Microsatellite instability testing: which analysis in routine practice for the pathologist?

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MMR system

• Major system of DNA repair
• MMR proteins:
  • MLH1, MSH2, MSH6, PMS2, PMS1, MSH3, MLH3
MMR system

- Major system of DNA repair
- MMR proteins:
  - MLH1, MSH2, MSH6, PMS2, PMS1, MSH3, MLH3
MMR system

• Major system of DNA repair
• MMR proteins:
  • MLH1, MSH2, MSH6, PMS2, PMS1, MSH3, MLH3
MMR deficiency

• Two causes of MMR deficiency
  • Genetic
  • Somatic

• MMR deficiency
  • MMR protein: loss of expression
  • Microsatellite instability
  • *High mutational burden (TMB)*
Somatic MMR testing

• To test function of the MMR (MisMatch Repair) proteins in the tumor: somatic MMR testing
  • MSI: microsatellite instability (tumor genome)
  • MMR immunohistochemistry: loss of expression (protein inactivation)
• dMMR tumor (deficient) / pMMR tumor (proficient)
• dMMR/MSI tumor
dMMR tumor (deficient MMR / MSI)

Microsatellite Instability (MSI)

Loss of protein MMR expression

5 microsatellites with repeats length alterations

Coupled MLH1 and PMS2 loss of staining
Indications for somatic MMR testing in GI cancers

• Triage test to identify Lynch syndrome
• Prognosis factor
• Predictive factor for immunotherapy (anti-PD-1/PD-L1) for metastatic cancer
1-MSI as a biomarker of Lynch Syndrome

« Narrow-spectrum »
- Colon- Rectum
- Endometrium
- Ovary
- Urinary tract
- Sebaceous tumor

« Broad spectrum »
- Small bowel
- Gastric
- Biliary tract
- Pancreas
- CNS tumors (glioblastoma, ..)

Rarely*
- Appendiceal
- Esophagus

*Dunlop 1997
Hampel 2005
Eriscam, 2010
*Latham L et al, JCO 2018
2- MSI: a factor of good prognosis for localized disease

**Colon cancer**
- Stage I-II-II: better survival
- Superficial cancer « pT1 » on polyp resection
  - MSI tumor: low grade
- Stage II cancer*
  - To challenge adjuvant chemoT with 5FU

**Gastric cancer**
- Stage I-II: better survival
- Standard treatment: peri-operative chemoT
- MSI testing:
  - To challenge pre-surgery chemotherapy for located tumor
    - MSI tumor: no neoadjuvant chemoT
    - MSS tumor: neoadjuvant chemoT

* With high risks: pT4, poorly differentiated, vascular emboles, perforation

K Polom et al, *BJS* 2018; **105**: 159–167
3- MSI as biomarker for response to immunotherapy

• dMMR tumor: good response to anti-PD-1 (Le et al, 2015)

• MSI testing: 1st « pan-cancer » companion biomarker approved by FDA (refractory metastatic cancer)
Questions for the pathologist in routine practice:

• Which method to use to detect MSI in tumor?
• For which indication? For which tumor?
• Which sample to test? And when?

Meet test quality criteria
Time limits for patients care
1- Immunohistochemistry

**Benefits:**
- Easy, rapid, low cost test
- Feasible in all labs
- Biopsies or surgical sample
- With few tumor cells
- At the diagnosis (« reflex testing »)

**Drawbacks:**
- False loss of staining without MMR deficiency: defective pre-analytical step
- False retained staining: missense mutation of MMR gene

**Reflex Testing:**
- Colorectal cancer, gastric cancer

**Technical calibration**
- Training ++++

www.nordiqc.org
Defective pre analytical step: surgical sample with a sub optimal fixation
Immunohistochemistry

Four proteins: MLH1, MSH2, MSH6 and PMS2

MMR protein expression patterns:

• Normal expression of 4 proteins (pMMR)
• Loss of protein expression (dMMR) :
  • MLH1 and PMS2
  • MSH6 and MSH2
  • PMS2 Isolated loss
  • MSH6 Isolated loss
  • Heterogeneous expression : subclonal
  • Atypical expression
• Equivocal (indefinite)
• Not assessable
Heterogeneous staining

One area with abrupt loss of staining (sub-clone) = loss of staining

MLH1/PMS2

MSH2/MSH6
Equivocal staining
2-Molecular biology: Microsatellite Instability (MSI)

• MSI-PCR: classical test

• 2 consensus microsatellites panels
  • Bethesda Panel, 1998:
    - 2 mononucleotides repeats (BAT25 and BAT26) + 3 dinucleotide repeats (D5S346, D2S123, D17S250)
    - Needs comparison with normal DNA
  • NCI Panel, 2004 (« Pentaplex -PCR »):
    - 5 from 7 mononucleotides repeats: Bat25, Bat26, Bat40, NR21, NR22, NR24 and NR27
    - Quasimonomorphous microsatellites: without comparison with normal DNA
    - Higher sensitivity than Bethesda panel
    - Commercial kit (Promega®, Bat25, Bat26, NR21, NR22, et Mono 27) or house made with EQC validation

• MSI: ≥ 2 out of 5 microsatellites are unstable
  • Do not apply MSI-low or MSI-high
Molecular biology: Microsatellite Instability (MSI)

• Benefits:
  • Robust, reproducible test
  • Good sensitivity for dMMR for colon cancer
    • Gastric ? Pancreas ?
  • Tumor DNA available for supplementary analyses

• Drawbacks:
  • Not easy to interpret (no colorectal cancer ++)
  • At least 20 % of tumor cells in the sample (false negative cases)*
  • False positive cases (rare) with Pentaplex-PCR : polymorphisms

*Danjoux, Selves et al, 2006
MSI: The alternatives

- **Test Idylla™:**
  - Easy to use, no molecular biology platform,
  - 7 markers, 3 possible results: Stable (MSS), Unstable (MSI), No valid
  - Good sensitivity for cancer colon (validation ongoing for other tumor types)

- **HSP 110 (HT17):** validation ongoing
  - One marker: homopolymere T17 of HSP110
  - Technic: PCR-based (fragment analysis /capillary electrophoresis), Cold PCR

- **Short mononucleotides repeats**

- **NGS:**
  - No limit on the number of markers (mononucleotides repeats, 2 to 6 pb, composite microsatellites)
  - Tumor Mutational Burden, genotyping
  - Not applicable in routine practice (cost)

*References:*
- How-Kit A, Duval A, Hum Mutat 2017
- Galon R, Hum Mutat 2018
Which analysis to perform? Which hierarchy? When to perform MMR testing?

• Indication of testing
  • Guidelines for Lynch syndrome and for immunotherapy
  • No guidelines for prognostic stratification of CRC and gastric cancer
• Tumor type
• Quality sample
1- Guidelines on microsatellite instability testing for Lynch syndrome

• European guidelines*
  • CRC + personal history or 1st degree relative with Lynch Spectrum tumor
  • CRC < 70 years, regardless of family history
  • Immunohistochemistry with the four antibodies MLH1, PMS2, MSH2, MSH6 or molecular biology

• Universal Screening with IHC using the four antibodies
  • All CRC independently of age/family history
  • Place of MSI molecular testing?
  • CAP: MSI test for all cases showing retained expression of all MMR proteins

• No recommendation for other GI cancer

* Provided that MLH1 methylation could be performed

Vasen HF et al, GUT 2013
Weissman SM et al, J Genet Couns 2012
Sepulveda , Arch Pathol Lab Med 2017
2- ESMO recommendations on MSI testing for immunotherapy (anti-PD1/PD-L1) in cancer¹

• 1st: Immunohistochemistry with the 4 antibodies MLH1, PMS2, MSH2 and MSH6
  • For all tumors belonging to Lynch spectrum: colon, rectum, endometrium, small bowel, urinary tract, glioblastoma, sebaceous tumor)

• MSI-PCR:
  • 2d step for equivocal IHC analysis and cases with isolated loss MSH6 or PMS2.

• Eligibility for immunotherapy:
  • dMMR with IHC and MSI with molecular testing¹,²

¹Luchini et al, Annals of Oncology, 2019
²Cohen et al, JAMA Oncol 2018
## 3- Tumor type

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Frequency of dMMR/MSI&lt;sup&gt;1&lt;/sup&gt;</th>
<th>MMR IHC/MSI testing performance</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer</td>
<td>16 %</td>
<td>High concordance (&gt;95 %)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>IHC: first method</td>
</tr>
<tr>
<td>GI High grade Neuroendocrine Carcinoma</td>
<td>12%</td>
<td>?</td>
<td>No recommendation</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2 %-4 %</td>
<td>MMR IHC &gt; MSI-PCR&lt;sup&gt;3&lt;/sup&gt;</td>
<td>No recommendation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IHC: first method ?</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>6 %</td>
<td>High concordance ?&lt;sup&gt;4&lt;/sup&gt;</td>
<td>No recommendation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IHC: first method ?</td>
</tr>
<tr>
<td>Esophageal carcinoma</td>
<td>8 %</td>
<td>?</td>
<td>No recommendation</td>
</tr>
<tr>
<td>Small bowel</td>
<td>30 %</td>
<td>?</td>
<td>No recommendation</td>
</tr>
<tr>
<td>Biliary tract</td>
<td>13 %</td>
<td>?</td>
<td>No recommendation</td>
</tr>
<tr>
<td>Appendiceal</td>
<td>3%</td>
<td>?</td>
<td>No recommendation</td>
</tr>
<tr>
<td>Pan-Cancer (&gt; 50 cancer types)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2,2 %</td>
<td>MMR IHC / MSI-NGS: High concordance (98,2 %)</td>
<td>No recommendation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IHC: first method ?</td>
</tr>
</tbody>
</table>

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<sup>1</sup>Latham A et al, JCO 2018  
<sup>2</sup>Selves et al, ECP 2019  
<sup>3</sup>Lupinacci RM et al, Gastroenterology 2018  
<sup>4</sup>Bae YS et al, Gut Liver 2015
Role of pathologist for identifying Lynch syndrome

- 16% to 18% of MSI tumor (pan-cancer) are Lynch syndrome:
  - CRC: 19%
  - Small bowel: 12%
  - Gastric and pancreatic: 15%

*Guidelines of somatic testing for screening of Lynch syndrome, French National Institute of Cancer, 2016*
Take Home messages

- Three indications for MMR/MSI testing in GI cancers
- IHC with four proteins MLH1, PMS2, MSH6 and MSH2 is a reliable method for MMR testing in tumor samples whatever the tumor type and the indication
  - Needs rigorous technical processing and efficient training for IHC analysis
  - Access to a molecular biology platform has to be guaranteed
- All dMMR has to be controlled by a second analysis before treatment
- « Reflex testing » for CRC and gastric cancer has to be developed
- Needs to ensure a rapid and efficient workflow from pathologists to clinicians and geneticists to perform MMR/MSI analysis
Somatic MMR testing

First: IHC (or MSI)

pMMR

STOP

dMMR

To be confirmed by a second technique (MSI or IHC)

MMR status difficult to determine

Atypical MMR profile

Move to a second technique (MSI or IHC)

Experienced pathologist and molecular biologist

*MSH2/MSH6 loss of expression, isolated PMS2 or MSH6 loss + MSI

Probable Lynch syndrome

Oncogeneticist advice

*MLH1/PMS2 loss of expression + MSI

MLH1 Methylation BRAF Mutation

yes

Sporadic cancer

*Eligible for ImmunoT

no

STOP

Universal screening of Lynch syndrome, pronostis stratification for CRC and gastric carcinoma, Immunotherapy

IHC and MSI for geneticist requirement