Formalin-fixed cell blocks prepared by the sodium alginate method


Objective
To propose a suitable method of receptor analysis for cytological specimens from breast cancer metastases.

Background
1. Receptor status may differ between primary and recurrent tumors.
2. Receptor analysis is recommended when choosing drug therapies.
3. Cytological procedure can be used to take specimens from several types of metastatic lesions.
4. Necessity of using receptor analysis on cytological specimens is increasing.
5. At Shikoku Cancer Center where the first author (RN) worked until March 2018, formalin-fixed cell blocks prepared by the sodium alginate method has been routinely used from August 2011.

Method
1. Receptor analysis was conducted by the above mentioned method in cytology specimens obtained from breast cancer metastases at the nine institutions to which the listed speakers are affiliated (registration period: April 1, 2015 to March 31, 2016).
2. The stained slides for evaluation were collected and independently evaluated by eight pathologists.
3. The discordance rates among the observers and the interobserver agreement level were assessed.
4. The types of discordant cases and the reasons for the discordance were discussed.
5. A suitable method of receptor analysis for cytological specimens from breast cancer metastases was proposed.

Cell block production by the Sodium Alginat

Use tubes with a pointed tip.

The cells should be mixed well with this solution.

Centrifuge for 5 min at 3000 rpm

Fix cells in 10% buffered formalin for 6 to 48 hours.

Add 3.5 ml of 1% sodium alginate solution and mix.

It takes approximately 20 min after the fixation until the cell sediment is formed.

If you would like to know details of this method, please email Rieko Nishimura at (nisshimura-path@umin.ac.jp).

I can send you a PDF file of the manual with this method and our recommendation of receptor status evaluation.

Results
1. Of 61 specimens, 57 (93.4%) contained tumor cells.
2. Interobserver discordance in the 57 cases was reviewed.

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Number of discordant cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>Discordant in two or more observers</td>
</tr>
<tr>
<td>Estrogen receptor (ER)</td>
<td>11 (19.3%)</td>
</tr>
<tr>
<td>Progesterone receptor (PR)</td>
<td>19 (31.6%)</td>
</tr>
<tr>
<td>HER2 protein</td>
<td>25 (43.8%)</td>
</tr>
</tbody>
</table>

3. Cases with discordant results of HER2 protein staining between two or more pathologists.

<table>
<thead>
<tr>
<th>HER2 protein staining results</th>
<th>No. of cases</th>
<th>DISH assay results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative/Equivocal</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Negative/Positive</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Equivocal/Positive</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>15</td>
</tr>
</tbody>
</table>

Reasons of Discordance
1. The discrepancies in the results of ER or PR analysis were considered to be attributable to the presence of only a small number of positively stained cells or only faint staining of cells.
2. For the cases of HER2 protein, the interobserver differences in the scoring criteria influenced the high interobserver discordance rate.

Conclusion
1. CB method is suitable for receptor analysis to cytological specimens from breast cancer metastases.
2. Use 10% buffered formalin as the fixative and set the fixation time between 6 and 48 hours.
3. Use categorical scoring into positive and negative for evaluating the hormone receptor expressions.
4. Use strict criteria for HER2 protein 2+ and 3+ cases.

Disclosure Statement
The authors have no conflicts of interests to declare.

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