mismatch repair deficiency but do not differentiate sporadic epigenetic defects, due to MLH1 promoter region methylation (13% of CRCs) from LS (4% of CRCs). A diagnostic biomarker capable of making this distinction would be valuable. This study compared two biomarkers in tumours with mismatch repair deficiency; quantification of methylation of the MLH1 promoter mutation analysis. Family history criteria and tumour-derived biomarkers are used to prescreen to select patients for germline testing. The Amsterdam II criteria were designed to select research families for linkage analysis. They are currently used, somewhat inappropriately, for clinical purposes to select individuals at high risk of having a MMR gene mutation. Patients who meet these criteria have at least 60% chance of a mutation.6 These criteria are inherently specific but consequently have low sensitivity. Much work has been done over the last decade to improve the identification of non-Amsterdam Lynch families. The revised Bethesda guidelines described in 20047 are sensitive but have low specificity. They have been criticised for being overly complicated and are little used in clinical practice.8 Tumour MSI and MMR protein
Standards and datasets for reporting cancers

Dataset for colorectal cancer histopathology reports

July 2014

Authors:
Dr Maurice B Loughrey, Consultant Pathologist, Royal Victoria Hospital, Belfast Trust, UK
Professor Philip Quirke, Head of Section, Professor of Pathology and Honorary Consultant, Pathology and Tumour Biology, Leeds University, UK
Professor Neil A Shepherd, Professor of Gastrointestinal Pathology, Gloucestershire Cellular Pathology Laboratory, Cheltenham, UK

<table>
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<td>Dataset for colorectal cancer histopathology reports</td>
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<tr>
<td>Version number</td>
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<td>Dr Maurice Loughrey, Professor Philip Quirke and Professor Neil Shepherd, Professor of Gastrointestinal Pathology, on behalf of the College’s Cancer Services Working Group. MBL leads the pathology QA process for Bowel Cancer Screening in Northern Ireland and sits on the National NHSCSP Pathology Steering Committee. PQ chairs the NHSCSP Pathology Committee, is on the NSPCC colorectal cancer study group, and the</td>
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... a strong case can now be made for performing MMR immunohistochemistry in all cases of CRC. However, given the resource implications of implementing this, it is not considered a core data item for all colorectal cancers currently.

We now consider MMR immunohistochemistry a core dataset item for

- all patients under 50 years at time of diagnosis
- for patients with adenocarcinomas classified as poorly differentiated morphologically or tumours showing other morphological features of MMR deficiency.

It should also be available upon request by either oncologist or geneticist on individual cases.
MRI MISMATCH REPAIR TESTING PATHWAY

The reporting Histopathologist identifies a patient fulfilling criteria for group 1 or is requested to refer by MDT based on individual factors.

The reporting Histopathologist will select representative tissue blocks containing tumour tissue and normal large bowel epithelium. This will be sent along with a representative H&E stained section for each block and a copy of the report to the IHC laboratory at MRI.

The tissue blocks and slides will be booked into the MRI laboratory database and tested for MLH1, PMS2, MSH2 and MSH6 protein loss by immunohistochemical analysis.

Samples demonstrating no protein loss.
Samples demonstrating MLH1 loss (+/- PMS2 loss).
Samples demonstrating isolated PMS2 loss or MSH2/MSH6 loss.

Sent to the regional genetics department for MLH1 promoter methylation and BRAF mutation status.
Sent to regional genetics department for MSI testing and further genetics referral.

MLH1 promoter methylation or BRAF Mutation.
No MLH1 promoter methylation & no BRAF Mutation.

No referral to Genetics.
Referral to Genetics for further testing.

A report for the MMR IHC results will be produced and sent to the referring Histopathologist. A separate report will be issued by St Mary’s Genetics department for any genetic testing that has been carried out.
MRI MISMATCH REPAIR TESTING PATHWAY

The reporting Histopathologist identifies a patient fulfilling criteria for group 1 or is requested to refer by MDT based on individual factors.

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CRITERIA FOR TESTING (Please tick)

☐ Direct Request from Histopathology. Patient <50 years old at time of diagnosis with colorectal adenocarcinoma.

The patient has been discussed at an MDT and;

☐ Is 50-60 years old and has a tumour with histological features suggestive of Lynch syndrome and a family history of Lynch syndrome related malignancies.

☐ MMR status will aid decision making regarding chemotherapy treatment (oncologist request).

No referral to Genetics. Referral to Genetics for further testing.

A report for the MMR IHC results will be produced and sent to the referring Histopathologist. A separate report will be issued by St Mary's Genetics department for any genetic testing that has been carried out.
Lynch Syndrome surveillance

- 1942 mutation carriers on surveillance colonoscopy (13782 observation yrs)
- 314 developed cancer: CRC (151), endo (72) and ovary (19)
- MLH1 and MSH2 carriers after 25 yrs; MSH6 and PMS2 carriers after 40 yrs
- Cumulative incidences for cancers detected at 70yrs
  - first CRC for MLH1 (46%), MSH2 (35%), MSH6 (20%) and PMS2 (10%)
  - endo were MLH1 (34%), MSH2 (51%), MSH6 (49%) and PMS2 (24%)
  - ovary were MLH1 (11%), MSH2 (15%), MSH6 (0%) and PMS2 (0%)
  - 10yrs crude survival were 87% (any), 91% (CRC), 98% (endo) and 89% (ovary)

Calculated cumulative incidences by age and mutated gene for any cancer.

Cumulative incidence any cancer (penetrance) by age and mutated gene

Calculated cumulative incidences by age and mutated gene for colorectal cancer (CRC) as the first cancer.

Calculated cumulative incidences by age and mutated gene for endometrial cancer as the first cancer by gene.
Lynch Syndrome Management

• CAPP2 Study Aspirin 600mg preventive for CRC in LS
• MLH1-Lynch: CRC 70% by 70yrs (mean age dx 45yrs); endo 20-50%; urinary tract 4-16%; breast ca also increased
• MSH2-Lynch: CRC/endo similar to MLH1; urinary tract increased ? prostate also
• EPCAM-Lynch: (MSH2 Methylation 10%); CRC similar to MSH2; endo reduced vs MSH2

Lynch Syndrome Management

- MSH6-Lynch: lower CRC risk with later onset; endo risk similar to MLH1/MSH2
- PMS2-Lynch: lower CRC/endo risk; same review as MSH6
- Probable Lynch: Amsterdam +, IHC+, MSI+ but mutation negative; possible biallelic somatic mutations in MLH1 or MSH2; same review as standard Lynch patients

Lynch Syndrome - Summary

• Contributes to our increasing understanding of molecular pathogenesis of colorectal cancer
• Recognition of MSI-H categories of tumours
• Need to be aware of hypermethylation as a cause of anomalous IHC results
• Varied surveillance programmes depending on specific gene mutation
• Extracolonic tumours an increasing issue (F>M)
• Generally good prognosis but treatment options may vary with mutation type
Lynch Syndrome - Future

• Currently awaiting NICE guidance on screening for Lynch Syndrome

• Likelihood that there will be a recommendation for universal testing of all new colorectal cancer diagnoses, by a combination of IHC +/- MSI with or without MLH1 promoter gene hypermethylation and/or BRAF testing

• Can we cope with workload?

• Who will pay?
Acknowledgements

Research Fellows
Doug Speake, Emma Barrow, Paul Barrow, Katy Newton

Surgeon
Jim Hill

Geneticists
Fiona Laloo, Gareth Evans, Andrew Wallace

Immunohistochemists
Judith Brierley, Cath Keeling, Emma Jagger

Pathologists
Lucy Foster, Mark Arends
Thank you for your attention.
Any questions?