Universal Screening for Lynch Syndrome in Women with Newly Diagnosed Endometrial Cancer

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Disclosures

• No financial relationships to disclose
Objectives

1. To review lifetime risk of gynecologic cancer in women with Lynch Syndrome
2. To understand current limitations of family history and tumour morphology in identifying Lynch syndrome in women with endometrial cancer
3. To understand the role of universal IHC for mismatch repair proteins (MMR) to screen women with endometrial cancer for Lynch Syndrome
4. To review our prospective data on screening for LS in women with endometrial cancer
Lynch Syndrome

- Lynch Syndrome (LS) is an inherited cancer susceptibility syndrome
- Characterized by familial clustering of cancers (e.g. colorectal, endometrial and ovarian cancer)
- Autosomal dominant genetic defects in mismatch repair genes (MMR)
  - $MLH1$ (42%)
  - $MSH2$ (33%)
  - $MSH6$ (18%)
  - $PMS2$ (7.5%)
  - $EPCAM$
Lynch Syndrome and Endometrial Cancer

- 2-3% of unselected CRC and EC population have germline mutation in MMR genes
- Since universal screening studies began up to 6% in unselected women with EC
Cumulative Risk of Cancer by Age for *MLH1, MSH2, MSH6*

Bonadona et al 2011, *JAMA*
Cumulative Risk of Cancer by Age 70 for *MLH1, MSH2, MSH6, PMS2*

- Cumulative incidence for any cancer by age 70 years was 75% in females and 58% in males:
  - *MLH1* = 80% (71-88); EC = 34%; CRC = 45%; OC = 11%
  - *MSH2* = 75% (65-85); EC = 51%; CRC = 33%; OC = 15%
  - *MSH6* = 71% (52-90); EC = 49%; CRC = 26%; OC = 0%
  - *PMS2* = 24% (0-53); EC = 24%; CRC = 0%; OV = 0%

Moller et al 2015, *Gut*
Why is it Important to Identify Women with Lynch Syndrome?

• EC is often sentinel cancer in LS\textsuperscript{1}
  • 101 women who fulfilled ACII criteria with metachronous CRC/gynecologic cancer
  • 51% had gynecologic cancer as sentinel cancer
  • Median age at diagnosis of EC, 45 (27-62)
  • Median age at diagnosis of OC, 39.5 (29-63)

• Significant lead time before 2\textsuperscript{nd} cancer\textsuperscript{1}
  • 11 years (1-39) after diagnosis of EC developed 2\textsuperscript{nd} cancer
  • 5.5 years (2-17) after diagnosis of OV developed 2\textsuperscript{nd} cancer

\textsuperscript{1}Lu \textit{et al. Obstet Gynecol}, 2005
Why is it Important to Identify Women with Lynch Syndrome?

- **Opportunity to impact patient and family members with CRC screening and risk-reducing surgery**\(^1,2,3\)

- **Colonoscopy Screening q 1-2 years reduction in:**
  - Cancer Incidence – 57-69% ↓
  - Cancer Death – 65-83% ↓

\(^1\)Jarvinen et al JCO 2009; \(^2\) de Jong Gastroenterology 2006; \(^3\)Schmeler et al. NEJM 2006
Current Steps to Identifying Women with EC for Lynch Syndrome Testing in Canada

1. Family History
2. Genetic Counselling
3. Tumor Immunohistochemistry (IHC)/ Microsatellite testing (MSI)
4. Germline Testing
## Criteria for Genetic Evaluation

<table>
<thead>
<tr>
<th>CRITERIA</th>
<th>SGO 20-25%</th>
<th>OMOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amsterdam II (AMSII) Criteria:</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>3 Relatives Affected with LS Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Degree Relative of Other 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 2 Generations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 1 Diagnosed Under Age 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMSII-Like Criteria:</td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td>3 Relatives Affected with LS Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 of 3 are 1&lt;sup&gt;st&lt;/sup&gt; Degree Relative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 1 Diagnosed Under Age 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Known LS Mutation in 1&lt;sup&gt;st&lt;/sup&gt;/2&lt;sup&gt;nd&lt;/sup&gt; Degree Relative</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>CRC + Synch/Metachronous LS Cancer with 1&lt;sup&gt;st&lt;/sup&gt; &lt; Age 50/55</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>CRC or EC with MMR Defect</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>CRC &lt; 50 with 1&lt;sup&gt;st&lt;/sup&gt;/2&lt;sup&gt;nd&lt;/sup&gt; Degree Relative with LS Cancer &lt; Age 50</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>CRC &lt; Age 35</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>
Clinical Sensitivity of Family History in Predicting LS in Mutation Positive Women with EC

• 76 mutation-positive women with EC - MSH Familial Gastrointestinal Cancer Registry and the BC Familial Cancer Registry
• Pedigrees were assessed to determine if they met:
  – Amsterdam II criteria
  – Revised Bethesda Guidelines
  – SGO Guidelines

Clinical Sensitivity of Family History in Predicting LS in Mutation Positive Women with EC

<table>
<thead>
<tr>
<th>Age</th>
<th>All N = 76 (%)</th>
<th>MLH1 N = 18 (%)</th>
<th>MSH2 N = 50 (%)</th>
<th>MSH6 N = 8 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC Sentinel Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC Sentinel &gt; 50 years</td>
<td></td>
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</tr>
</tbody>
</table>

### Age

- **Mean**: 47.3, 49.3, 46, 50.6
- **Range**: 31-73, 36-61, 31-73, 34-61
- **> 50 years**: 28 (37), 8 (44), 15 (30), 6 (75)
- **EC Sentinel Cancer**: 52 (68), 11 (61), 34 (68), 7 (88)
- **EC Sentinel > 50 years**: 20/52 (39), 11 (61), 34 (68), 7 (88)

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Clinical Sensitivity of Family History in Predicting LS in Mutation Positive Women with Endometrial Cancer

<table>
<thead>
<tr>
<th></th>
<th>All N = 76 (%)</th>
<th>MLH1 N = 18 (%)</th>
<th>MSH2 N = 50 (%)</th>
<th>MSH6 N = 8 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amsterdam II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44 (58)</td>
<td>10 (56)</td>
<td>31 (62)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Revised Bethesda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27 (36)</td>
<td>7 (39)</td>
<td>19 (38)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>SGO 20-25%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>54 (71)</td>
<td>11 (61)</td>
<td>39 (78)</td>
<td>4 (50)</td>
</tr>
</tbody>
</table>

LS in 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
Rationale for Study

• Difficult to identify women with EC at risk for LS:
  – Criteria for LS colorectal-based
  – Dependence on detailed family history
  – 2/3 with EC would not be identified

• A reliable screening method required to identify women at significant risk for LS
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

• Prospective cohort study
  – July 2010 to June 2011
  – All consecutive cases of newly diagnosed EC
  – REB approved at Princess Margaret Cancer Centre, Toronto, Canada

• Eligibility Criteria:
  – Histologically confirmed EC
  – All histologic subtypes
  – FIGO stage I-IV
Methods

• Extended Family History Questionnaire (eFHQ)
• Blood samples for research germline testing:
  • *MLH1, MSH2, MSH6, PMS2, EPCAM*
• Tumour assessment:
  • Tumour morphology
  • IHC for MMR proteins
• Microsatellite testing:
  • 2 mononucleotide (BAT 25 and BAT 26)
  • 3 dinucleotide (D17S250, D5S346 and D2S123)
  • ≥ 2 markers were positive for MSI
Consecutive EC N = 182

Consented N=119 (65%)

1 excluded – ovarian cancer

118 EC

MSI N = 117

MSI+ N = 27 (23%)

Meets OMOH MSI+ N = 13

eFHQ N=105

Meets OMOH FHx N = 14

IHC N=118

IHC deficient N = 34 (29%)

Meets OMOH IHC N = 20

Universal Germline Testing N = 89 (75%)

Pathogenic Mutation N = 7

*N = 20 possible LS
Genetic counselling/ Confirmatory GLT

Ferguson, 2014 Cancer
### Demographics

<table>
<thead>
<tr>
<th>Patient Demographics</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age (range) N = 118</td>
<td>61 (26 – 91)</td>
</tr>
<tr>
<td>Postmenopausal (%) N = 117</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>94 (80)</td>
</tr>
<tr>
<td>No</td>
<td>23 (20)</td>
</tr>
<tr>
<td>FIGO Stage (%) N = 118</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>96 (81.3)</td>
</tr>
<tr>
<td>&gt;II</td>
<td>22 (18.7)</td>
</tr>
<tr>
<td>Histology (%) N = 118</td>
<td></td>
</tr>
<tr>
<td>High-risk</td>
<td>40 (33.9)</td>
</tr>
<tr>
<td>Low-risk (grade I/II endometrioid)</td>
<td>78 (66.1)</td>
</tr>
<tr>
<td>Fulfills clinical criteria</td>
<td></td>
</tr>
<tr>
<td>Ontario Ministry of Health</td>
<td>16 (15.2)</td>
</tr>
<tr>
<td>Amsterdam II</td>
<td>7 (5.9)</td>
</tr>
<tr>
<td>SGO 20-25%</td>
<td>8 (6.8)</td>
</tr>
</tbody>
</table>

Ferguson, 2014 *Cancer*
# IHC Results

<table>
<thead>
<tr>
<th>MMR Protein IHC Status</th>
<th>N= 118 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall</strong></td>
<td>34 (29)</td>
</tr>
<tr>
<td><strong>Overall IHC Meeting Ontario MOH Criteria:</strong></td>
<td>20 (17)</td>
</tr>
<tr>
<td>MSH2/MSH6</td>
<td>6 (5)</td>
</tr>
<tr>
<td>MLH1/PMS2</td>
<td>23 (20)</td>
</tr>
<tr>
<td>&lt; 60</td>
<td>7 (6)</td>
</tr>
<tr>
<td>&gt; 60 with + Family History</td>
<td>2 (2)</td>
</tr>
<tr>
<td>MSH6</td>
<td>5 (4)</td>
</tr>
<tr>
<td>PMS2</td>
<td>0</td>
</tr>
</tbody>
</table>

Ferguson, 2014 *Cancer*
# MSI Results

<table>
<thead>
<tr>
<th>MSI Testing</th>
<th>N= 117 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td></td>
</tr>
<tr>
<td>MSI+</td>
<td>27 (23)</td>
</tr>
<tr>
<td>MSS</td>
<td>86 (74)</td>
</tr>
<tr>
<td>Equivocal</td>
<td>4 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall MSI Meeting OMOH Criteria:</td>
<td>13 (11)</td>
</tr>
</tbody>
</table>

Ferguson, 2014 *Cancer*
# Tumour Morphology

<table>
<thead>
<tr>
<th>Morphology Feature</th>
<th>N= 111 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One or more LS-Associated Feature</td>
<td>67 (60)</td>
</tr>
<tr>
<td>Tumour Infiltrating lymphocytes &gt; 40 per 10 HPF</td>
<td>29 (25)</td>
</tr>
<tr>
<td>Peritumoural lymphocytes and lymphoid follicles</td>
<td>19 (16)</td>
</tr>
<tr>
<td>Tumour heterogeneity</td>
<td>14 (12)</td>
</tr>
<tr>
<td>Lower Uterine Segment Tumour</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Mucinuous Differentiation</td>
<td>39 (33)</td>
</tr>
</tbody>
</table>

Ferguson, 2014 *Cancer*
Results Summary

20/118 (17%) Possible Lynch Syndrome

Pathogenic Mutations Detected N = 7 (6%)
- 3 MSH2/MSH6 (ages 45, 53*, 60)
- 2 MSH6 (ages 39*, 63)
- 4 MLH1 (age 57, 59, 74*, 77*)

No Mutation Detected N = 9
- 2 MSH2/MSH6 (ages 57, 62)
- 1 MSH6 (age 63)
- 1 MLH1 (age 48)

No Results N = 4
- 2 MSH2/MSH6 (ages 57, 62)
- 1 MSH6 (age 63)
- 1 MLH1 (age 48)

* Positive Family history

Ferguson, 2014 Cancer
# Clinical Characteristics of LS Patients

<table>
<thead>
<tr>
<th>Mutation Status</th>
<th>Age</th>
<th>Referred GC</th>
<th>eFHQ Criteria</th>
<th>History of LS Cancer</th>
<th>Final Histology</th>
<th>FIGO Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1</td>
<td>50</td>
<td>FH</td>
<td>AMSII, SGO, OMOH</td>
<td></td>
<td>Grade 3 Endometrioid</td>
<td>IIIIC1</td>
</tr>
<tr>
<td>MLH1</td>
<td>43</td>
<td>FH</td>
<td>OMOH</td>
<td></td>
<td>Grade 1 Endometrioid</td>
<td>Ia</td>
</tr>
<tr>
<td>MLH1</td>
<td>39</td>
<td>FH</td>
<td>AMSII, SGO, OMOH</td>
<td>Grade 1 Endometrioid</td>
<td>Clinical stage I</td>
<td></td>
</tr>
<tr>
<td>MLH1</td>
<td>49</td>
<td>FH</td>
<td>SGO, OMOH</td>
<td>Colon 48 yo</td>
<td>Grade 2 endometrioid</td>
<td>IIIa</td>
</tr>
<tr>
<td>MSH6</td>
<td>45</td>
<td>FH</td>
<td>AMSII, SGO, OMOH</td>
<td>Clear Cell</td>
<td>IIIa</td>
<td></td>
</tr>
<tr>
<td>MSH6</td>
<td>26</td>
<td>IHC</td>
<td>None</td>
<td>Ovary 25 yo</td>
<td>Mixed Serous/Endometrioid</td>
<td>Ia</td>
</tr>
<tr>
<td>MSH2</td>
<td>57</td>
<td>IHC</td>
<td>None</td>
<td></td>
<td>Mixed serous/Endometrioid</td>
<td>Ia</td>
</tr>
</tbody>
</table>
## 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>89</td>
<td>85.7</td>
<td>81.7</td>
<td>28.6</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>

Ferguson, 2014 *Cancer*
Conclusions

• 17% suspected Lynch Syndrome
• Germline mutation rate at least 6%
• LS Morphologic features not adequate screening strategy
• Current family history criteria miss significant proportion of women with LS
• EC was the sentinel cancer in majority of women with LS
• 45% of those eligible did not follow through with genetic testing
• IHC most sensitive and specific method of screening
  – Directs germline analysis
  – Specificity may be improved in (age < 60)
  – Technically easier and less expensive
• UHN/MSH now do IHC on all EC < age 70

Ferguson, 2014 Cancer
Cancer Prevention

• Benefit of IHC if individual and FDR go to GC
• Uptake of GT high (77-90%) if get to genetic counsellor
• Our pilot had only 55% up take of GT (moderate)
• Review of US centers initiating IHC in CRC:
  – 67% had low uptake of genetic testing (<40% of eligible patients)
  – 20% had moderate uptake (40-70%)
  – 13% had high uptake (> 70% of eligible patients)
Barriers to Genetic Assessment

• Barriers:
  – Lack of IHC expertise and/or reflex IHC process
  – Lack of process for disclosure of results
  – Lack of resources for genetic counselling
  – Delay between IHC and cancer diagnosis
  – Distance from genetic counselling
  – Lack of perceived relevance to patient
  – Feeling overwhelmed by a new cancer diagnosis
  – Lack of knowledge of the implication of screen positive test by patient, FDR and physician’s

Facilitators to Genetic Assessment

- Facilitators:
  - Multidisciplinary collaboration (pathology, genetic counselling, surgeons)
  - Institutional champion
  - Guidance from institution that had implemented IHC (lessons learned)
  - Stream-lined GC referrals
  - Disclosure by someone trained in GC
  - Support from treating physician reinforcing the importance of genetic testing for patient and their FDR
  - Education to stake-holders

Maximizing Cancer Prevention:
Enhanced Genetic Uptake Program for Lynch Syndrome in Endometrial and Non-serous Ovarian Cancer

• **Aim 1:** Assess impact of our program on uptake of genetic testing and cancer incidence

• **Aim 2:** Determine the frequency of LS in women with non-serous OC.

• **Aim 3:** Model the cost-effectiveness of IHC in EC/OC.
Enhanced Genetic Uptake Program

Patient consents to study
- Endometrial Cancer - all stages, histology, < age 70
- Non-serous, non-mucinous ovarian cancer

? Meets Ontario MOH Family History Criteria for Genetic Assessment

IHC for MMR Proteins on Tumour

Positive Family History AND/OR

MMR-deficient
- MSH2, MSH6, PMS2 any age
- MLH1 < 60 years old

Eligible for Genetic Testing

Letter to Treating Physician
- Inform them of patient participation in study + eligibility for genetic testing
- Provide IHC results + education on clinical implications of results
- Ask them to review results with patient

Streamlined Genetic Referral
- Genetic counsellor-trained coordinator calls patient
- Study PI issues referral to Genetics for clinical germline testing

Germline Mutation Positive (Lynch Syndrome)
- Materials sent to patients to invite their FDRs to study
- FDR consents to study

Germline Mutation Negative
- Screening Recommendation Letter
  - Colonoscopy C.1-2 year
  - RR5
- Annual Follow-Up
  - Colonoscopy uptake
  - Personal and family history of cancer

Annual Follow-Up
- Annual Follow-up for 10 years
Impact of our Enhanced Genetic Uptake Program

A. 5600 EC + 520 non-serous OC in Canada/year  
   80% uptake  
   5% LS in unselected  
   245 probands with LS  
   735 FDRs  
   50% risk of LS  
   368 FDRs with LS  
   50% female  
   613 LS patients unaffected by CRC  
   60% risk of CRC  
   368 at-risk for CRC  
   184 female FDRs with LS  
   50% risk of EC  
   92 at-risk for EC/preventable by RRS  
   155CRC prevented  
   22 at-risk for OC/preventable by RRS

Up to 269 cancers prevented in 1 year if adopted nationally for newly diagnosed EC and non-serous OC

B. 850 EC and non-serous OC  
   80% uptake  
   5% LS in unselected  
   34 probands with LS  
   102 FDRs  
   50% risk of LS  
   51 FDRs with LS  
   50% female  
   85 LS patients unaffected by CRC  
   60% risk of CRC  
   51 at-risk for CRC  
   26 female FDRs with LS  
   50% risk of EC  
   13 at-risk for EC/preventable by RRS  
   21 CRC prevented

Predicted 37 cancers prevented in our study cohort based on 80% uptake of genetic testing and 70% compliance to colonoscopy guidelines in eligible individuals

CCSRI Prevention Grant - Ferguson
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

No Further Action

Family Hx

Genetic Counselling

Not Hypermethylated

Genetic Counselling

CCO Pollett et al 2015
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