Assay result variability during determination of mismatch repair deficiency status using immunohistochemistry – a transatlantic comparative study

**Purpose**

Colorectal cancer (CRC) patients with deficient mismatch repair (dMMR) have a significantly reduced risk of tumour recurrence and may respond less well to chemotherapy. Determination of MMR status is therefore advocated in patients whose adjuvant therapy is not indicated because of their low recurrence risk.

Despite substantial evidence to support the use of immunohistochemistry (IHC) to determine MMR status, little is known regarding the variability aspects – how reproducible is IHC in the determination of MMR status? We aimed to define MMR IHC assay reproducibility using formalin-fixed, paraffin-embedded (FFPE) material from the QUASAR randomised control trial (ISRCTN28375386) contained within heterogeneity-prone tissue microarray (TMA) material. Single observer reproducibility (intra-observer agreement) was also assessed.

**Methods**

Resected pathological material was obtained from 3239 patients (91% stage II) entered into the QUASAR randomised control trial of 5-fluorouracil (5-FU) / folinic acid (FA) chemotherapy versus observation alone (ISRCTN28375386). Material from 2007 patients was suitable for TMA construction. Tissue sections derived from identical TMAs were distributed to Leeds Institute of Molecular Medicine (LIMM) in the United Kingdom (UK) and Vitro Molecular Laboratories (VML) in the United States (US) for MMR testing using IHC techniques.

Expression of MMR proteins MLH1 and MSH2 was independently evaluated by LIMM and VML using IHC. Exact IHC methodologies employed by each laboratory were variable and blinded to the other unit. For **inter-laboratory agreement** analyses (table 1 a,b,c), following case exclusions or losses, the MMR status for 1224 stage II colon cancer patients was determined independently in both laboratories and compared; outcomes assessed included anatomical distribution of discordant cases, % agreement of scores and kappa coefficients. For **intra-observer agreement** analyses (table 2 a,b,c), following losses, MMR status was comparable in 1826 stage II / III CRC patients. **Intra-observer agreement** was determined by a single pathologist assessment of MMR-stained slides from both VML and LIMM. Again outcomes assessed included the anatomical distribution of discordant cases, % agreement and kappa coefficients.

**Results – discordant cases**

<table>
<thead>
<tr>
<th>LIMM MLH1 STATUS (%)</th>
<th>VML MLH1 STATUS (%)</th>
<th>Discordant MMR cases (n, %)</th>
<th>VML dMMR</th>
<th>LIMM dMMR</th>
<th>Totals</th>
<th>Left colon / rectum</th>
<th>Right colon</th>
<th>No data</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>dMMR</td>
<td>µMMR</td>
<td>dMMR</td>
<td></td>
<td></td>
<td></td>
<td>11 (55)</td>
<td>5 (12.8)</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>µMMR</td>
<td></td>
<td>µMMR</td>
<td></td>
<td></td>
<td></td>
<td>175 (86)</td>
<td>18 (1)</td>
<td>193</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>TOTAL</td>
<td>160</td>
<td>1064</td>
<td>1224</td>
<td>28 39 59</td>
<td>20 36 56</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion**

- Independent determination of MMR status by IHC on CRC TMA material is associated with good to excellent **inter-laboratory** and **intra-observer** agreement with the latter demonstrating excellent assay reproducibility (tables 1 / 2).
- The anatomical distribution of **inter-laboratory** discordant MMR cases may highlight possible false-negative cases as the dMMR phenotype is associated with the right colon.
- These data validate the routine use of IHC to determine MMR status, particularly as a result of whole tissue section IHC being less vulnerable to sampling heterogeneity when compared to TMAs.
- The precise reasons for dMMR / MLH1 / MSH2 discordance are currently under investigation.