Pathology of Lynch Syndrome associated Gynaecologic Cancers: implementation of reflex testing and its implications

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OVERVIEW/ OBJECTIVES

- Screening strategies
- Laboratory driven reflex testing algorithms
- Pre-requisites
- Consequences
- Circle of care
- Emerging issues
# Lynch Syndrome

<table>
<thead>
<tr>
<th>Type of Cancer</th>
<th>Population Lifetime Risk (%)</th>
<th>LS Lifetime Risk (%)</th>
<th>Average Age Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colon</strong></td>
<td>5.5</td>
<td>65-80</td>
<td>44</td>
</tr>
<tr>
<td><strong>Endometrial</strong></td>
<td>2.7</td>
<td>60</td>
<td>46*</td>
</tr>
<tr>
<td><strong>Ovary</strong></td>
<td>1.6</td>
<td>12</td>
<td>43</td>
</tr>
<tr>
<td><strong>Stomach</strong></td>
<td>&lt;1</td>
<td>11-19</td>
<td>56</td>
</tr>
<tr>
<td><strong>Urinary tract</strong></td>
<td>&lt;1</td>
<td>4-10</td>
<td>55</td>
</tr>
<tr>
<td><strong>Hepatobiliary</strong></td>
<td>&lt;1</td>
<td>2-7</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Small bowel</strong></td>
<td>&lt;1</td>
<td>1-4</td>
<td>49</td>
</tr>
<tr>
<td><strong>Brain (Turcot)</strong></td>
<td>&lt;1</td>
<td>1-3</td>
<td>50</td>
</tr>
<tr>
<td><strong>Sebaceous</strong></td>
<td>&lt;1</td>
<td>9</td>
<td>52</td>
</tr>
<tr>
<td><strong>Keratoacanthoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Muir-Torre)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Average age of general population = 60
Lynch Syndrome and Endometrial Cancer

51% (n= 52) of women with metachronous tumours present with gynecologic cancer as the sentinel cancer

Lu et al., Obstetrics and Gynecology, 2005
Steps to Identifying LS in cancer patients

1. Family History
2. Amsterdam/Bethesda
3. Genetic Counselling
4. Tumor Immunohistochemistry (IHC)/Microsatellite testing (MSI)
5. Germline Testing
LS Unselected Endometrial Cancer

- 562 incident cases endometrial cancer
- 118 (21.7%) were MSI positive
- 13 (2.3%) positive germline mutation
- 2/13 (15%) missed by MSI testing; both were deficient in protein expression MSH6
- 5 (38%) diagnosed > age 50
- 8 (62%) did not meet family history criteria in Amsterdam II or Bethesda Guidelines
- **Current screening guidelines for LS not adequate for EC population**

Hampel et al 2006, Cancer Research
### SGO- Statement on Risk Assessment for Inherited Gynecologic Cancer Predispositions

#### 20-25% risk of LS and genetic risk assessment is recommended:
1. Meet Amsterdam II criteria
2. Synchronous or metachronous EC and CRC with first cancer < 50
3. Synchronous or metachronous ovarian cancer and CRC with first cancer < 50
4. CRC or EC with evidence of a MMR defect (MSI or loss of expression of MMR gene on IHC)
5. Patient with a 1\textsuperscript{st} or 2\textsuperscript{nd} degree relative with known MMR gene mutation

#### 5-10% risk of LS and genetic risk assessment maybe helpful:
1. CRC or EC diagnosed < 50 years
2. EC or ovarian cancer with synchronous or metachronous CRC or other LS-associated tumour at any age
3. EC or CRC and a 1\textsuperscript{st} degree relative with a LS-associated tumour diagnosed < 50 years
4. CRC or EC with 2 or more 1\textsuperscript{st} or 2\textsuperscript{nd} degree relatives with LS-associated tumour, regardless of age
5. 1\textsuperscript{st} or 2\textsuperscript{nd} degree relative that meets the above criteria
*Histologic Features Suggestive of Microsatellite Instability (Note I)

*Intratumoral Lymphocytic Response (tumor-infiltrating lymphocytes)
  *___ None
  *___ Mild to moderate (0-2 per high-power [X400] field)
  *___ Marked (3 or more per high-power field)

*Peritumor Lymphocytic Response (Crohn-like response)
  *___ None
  *___ Mild to moderate
  *___ Marked

*Tumor Subtype and Differentiation (select all that apply)
  *___ Mucinous tumor component (specify percentage: ___)
  *___ Medullary tumor component
  *___ High histologic grade (poorly differentiated)
Reflex Immunohistochemistry and Microsatellite Instability Testing of Colorectal Tumors for Lynch Syndrome Among US Cancer Programs and Follow-Up of Abnormal Results

Laura C. Beamer, Marcia L. Grant, Carin R. Espenschied, Kathleen R. Blazer, Heather L. Hampel, Jeffrey N. Weitzel, and Deborah J. MacDonald

Results
The overall response rate was 50%. Seventy-one percent of NCI-CCCs, 36% of COMPs, and 15% of CHCPs were conducting reflex IHC/MSI for LS; 48% of the programs used IHC, 14% of the programs used MSI, and 38% of the programs used both IHC and MSI. One program used a presurgical information packet, four programs offered an opt-out option, and none of the programs required written consent.

Conclusion
Although most NCI-CCCs use reflex IHC/MSI to screen for LS, this practice is not well-adopted by community hospitals. These findings may indicate an emerging standard of care and diffusion from NCI-CCC to community cancer programs. Our findings also described an important trend away from requiring written patient consent for screening.
Clinical Sensitivity of Family History in Predicting Lynch Syndrome

<table>
<thead>
<tr>
<th>EC in Mutation proven LS</th>
<th>N = 76 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amsterdam II</td>
<td>44 (58)</td>
</tr>
<tr>
<td>rBethesda</td>
<td>27 (36)</td>
</tr>
<tr>
<td>SGO 20-25%</td>
<td>54 (71)</td>
</tr>
<tr>
<td>SGO 5-10%</td>
<td>71 (93)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EC in population based cohort</th>
<th>N=412</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity SGO</td>
<td>32.6% (95% CI 19.2-48.5)</td>
</tr>
<tr>
<td>Specificity SGO</td>
<td>77%</td>
</tr>
</tbody>
</table>

They acknowledged that a large proportion of probable LS patients which are older and have less significant family history are not detected.
# Age based criteria

<table>
<thead>
<tr>
<th>Study</th>
<th>Total Cohort</th>
<th>EC Mean Age and Range</th>
<th>NEEC Mean Age and Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broaddus et al¹⁷</td>
<td>46.8 y</td>
<td>—</td>
<td>46.4 y</td>
</tr>
<tr>
<td>n = 50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcingau et al¹⁹</td>
<td>46.2 y</td>
<td>47.8 y</td>
<td>44.2 y</td>
</tr>
<tr>
<td>n = 23</td>
<td>R: 30-67 y</td>
<td>R: 30-67 y</td>
<td>R: 35-54 y</td>
</tr>
<tr>
<td>Ryan et al¹⁴</td>
<td>47.1 y</td>
<td>46.8 y</td>
<td>48.1 y</td>
</tr>
<tr>
<td>n = 76</td>
<td>R: 31-65 y</td>
<td>R: 31-65 y</td>
<td>R: 40-61 y</td>
</tr>
<tr>
<td>Palacios et al¹⁸</td>
<td>44.9 y</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>n = 16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van den Bos et al²⁰</td>
<td>44</td>
<td>44</td>
<td>NA</td>
</tr>
<tr>
<td>n = 6</td>
<td>R: 38-53 y</td>
<td>R: 38-53 y</td>
<td></td>
</tr>
</tbody>
</table>

NA indicates not applicable; R, range.
### TABLE 4. Mean Age According to Gene Mutated

<table>
<thead>
<tr>
<th>Study</th>
<th>All</th>
<th>MLH1</th>
<th>MSH2</th>
<th>MSH6</th>
<th>PMS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryan et al*14, n = 76</td>
<td>47.3 y</td>
<td>49.3 y (18 cases)</td>
<td>46 y (50 cases)</td>
<td>50.6 y (8 cases)</td>
<td>—</td>
</tr>
<tr>
<td>Hampel et al12,13, n = 13</td>
<td>54.9 y</td>
<td>39 (1 case)</td>
<td>46.3 (3 cases)</td>
<td>59.5 y (9 cases)</td>
<td>—</td>
</tr>
<tr>
<td>De Leeuw et al21, n = 23</td>
<td>48 y</td>
<td>49.5 (8 cases)</td>
<td>41 (4 cases)</td>
<td>55.5 y (11 cases)</td>
<td>—</td>
</tr>
<tr>
<td>Berends et al23, MSH6 only, n = 9</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>52.2 y (9 cases)</td>
<td>—</td>
</tr>
<tr>
<td>Goodfellow et al29, MSH6 only, n = 7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>54.9 y (7 cases)</td>
<td>—</td>
</tr>
<tr>
<td>Djordjevic and Broaddus26, PMS2 only, n = 7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>64.6 y</td>
</tr>
</tbody>
</table>
| *Ryan et al, total of 76 patient pedigree’s available.
R indicates range.
## Histotype and Lynch Syndrome

<table>
<thead>
<tr>
<th>Study</th>
<th>Endometrioid</th>
<th>Serous</th>
<th>Clear cell</th>
<th>Mixed/other/non-endometriod</th>
<th>MMMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broddus (50)</td>
<td>86%</td>
<td>6%</td>
<td></td>
<td>6%</td>
<td>2%</td>
</tr>
<tr>
<td>Ryan (38)</td>
<td>76%</td>
<td>5%</td>
<td>3%</td>
<td>13%</td>
<td>3%</td>
</tr>
<tr>
<td>De Leeuw (23)</td>
<td>87%</td>
<td>6.5%</td>
<td></td>
<td>6.5%</td>
<td></td>
</tr>
<tr>
<td>Berends (17)</td>
<td>80%</td>
<td></td>
<td></td>
<td></td>
<td>20%</td>
</tr>
<tr>
<td>Van den Bos (6)</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcangiu (23)</td>
<td>56.5%</td>
<td>8%</td>
<td>21%</td>
<td>4%</td>
<td>8%</td>
</tr>
<tr>
<td>Palacois (16)</td>
<td>62.5%</td>
<td></td>
<td></td>
<td></td>
<td>37.5%</td>
</tr>
</tbody>
</table>
**Assessment of MSI histologic features**

<table>
<thead>
<tr>
<th></th>
<th>TILS</th>
<th>Peritumoral</th>
<th>Mucinous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Honore et al(^{35})</td>
<td>0.4864</td>
<td>0.4864</td>
<td>0.4864</td>
</tr>
<tr>
<td>Van den Bos et al(^{20})</td>
<td>0.002</td>
<td>0.001</td>
<td>—</td>
</tr>
<tr>
<td>Parc et al(^{36})</td>
<td>0.102</td>
<td>—</td>
<td>0.046</td>
</tr>
<tr>
<td>Catasus et al(^{37})</td>
<td>—</td>
<td>—</td>
<td>No correlation</td>
</tr>
<tr>
<td>Shia et al(^{25})</td>
<td>0.002</td>
<td>0.004</td>
<td>—</td>
</tr>
<tr>
<td>Toledo et al(^{39})</td>
<td>&gt;0.05</td>
<td>—</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Walsh et al(^{11})</td>
<td>0.004</td>
<td>&gt;0.05</td>
<td>—</td>
</tr>
</tbody>
</table>

EC indicates endometrial cancer; MSI-H, microsatellite instability-high; TILS, tumor infiltrating lymphocytes.

*Clarke and Cooper  Adv Anat Pathol  •  Volume 19, Number 4, July 2012*
Objective

• To compare IHC, MSI testing, tumour morphology and family history to germline mutation status to determine which screening strategy is superior at identifying those at risk for Lynch Syndrome in unselected women with endometrial cancer
Methods

- Extended Family History Questionnaire (eFHQ)
- Brief Family History Questionnaire (bFHQ)
  - “Flagged” if have one of predetermined criteria:
    1. Family member with LS-associated cancer < 50 yo
    2. Patient/family member has 2 LS-associated cancer
    3. ≥ 2 1st/2nd degree relative with LS-associated cancer
    4. Patient diagnosed EC < 35 yo

- Blood samples for research germline testing-MLH1/PMS2/MSH2/MSH6/EPCAM:
- Tumour assessment:
  - Tumour morphology
  - IHC for MMR proteins
  - MSI
Study Schema

Consecutive Incident EC
- All histologic types
- All FIGO stages

All participants

- eFHQ
- bFHQ
- IHC
- MSI
- Tumour Morphology
- Universal Germline Testing

Ontario MOH, SGO, AMS II Criteria +

Deficient MSH2, MSH6, PMS2 or MLH1 < 60 Without Family History

Genetic Counselling and Clinical Germline Testing

Eligible participants

MSI-High < 60
Germline Results Summary

- 20/118 (17%) possible LS
- 7/118 (6%) tested positive in entire cohort
- Mutation rate was higher in younger women
- Women with LS had a lower BMI than those without LS
## Comparison of Screening Strategies

<table>
<thead>
<tr>
<th>Screening Strategy</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC</td>
<td>89</td>
<td>100</td>
<td>78.1</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>IHC &lt; 60</td>
<td>43</td>
<td>100</td>
<td>85.7</td>
<td>58.3</td>
<td>100</td>
</tr>
<tr>
<td>MSI Testing</td>
<td>87</td>
<td>100</td>
<td>81.5</td>
<td>28.6</td>
<td>100</td>
</tr>
<tr>
<td>bFHQ</td>
<td>87</td>
<td>100</td>
<td>73.8</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Morphology</td>
<td>83</td>
<td>71.4</td>
<td>42.1</td>
<td>10.2</td>
<td>94.1</td>
</tr>
<tr>
<td>eFHQ</td>
<td>82</td>
<td>71.4</td>
<td>86.7</td>
<td>33.3</td>
<td>97</td>
</tr>
</tbody>
</table>
Conclusions

- Germline mutation rate at least 6%
- Morphologic features inadequate
  Current family history criteria miss significant proportion of women with LS
- EC was the sentinel cancer in majority of women with LS
- IHC most sensitive and specific method of screening
  - Directs germline analysis
Screening Algorithm for Endometrial Cancer

Endometrial Cancer < 70 years

IHC

Intact

No Family Hx

No Further Action

Family Hx

Genetic Counselling

Deficient

PMS2, MSH2, MSH6 Deficient

Genetic Counselling

MLH1 Deficient

Methylation

Hypermethylated

No Family Hx

< 50 years or Family Hx

Genetic Counselling

Not Hypermethylated

No Family Hx

< 50 years or Family Hx

No Further Action

University Health Network
Lynch Syndrome Screening Should Be Considered for All Patients With Newly Diagnosed Endometrial Cancer


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**IHC testing for loss of MMR protein expression for all endometrial carcinomas**

**MMR intact by IHC but clinical suspicion of LS**
- Order LS microsatellite instability by PCR
  - Instability at ≥ 2/5 of microsatellite markers
    - High
      - Consider germline testing of LS mismatch repair genes
    - Indeterminate
  - No instability present
    - Low

**Loss of MMR by IHC**
- Loss of MLH1 & PMS2
  - Test for MLH1 promoter methylation
    - Methylation present
      - Likely sporadic endometrial carcinoma
    - Methylation absent

- Loss of MSH2 & MSH6
  - Genetic mutation testing for LS: recommend LS MSH2 sequencing and deletion/duplication as first test

- Loss of MSH6
  - Genetic mutation testing for LS: recommend LS MSH6 sequencing and deletion/duplication as first test

- Loss of PMS2
  - Genetic mutation testing for LS: recommend LS PMS2 sequencing and deletion/duplication as first test
Hysterectomy with endometrial cancer

Evaluate for criteria to trigger screening

- Age 50 or younger
- Bethesda guideline criteria
- MMR-morphology

One or more criteria present

MLH1, PMS2, MSH2, MSH6 IHC

Loss of any one:
PMS2 (intact MLH1)
MSH2
MSH6

Loss of MLH1

MLH1 promoter methylation test

Methylation Absent

- Formal genetic counseling
- Test for germline MMR mutations

Methylation present

Not likely to be at elevated risk for Lynch syndrome

Loss of MSH6

- Formal genetic counseling
- Test for germline MMR mutations

No criteria present

only MSH6 IHC

Intact

Association of Tumor Morphology With Mismatch-repair Protein Status in Older Endometrial Cancer Patients: Implications for Universal Versus Selective Screening Strategies for Lynch Syndrome

## Ovarian Cancer and Lynch Syndrome

<table>
<thead>
<tr>
<th>Histology</th>
<th>Percentage</th>
<th>Comparison cases, percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial</td>
<td>93.7</td>
<td>83.6</td>
</tr>
<tr>
<td>Nonepithelial</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Among epithelial cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frankly invasive</td>
<td>95.9</td>
<td></td>
</tr>
<tr>
<td>Borderline</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Among frankly invasive epithelial cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pap/epithelial nos</td>
<td>32.4</td>
<td>35.4</td>
</tr>
<tr>
<td>Serous</td>
<td>23.9</td>
<td>29.8</td>
</tr>
<tr>
<td>Mucinous</td>
<td>9.9</td>
<td>13.4</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>18.3</td>
<td>9.6</td>
</tr>
<tr>
<td>Clear cell</td>
<td>9.9</td>
<td>3.6</td>
</tr>
<tr>
<td>Mixed (other)</td>
<td>5.6</td>
<td>8.2</td>
</tr>
<tr>
<td>Among frankly invasive epithelial cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>30.6</td>
<td>16.1</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>41.7</td>
<td>25.0</td>
</tr>
<tr>
<td>Poorly and undifferentiated</td>
<td>27.8</td>
<td>58.9</td>
</tr>
</tbody>
</table>

The Clinical Features of Ovarian Cancer in Hereditary Nonpolyposis Colorectal Cancer

Gynecologic Oncology 82, 223–228 (2001)
# Ovarian Cancer and Lynch Syndrome

## TABLE 2. Histologic Subtypes of Invasive OC in LS Cohorts

<table>
<thead>
<tr>
<th>References</th>
<th>No. LS-confirmed OC</th>
<th>Cohort Characteristics</th>
<th>Central Pathology Review</th>
<th>Mucinous</th>
<th>Serous</th>
<th>Endometrioid</th>
<th>Clear Cell</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bewtra et al.</td>
<td>3</td>
<td>3 HNPCC families</td>
<td>Yes</td>
<td>0/3 (0)</td>
<td>0/3 (0)</td>
<td>1/3 (33.3)</td>
<td>2/3 (66.7)</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>Aarnio et al.</td>
<td>13</td>
<td>50 HNPCC families, MLH1, MSH2 germline mutation detected</td>
<td>Partial</td>
<td>2/13 (15.4)</td>
<td>4/13 (30.8)</td>
<td>1/13 (7.7)</td>
<td>2/13 (15.4)</td>
<td>4/13 (30.8) NOS</td>
</tr>
<tr>
<td>Ichikawa et al.</td>
<td>4</td>
<td>7 HNPCC families, germline mutation confirmed</td>
<td>Yes</td>
<td>1/4 (25.0)</td>
<td>2/4 (50.0)</td>
<td>1/4 (25.0)</td>
<td>0/4 (0)</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>Stratton et al.</td>
<td>2</td>
<td>&lt; 30 y, population-based study</td>
<td>Yes</td>
<td>1/2 (50.0)</td>
<td>0/2 (0)</td>
<td>1/2 (50.0)</td>
<td>0/2 (0)</td>
<td>0/2 (0)</td>
</tr>
<tr>
<td>Watson et al.</td>
<td>71</td>
<td>HNPCC families (International Collaborative Group on HNPCC)</td>
<td>No (from questionnaire sent to centers)</td>
<td>7/71 (9.9)</td>
<td>17/71 (23.9)</td>
<td>13/71 (18.3)</td>
<td>7/71 (9.9)</td>
<td>4/71 (5.6) mixed, 23/71 (32.4) NOS</td>
</tr>
<tr>
<td>Crijnen et al.</td>
<td>20</td>
<td>Dutch HNPCC registry</td>
<td>No (extracted from path reports only)</td>
<td>1/20 (5.0)</td>
<td>12/20 (60.0)</td>
<td>4/20 (20.0)</td>
<td>1/20 (5.0)</td>
<td>1/20 (5.0) NOS, 1/20 (5.0) undifferentiated (0/2)</td>
</tr>
<tr>
<td>Melander et al.</td>
<td>2</td>
<td>128 unselected cohort</td>
<td>Yes</td>
<td>0/2 (0)</td>
<td>0/2 (0)</td>
<td>1/2 (50.0)</td>
<td>1/2 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Ketabi et al.</td>
<td>44</td>
<td>Swedish and Danish cancer registries</td>
<td>No (extracted from path reports only)</td>
<td>2/44 (4.5)</td>
<td>17/44 (38.6)</td>
<td>16/44 (36.4)</td>
<td>9/44 (20.5)</td>
<td>0/44 (0)</td>
</tr>
<tr>
<td>Pal et al.</td>
<td>9</td>
<td>Unselected cohort</td>
<td>No (from medical records)</td>
<td>0/9 (0)</td>
<td>2/9 (22.2)</td>
<td>5/9 (55.6)</td>
<td>2/9 (22.2)</td>
<td>0/9 (0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td>14/168 (8.3)</td>
<td>54/168 (32.1)</td>
<td>43/168 (25.6)</td>
<td>24/168 (14.3)</td>
<td>33/168 (19.6)</td>
</tr>
</tbody>
</table>

Identifying Lynch Syndrome in Patients With Ovarian Carcinoma: The Significance of Tumor Subtype

*Adv Anat Pathol* • Volume 20, Number 6, November 2013
**MMRd in Epithelial ovarian tumors**

**TABLE 2. Expression of DNA mismatch repair (MMR) proteins among primary epithelial ovarian tumors**

<table>
<thead>
<tr>
<th>Histologic subtypes</th>
<th>MMR−/total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrioid carcinoma</td>
<td>2/29</td>
<td>6.9</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>1/27</td>
<td>3.7</td>
</tr>
<tr>
<td>Undifferentiated carcinoma</td>
<td>1/8</td>
<td>12.5</td>
</tr>
<tr>
<td>Mixed carcinoma</td>
<td>3/5</td>
<td>60</td>
</tr>
<tr>
<td>High-grade serous carcinoma</td>
<td>2/182</td>
<td>1.1</td>
</tr>
<tr>
<td>Borderline tumors</td>
<td>0/22</td>
<td>0</td>
</tr>
<tr>
<td>Low-grade serous carcinoma</td>
<td>0/7</td>
<td>0</td>
</tr>
<tr>
<td>Mucinous carcinoma</td>
<td>0/7</td>
<td>0</td>
</tr>
<tr>
<td>Brenner tumor</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td><strong>All epithelial tumors</strong></td>
<td><strong>9/290</strong></td>
<td><strong>3.1</strong></td>
</tr>
</tbody>
</table>

**Prevalence of Loss of Expression of DNA Mismatch Repair Proteins in Primary Epithelial Ovarian Tumors**

*International Journal of Gynecological Pathology*  
00:1–9, Lippincott Williams & Wilkins, Baltimore  
© 2012 International Society of Gynecological Pathologists
## Family history and cell type in LS-OC

<table>
<thead>
<tr>
<th>LS-OC</th>
<th>Endometrioid</th>
<th>Clear</th>
<th>EC/CC</th>
<th>Dediff</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 cases</td>
<td>15</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmII</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>rBethesda</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>SGO20-25%</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>SGo5-10%</td>
<td>16 (80%)</td>
</tr>
</tbody>
</table>

The Histomorphology of Lynch Syndrome–associated Ovarian Carcinomas

Towards a Subtype-specific Screening Strategy

*Am J Surg Pathol* • Volume 38, Number 9, September 2014
Screening Algorithm for Ovarian Cancer

Non-mucinous, non-serous ovarian cancer < 70 years

IHC

- Intact
  - No Family Hx: No Further Action
  - Family Hx: Genetic Counselling

- Deficient
  - PMS2, MSH2, MSH6 Deficient: Methylation
    - Hypermethylated: No Family Hx: No Further Action
    - Not Hypermethylated: < 50 years or Family Hx: Genetic Counselling
  - MLH1 Deficient: No Family Hx: No Further Action
  - Not Hypermethylated: Genetic Counselling

University Health Network
IHC interpretation – These findings indicate that it is unlikely that this carcinoma associated with Lynch Syndrome (LS) since tumours in patients with LS typically show abnormal mismatch repair protein expression. These immunohistochemical findings cannot entirely exclude the possibility of LS and family/personal history of cancers is still important.

Clinical Guidance - If there is a clinical suspicion of LS based on personal or family history referral to a genetic counsellor is indicated according to Ontario Ministry of Health guidelines. Features associated with LS include young age of onset (age < 50), strong family history of certain cancer types (including colorectal, small bowel, endometrial, ureter, renal pelvis and ovarian cancer) and multiple primary LS cancers in one individual.

IHC interpretation- The patient’s tumour has abnormal MSH2 expression (MSH2 Deficient tumour). Abnormal MSH2 expression can be associated with Lynch Syndrome (LS). The expression of MSH6 is lost in MSH2 deficient tumours. As a result, loss of MSH6 expression by immunohistochemistry in this patient is not informative of possible alterations of the MSH6 gene.

Clinical Guidance- Referral to a genetic counsellor for LS assessment is indicated according to Ontario Ministry of Health guidelines.

This is a worksheet. It is NOT the final diagnosis.
Suspected Lynch Syndrome

• Growing clinical problem
• MMRd tumor but patient found not to have germline alteration or MLH1 methylation using current diagnostic approaches
• Meta-analysis: 59% of CRC and 52% of EC with MMRd were identified as suspected LS - Buchanan et al
• Cancer risk intermediated vs LS and sporadic ?screening
Potential causes of MMRd in sLS

• Germline inactivation
  – Complex or cryptic mutations in MMR genes not detected using Sanger and MLPA
  – Low penetrance variant in MLH1 in regulatory/promoter region
  – Mutation in 3’ UTR of MLH1
  – Intronic mutation in MSH2
  – Complex structural variants: inversion of exons 1-7 in MSH2; interstitial deletion on ch3 with fusion of MLH1 and ITGA9
Immunostable MLH1 and MSH2 mutations

Germline *MLH1* Mutations Are Frequently Identified in Lynch Syndrome Patients With Colorectal and Endometrial Carcinoma Demonstrating Isolated Loss of PMS2 Immunohistochemical Expression

*(Am J Surg Pathol 2015;00:000–000)*
Biallelic somatic mutation in MMR genes

Colon and Endometrial Cancers With Mismatch Repair Deficiency Can Arise From Somatic, Rather Than Germline, Mutations

Sigurdis Haraldsdottir,1 Heather Hampel,2 Jerneja Tomsic,3 Wendy L. Frankel,4 Rachel Pearlman,2 Albert de la Chapelle,3 and Colin C. Pritchard5

Somatic aberrations of mismatch repair genes as a cause of microsatellite unstable cancers
Geurts-Giele, Journal of Pathology, 2014
Screening Algorithm for Endometrial Cancer

Endometrial Cancer < 70 years

IHC

Intact

No Family Hx

No Further Action

Family Hx

Genetic Counselling

Deficient

PMS2, MSH2, MSH6 Deficient

MLH1 Deficient

Methylation

Hypermethylated

No Family Hx

< 50 years or Family Hx

Genetic Counselling

Not Hypermethylated

No Family Hx

No Further Action

< 50 years or Family Hx

Genetic Counselling

University Health Network
Secondary mutation in a coding mononucleotide tract in \textit{MSH6} causes loss of immunoexpression of MSH6 in colorectal carcinomas with MLH1/PMS2 deficiency
Abrupt Loss of MLH1 and PMS2 Expression in Endometrial Carcinoma

Molecular and Morphologic Analysis of 6 Cases

(Am J Surg Pathol 2015;00:000–000)
Patchy loss of MLH1
Constitutional MMRd (CMMRD)

Biallelic germline mutation - in any of the MMR genes

Hematologic
Brain
Intestinal tumors
What are the implications of the study?

Testing everyone with colon cancer to identify families with the Lynch syndrome seems to prevent many cancers in those relatives at very high risk for cancer at an acceptable cost. However, the findings hold true only if many close relatives of all people with colon cancer and the Lynch syndrome have gene testing, and if healthy relatives with the gene defect undergo intensive cancer screening and possibly surgery to prevent cancer. The genetic testing is not something most doctors and clinics currently do, but testing for colon cancer could be done as a first step in many hospitals and laboratories. Genetic testing could then be done at specialty clinics or by genetic counselors.
Who is responsible?
Ethics

• Ultimate goal:
  – Restoration of patient health
• Key internal goods
  • Technical competence /excellence
  • Proper patient-pathologist relationship

• Changing ethical Landscape
  – Technical growth in pathology requires continued articulation of the goals and internal goods of the discipline
  – Prevention of disease
  – Complexity of the patient-clinician-pathologist relationship

The Virtuous Pathologist W E Stempsey
MMRd Prognostic and predictive biomarker

- The New England Journal of Medicine

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency


The Vigorous Immune Microenvironment of Microsatellite Instable Colon Cancer Is Balanced by Multiple Counter-Inhibitory Checkpoints


University Health Network
Figure 8: Next-generation sequencing data generated using a targeted MethylSeq panel to profile sequence variants and methylation status from a panel of genes associated with Lynch syndrome. Grey segments represent sequencing reads aligned to a human genome reference. The “Bisulphite-treated DNA” track shows MSH2 exon 1 is not methylated in this case (blue bases), although there are methylcytosines within the upstream region and downstream intronic bases (red). The “Untreated DNA” track contains data supporting calls at known germline SNP sites.
As laboratory techniques evolve, it is conceivable that gene “panel testing” or even some version of whole-genome analysis could be preferred on a technical basis to focused studies of a single or a few genes associated with a specific syndrome. Already, panel tests have come into clinical use, especially in cases where the differential diagnosis includes several cancer predisposition syndromes.
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Detecting Lynch Syndrome in Women with Endometrial and Ovarian Cancer

Blaise Clarke, MD
Princess Margaret Hospital/University Health Network
Ethics of MMR-IHC and MSI testing

University Health Network
Toronto General Hospital  Toronto Western Hospital  Princess Margaret Hospital
MMR-IHC and MSI testing

**MSI**
- No compelling grounds for informed consent for MSI testing
- MSI does not describe the patients body or inherited properties of relatives
- Test on tumor not germline
- MSI testing is similar to ER/PR in breast cancer

**IHC**
- IHC is generally not viewed as requiring consent
- MMR-IHC especially MSH2/6 can suggest genetic information about patient and family
- IHC has more in common with a genetic test than MSI testing
- IHC results cannot be considered synonymous with germline mutation
- Further study to better assess risk-benefit ratio

Chubak et al, Genetics in Medicine, 2011
<table>
<thead>
<tr>
<th>Test characteristic</th>
<th>Genetic testing</th>
<th>MSI testing</th>
<th>IHC testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physically invasive or burdensome on the proband</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Significant implications for patient management (including medical monitoring)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Potential to redefine a patient’s view of themselves and their health status</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Potential for psychosocial harm (anxiety, stigmatization, discrimination, etc.)</td>
<td>Low</td>
<td>Very low</td>
<td>Very low</td>
</tr>
<tr>
<td>Can create moral obligations to share results with the proband’s family</td>
<td>Yes</td>
<td>No</td>
<td>Unclear</td>
</tr>
</tbody>
</table>
Is MMR-IHC a Germline Test

- Two possible options about informing EC or CRC patients about testing for LS
  
  1. Counsel everyone at outset
     (97% unnecessary counselling)
  
  2. Perform MSI or IHC testing as part of routine practice without consent and reserve counselling for those whose results indicate that mutation testing be considered
     (counselling only directed at those with abnormal result; patients with MSH2 and/or MSH6 highly likely but not obliged to pursue testing)

- “It has been proposed that a waiver of consent to perform MSI and IHC screening be considered for individuals with CRC”.

University Health Network
Attitude Toward MMR-IHC

- 48.65% Never thought about it
- 31.08% Not germline
- 20.27% Germline
Conclusions

• The ethical and regulatory issues are currently unresolved

• Some institutions require consent- either specific or general for IHC testing

• Some do not currently require any patient consent, the idea being that IHC testing in this setting is not a direct test of a patient’s genome.

• Whatever approach is chosen, it is chain of command that guarantees that all targeted patient material is tested and that all applicable patients are referred for the appropriate counselling.
1: Access to MMR-IHC
2: Access to MSI testing
3: Genetic counseling services available
4: Multi-disciplinary approach to the identification of Lynch Syndrome in use
Progress and Test Development

- MLH1, PMS2, MSh2, MSH6 immunohistochemistry
- MSI – Bethesda panel
- Consortia and collaboration- genetic uncertainty
  - 1998: MSH2 deletions (MLPA)
  - 2009: EPCAM- “heritable somatic inactivation of MSH2”
  - 2014: Inversions of exons1-7 in MSH2 gene

- **Autosomal dominant genetic defects in mismatch repair genes (MMR)**
  - MLH1, MSH2, MSH6, PMS2, EPCAM