Importance of PCR-Based Tumor Testing in the Evaluation of Lynch Syndrome

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ACCME/Disclosures

Dr. Broaddus declares he has no conflict of interest to disclose.

Value of Microsatellite Instability Analysis

- 56-year old woman with endometrial endometrioid adenocarcinoma

- Significant family history of colon cancer
  1. Father died of colon cancer
  2. Paternal uncle died of colon cancer
  3. Maternal grandmother has colon cancer

MSH6 IHC
What I wish I was seeing

Microsatellite Instability Analysis

- PCR-based test measures errors in DNA replication resulting from absence of MMR protein function
- Requires tumor and normal
- 5 markers recommended by the NCI (+ 2 markers (BAT25, BAT26, D2S123, D5S346, D17S250, BAT40 and TGFBRII)) to detect changes in the number of microsatellite repeats between normal tissue and tumor

IHC Problems – Errors in IHC Interpretation

MSI-High colorectal adenocarcinoma, but IHC was initially interpreted as retained expression of MLH1, MSH2, MSH6, and PMS2
IHC Only Tissue Testing
Unsettling Issues for Pathologists

- 365 EC patients had tissue testing (MMR IHC, MLH1 methylation, MSI)
- 51/365 (14%) tissue testing suggestive of Lynch (compare to germline mutation detected in 5.8%)
- 20/22 germline Lynch mutation had tissue testing
- 2/20 (10%) had tissue testing = sporadic (PMS2 and MSH6)
  - Each older than 50 y.o.; each with no family history of Lynch-associated cancer

<table>
<thead>
<tr>
<th>MSI-High (N = 102)</th>
</tr>
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<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>%</td>
</tr>
<tr>
<td>90</td>
</tr>
<tr>
<td>88.24</td>
</tr>
<tr>
<td>80.35 – 93.77</td>
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</table>

<table>
<thead>
<tr>
<th>MSI-Low (N = 591)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>%</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>11.76</td>
</tr>
<tr>
<td>6.23 – 19.65</td>
</tr>
</tbody>
</table>

2012; 5(2): 320-327
n=591 CRC and EC
Benefit of large sample size – can begin to identify the zebras which do pop up in population based studies

MMR+, MSI-high – estimate that 1/150 women with EC have LS with this tissue testing result

MSI Patterns Colorectal vs. Endometrial


44 colorectal cancers and 57 endometrial cancers from 8 families with known MLH1 or MSH2 mutations

MSS: EC 23%; CRC 11%

Amongst the MSI-High tumors, EC had fewer microsatellites affected

Young patient with both colorectal and adrenal cortical carcinoma
MSI-Low: Significance?

- 55 colorectal cancer patients
- 53/55 had positive MMR IHC

Patient 1: 53 year old with MSI-Low rectal adenocarcinoma and loss of MSH6 by IHC. Pathologic MSH6 mutation detected
Patient 2: 68 year old MSI-Low rectal adenocarcinoma with IHC loss of MSH2 and MSH6; declined genetic testing

Problematic – What is an IHC-MSI discordance? Are we missing any mutations in patients with MSI-Low tumors with retained IHC expression of MMR proteins? (Yes)

MLH1 Methylation Assay

Treat DNA with bisulfite – converts C to U (methylated C is resistant)

K562 negative control
RKO positive control
Tumor

Importance of MLH1 Methylation Assay

MLH1 Methylation Analysis in the Evaluation of Lynch Syndrome

MLH1 Methylation

IHC Loss of MLH1

No

Yes

No

Yes

Methylation

Not Methylation

N = 20

MLH1 Methylation Analysis

N = 20

N = 20

N = 20

N = 20

N = 20

N = 20
## Methylated MLH1 n (%) | Unmethylated MLH1 n (%) | P - value
--- | --- | ---
Age | 57 | 52 | 0.2505
Age range | 31-92 | 42-99 | > 0.999
Median Body Mass Index | 25 (13.7) | 12 (7.1) | > 0.999
Family history of GC | 7 (13.6) | 2 (1.2) | 0.299
Family history of CRC | 22 (31.2) | 12 (7.4) | 0.0315
Hypertension | 10 (14.3) | 2 (1.2) | 0.299
Family History of EC | 4 (10.5) | 3 (21.4) | 0.370
Family History of CRC | 7 (18.4) | 3 (21.4) | 0.999
Diabetes | 4 (10) | 6 (42.9) | 0.013
Hypertension | 23 (57.5) | 6 (42.9) | 0.371
Histology | 35 (87.5) | 5 (12.5) | > 0.999
Endometrioid | 32.9 | 26 (66.7) | > 0.999
Non-Endometrioid | 5 (35.7) | 9 (64.3) | > 0.999
FIGO Stage | 27 (67.5) | 13 (32.5) | > 0.999
I & II | 5 (35.7) | 9 (64.3) | > 0.999
III & IV | 11 (78.6) | 3 (21.4) | > 0.999
Endometrial Tumor Grade | 26 (74.3) | 9 (25.7) | 0.035
1 or 2 | 9 (18.1) | 2 (18.1) | > 0.999
> 3 | 3 (60) | 11 (61.1) | > 0.999
Lymphovascular space invasion | 20 (38.4) | 1 (1.7) | > 0.999
Tumor location | 37 (92.5) | 3 (7.5) | > 0.999
Corpus | 21 (52.5) | 9 (21.4) | > 0.999
Lower uterine segment | 25 (62.5) | 15 (37.5) | > 0.999
Tumor Size | 21 (52.5) | 9 (21.4) | > 0.999
< 4 cm | 19 (47.5) | 6 (42.9) | > 0.999
≥ 4 cm | 26 (74.3) | 9 (25.7) | > 0.999

## Sensitivity and Specificity for Accurately Predicting MLH1 Methylation

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
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<tbody>
<tr>
<td>Age &lt; 50</td>
<td>50.0</td>
</tr>
<tr>
<td>Body mass index &lt; 30</td>
<td>33.3</td>
</tr>
<tr>
<td>History of diabetes</td>
<td>42.8</td>
</tr>
<tr>
<td>Myometrial invasion &gt; 50%</td>
<td>71.4</td>
</tr>
<tr>
<td>Family history colorectal cancer</td>
<td>21.4</td>
</tr>
<tr>
<td>Family history endometrial cancer</td>
<td>21.4</td>
</tr>
<tr>
<td>Amsterdam II Criteria</td>
<td>14.3</td>
</tr>
<tr>
<td>SGO Criteria</td>
<td>71.4</td>
</tr>
<tr>
<td>SGO Criteria or ≥ 50% myometrial invasion or diabetes</td>
<td>100</td>
</tr>
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## Cost Comparison – Universal Tissue Testing vs Family History

<table>
<thead>
<tr>
<th>Screening strategy</th>
<th>SGO</th>
<th>Universal</th>
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<tbody>
<tr>
<td>who undergo MMR IHC testing</td>
<td>97</td>
<td>118</td>
</tr>
<tr>
<td>who undergo MLH1 methylation testing</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>who undergo MSI testing</td>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>PLH identified by strategy</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>PLH with the positive germline test (detected rate of 30%)</td>
<td>6.8</td>
<td>6.8</td>
</tr>
<tr>
<td>PLH with the positive germline test (detected rate of 50%)</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>PLH with the positive germline test (detected rate of 75%)</td>
<td>1.5</td>
<td>1.5</td>
</tr>
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### Estimated costs for screening strategies

<table>
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<th>Estimated cost per case identified</th>
<th>$41,655</th>
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<td>Average cost per case identified</td>
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### Unsettling Issues for Pathologists

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- 51/365 (14%) tissue testing suggestive of Lynch (compare to germline mutation detected in 5.8%)
- 2/20 germline Lynch mutation had tissue testing
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## Germline Testing vs. Tissue Testing

### Estimated costs for screening strategies

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No Germline Mutation Detected - Ohio State Gastroenterology 2014

- 32 patients with IHC loss of MMR protein, no MLH1 methylation, and no germline mutation detected
- 22/32 – 2 somatic mutations detected; less than 1/3 were previously classified by InSight
- All 22 were in ultra-mutated tumors; 5 had mutation in POLE

No Germline Mutation Detected – Netherlands Journal of Pathology 2014

- 40 colorectal cancers suspicious for Lynch Syndrome based on IHC and MS
- 5/40 had 2 somatic mutations
- 16/40 had 1 somatic mutation and suspected LOH

Conclusions and Recommendations

- Clinical and pathological characteristics capture less than 50% of patients with Lynch germline mutations
- Tissue testing approach as a screen - MMR IHC by itself is OK, but better if MLH1 methylation is added
- Adding MSI captures MMR proficient cases
- Adding somatic sequencing of MMR genes helps in ruling out hereditary basis of cancer