Immunohistochemistry and Breast Cancer
Diagnosis, Therapy and Prognosis

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**Introduction**

Immunohistochemistry is now a standard methodology in pathology laboratories worldwide and can be used to provide substantial information with regard to diagnosis, therapeutic prediction and prognosis of breast cancer.

**Diagnosis and Prognosis of Breast Cancer**

There are an ever-increasing number of markers that can be of value but it is important to ensure that they have been validated in published studies. The majority of these markers are antibodies that are suitable for use with formalin-fixed, paraffin-embedded (FFPE) tissue. Changes in the non-operative diagnosis of breast cancer, with the greater use of tissue core biopsies, have also had an impact. The nature of the lesions identified by screening may cause diagnostic difficulties that can be aided by immunohistochemistry. Core biopsies also allow evaluation of predictive markers that can help in the selection of a more appropriate form of management for advanced disease or for the elderly. Table 1 summarizes those markers that are frequently of use in breast cancer.

<table>
<thead>
<tr>
<th>Table 1. Markers of frequent use in breast cancer.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnosis</strong></td>
</tr>
<tr>
<td>Myoepithelial markers</td>
</tr>
<tr>
<td>- Smooth muscle actin</td>
</tr>
<tr>
<td>- CK14</td>
</tr>
<tr>
<td>- P-cadherin</td>
</tr>
<tr>
<td>- Calponin</td>
</tr>
<tr>
<td>- Caldesmon</td>
</tr>
<tr>
<td>- p63</td>
</tr>
<tr>
<td>Basement membrane – collagen IV</td>
</tr>
<tr>
<td>Cytokeratin 5/6</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>E-cadherin</td>
</tr>
<tr>
<td>Epithelial membrane antigen</td>
</tr>
<tr>
<td>Low molecular weight cytokeratins</td>
</tr>
</tbody>
</table>

**Immunohistochemistry can be of value in several problem areas**

- **Is it metastatic breast cancer?**
  Situations where this question can arise are; large axillary nodal metastasis with nothing palpable or detected mammographically within the breast; lung mass in women with a previous history of breast cancer. There are no markers specific for breast cancer although prominent staining for estrogen receptor (ER) α can be very supportive (1). A combination of markers such as low molecular weight cytokeratins, cytokeratin 7, cytokeratin 20, epithelial membrane antigen, thyroid transcription factor and melanosome (Melan –A) may help to discriminate on the basis of their presence in other types of cancers (1).
  Breast and ovarian malignancies are common in the same patient population. Positive reaction for GCDFP-15 is consistent with primary breast cancer, but a negative reaction is noninformative. A positive reaction for CA-125 is not helpful in distinguishing breast from ovarian carcinoma, but a negative reaction tends to favor breast origin.

- The detection of basement membrane collagen IV can help in determining whether or not there is invasion in cases of ductal carcinoma in situ (2).
The identification of myoepithelial cells around small glands, particularly in sclerotic lesions such as radial scars, can differentiate between a tubular or grade I infiltrating ductal carcinoma and a benign lesion. It may also be of value in assessing invasion. Smooth muscle actin is expressed in myoepithelial cells and is certainly useful, but it is also present in stromal myofibroblasts (3). Markers present in just myoepithelial cells include cytokeratin 14, common acute lymphoblastic leukaemia antigen (CALLA), P-cadherin, p63 and caldesmon (3-5). S100, however, lacks specificity, being expressed in epithelial cells as well (6).

Table 2. Staining of myoepithelial and epithelial cells.

<table>
<thead>
<tr>
<th></th>
<th>Myoepithelial cells</th>
<th>Epithelial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>S100</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Cytokeratin 7</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Actin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cytokeratin 14</td>
<td>+</td>
<td>-/+</td>
</tr>
</tbody>
</table>

The use of endothelial cell markers, e.g. CD31, may be of help in determining whether or not there is vascular invasion (7).

Concerning discrimination of usual type hyperplasia, atypical hyperplasia and ductal carcinoma in situ. Although there are no markers that can distinguish between atypical ductal hyperplasia and low-grade ductal carcinoma in situ, cytokeratin 5/6 expression is seen in usual type hyperplasia but not atypical hyperplasia or ductal carcinoma in situ, and can assist in differential diagnosis (8).

The cells of Paget’s disease of the nipple express low molecular weight cytokeratins and epithelial membrane antigen, unlike the surrounding squamous cells (9). CEA is also useful in showing the Paget cells. S100 and Melan-a is negative in Paget cells.

Both lobular neoplasia and infiltrating lobular carcinoma lack E-cadherin (10-12), which will be present in adjacent normal epithelium. This absence of a marker can be of value in distinguishing between expanded lobular carcinoma in situ and ductal carcinoma in situ, and infiltrating lobular and infiltrating ductal carcinoma.

Table 3. Distinguishing between expanded lobular and ductal carcinoma in situ.

<table>
<thead>
<tr>
<th></th>
<th>Cytokeratin</th>
<th>E-cadherin</th>
<th>Cytokeratin 7</th>
<th>Epithelial Membrane Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive ductal carcinoma</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ductal carcinoma in situ</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lobular</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lobular carcinoma in situ</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Paget’s</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Prediction
There are many markers that can help us to characterize breast cancer and provide us with information about choice of therapy and prognosis.
Estrogen receptor α

The determination of ER α by immunohistochemistry is now a standard method for selecting those patients who would benefit from endocrine treatment (13, 14). The ER antibody, clone 1D5, in combination with heat-induced epitope retrieval (HIER) methods can give clear reproducible results in FFPE tissue (please see Figure 1).

This type of method is also suitable for automation in larger laboratories. Results may be affected by delayed fixation (15) and because of this, some centers prefer to determine ER status on core biopsies. Inadequate antigen retrieval can also affect results (16).

In comparison to the original biochemical methods, immunohistochemistry cannot provide a quantified receptor level; however, semi-quantitative systems have been developed that are based on the percentage of cells stained and the intensity of staining. These include the H score, which is based on the summation of percentage of cells of different intensities, and the quick score that is now used more frequently (15). Both are shown in Table 4. In Denmark we use semiquantitative scoring only. 0-9 % of the nuclei is classified as negative and above 10 % is scored positive indicating a possibility of response when treated with Tamoxifen.

Between 70% and 80% of breast cancers are ER α-positive. There is a correlation between the presence of ER and the type and grade of the carcinoma, and as normal breast should express ER (the extent depending on the age of the woman) it can act as an internal positive control. Both of these factors should be taken into account when interpreting staining. Much of the data in predicting response and defining cut-off points relates to metastatic disease; the higher the score, the more likely the response (14, 17) and as such there is evidence that even low quick scores can predict a favourable response to adjuvant treatment (13).

Table 4. Scoring systems for estrogen and progesterone receptor: H and quick score.

<table>
<thead>
<tr>
<th>H Score</th>
<th>% cells with weak staining x 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% cells with moderate staining x 2</td>
</tr>
<tr>
<td></td>
<td>% cells with strong staining x 3</td>
</tr>
</tbody>
</table>

Total Score = 300

- 0 – 50: Negative
- 51 – 100: Low positive
- 101 – 200: Moderate positive
- 201 – 300: Strong positive

<table>
<thead>
<tr>
<th>Quick Score</th>
<th>Extent</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No staining</td>
<td>0</td>
</tr>
<tr>
<td>&lt; 1%</td>
<td>1</td>
<td>Moderate</td>
</tr>
<tr>
<td>1 – 10%</td>
<td>2</td>
<td>Strong</td>
</tr>
<tr>
<td>11 – 33%</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>34 – 66%</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>67 – 100%</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Summated to give maximum score 8

Likely response to endocrine treatment for advanced disease:

- 0 – 1 = endocrine treatment will not work
- 2 – 3 = small (20%) chance of response
- 4 – 6 = even (50%) chance of response
- 7 – 8 = good (75%) chance of response
In Denmark the cut off point is 1%, and all below is considered negative. Estrogen is read semi-quantitatively as an average of the positive nuclei of the carcinoma. In USA the patients is considered positive if but one nuclei is positive.

**Progesterone receptor**

Progesterone receptor (PR) is induced by estrogen and is, therefore, a marker of a functioning ER. Some investigators have found that Progesterone status has a greater power than ER status in predicting the disease free interval.

It can be determined in FFPE tissue using similar methods to ER detection and evaluated in the same way. Between 55% and 65% of breast cancers are likely to be positive for PR with two thirds of ER α-positive cases expressing it (18). PR will also be present in normal breast. The extent of reactivity in cancers may be similar to or less than ER α. In addition, a small proportion (< 5%) of breast cancers are PR-positive, ER receptor α-negative (18).

**pS2 protein**

pS2 is another estrogen-induced protein. It is a small (6.4 kDa) treefoil protein that is also present in the gastro-intestinal tract. pS2 can be detected using immunohistochemistry in 45-70% of breast cancers and may be of value in predicting hormone responsiveness (14). pS2 is used less frequently as a marker than PR.

**Epidermal growth factor receptor (EGFR)**

This is a member of the type1 tyrosine kinase receptor family and is also known as HER-1. It is present in normal breast. 30-40% of breast cancers have high levels with an inverse relationship with ER α. It can be detected immunohistochemically in FFPE tissue. Interest in this receptor has grown the development of inhibitors that can be used therapeutically, due to the recognized interaction between it and ER, and its possible role in endocrine response / resistance (19).

**HER2 protein**

This is the second member of the type 1 tyrosine kinase family, also known as c-erbB-2 and neu. It is an oncoprotein that is overexpressed in 20% of invasive primary breast cancers. There is a good correlation between amplification of the gene and overexpression of mRNA and protein. The latter can be detected immunohistochemically in FFPE tissue. Whilst initial interest in HER2 was as a marker for poor prognosis (20), its value now is in relation to the selection of patients who could benefit from Herceptin™ treatment, which is a humanized monoclonal antibody directed against the protein (21). This selection can be determined by the use of the HercepTest™. Staining should only be membrane and is assessed on a scale of 0 to 3+, dependent on the intensity of staining in more than 10% of invasive tumor cells. Any cytoplasmic staining is ignored. 3+ is strongly positive (please see Figure 3).

HER2 is detected at a higher frequency in ductal carcinoma in situ, particularly high grade, but is not found in usual and atypical hyperplasia. Paget’s disease of the nipple has a high frequency of staining.

Reading of the results of the immunohistochemical reaction of HER2 can be done using a grading system in which the degree of gene amplification corresponds to the staining reaction of the HercepTest™. Today, HercepTest™ is relied upon worldwide as the assay for HER2 overexpression because it has proven its worth as a useful predictor of response to Herceptin™. Yet, uncertainty remains an issue for the sub-group of HER2 IHC 2+ cases. To clarify these equivocal HER2 cases, HER2 FISH pharmDx™. Using the FISH test it can be shown at the gene level if the HER2 is amplified. If the ratio between FISH HER2/centromere 17 is above or equal to 2 the gene is amplified and treatment will be of benefit.
Table 5. Grading system for the evaluation of the immunohistochemical HercepTest™ reaction.

<table>
<thead>
<tr>
<th>Staining Pattern</th>
<th>Score</th>
<th>HER2 Protein Overexpression Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>No staining is observed or membrane staining is observed in less than 10% of the tumor cells</td>
<td>0</td>
<td>Negative</td>
</tr>
<tr>
<td>A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane</td>
<td>1+</td>
<td>Negative</td>
</tr>
<tr>
<td>A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells</td>
<td>2+</td>
<td>equivocal</td>
</tr>
<tr>
<td>A strong complete membrane staining is observed in more than 10% (30)% of the tumor cells</td>
<td>3+</td>
<td>Strongly positive</td>
</tr>
</tbody>
</table>

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**Topoisomerase - TOP2A**

The TOP2A gene is located on chromosome 17 close to the HER2 gene. It encodes an enzyme involved in the regulation of cell proliferation. Using fluorescence in situ hybridization (FISH) the number of genes can be counted. Tumor cells with TOP2A genetic changes seem to have increased sensitivity to antracyclines and is therefore a predictive factor. Around 20% of breast cancers show either deletion or amplification. Increased expression of TOP2A is associated with HER2 overexpression in most studies (24). TOP2A is a frequent finding in breast cancer and is often but not exclusively accompanied by HER2 gene amplification. It is associated with histological high grade and loss of estrogen receptor. TOP2A deletion may also be associated with worse prognosis of breast cancer and resistance to anthacycline.

**p53 protein**

Abnormalities of the p53 gene are important in many cancers, including breast. Detection of p53 protein by immunohistochemistry may be due to a mutation or other factors that result in stabilization of the protein, but can be affected by fixation, temperature treatment and visualization methods (25). The presence of p53 protein has been independently associated with poorer prognosis, since those with detectable p53 protein do worse (26).

**MIB-1 (Ki-67)**

The Ki-67 antigen is expressed in cells that are in the cell cycle and is used as a broad measure of proliferation. The antibodies now available are suitable for use with FFPE tissue when used in conjunction with antigen retrieval (please see Figure 4). Quantification is required with 500 to 1000 nuclei being counted (27). Several studies have found it to be of
prognostic value, and differences in counts before and after chemotherapy can indicate the likelihood of a good response (28).

**Other potential markers**

**Cyclin D1 protein**

Cyclin D1 is a cell cycle protein that regulates the progression through G₁, the protein being overexpressed in 50% of breast cancers. There is an association between this, presence of ER α and response to endocrine treatment (29), although if Cyclin D1 is present in ER-negative cases, this links with poor prognosis (30).

**p27 protein**

p27 is a cell cycle protein regulating G₁ to S transition that may be down-regulated in many cancers. In breast cancer high levels are associated with better outcome, whilst low levels can predict endocrine resistance (31).

**p21WAF1 protein**

p21 is a cyclin-dependent kinase that is regulated by p53; lack of p21 staining in the presence of p53 reactivity can predict poorer survival in patients treated with adjuvant chemotherapy (32).

**ERK1/2 protein**

ERK1/2 is a member of the mitogen-activated protein (MAP) kinase pathway that plays a central role in cell proliferation, ERK1/2 are thought to be key regulatory elements in the interactions between estrogen and growth factor cross talk. Detection of the active (phosphorylated) forms immunohistochemically relates to endocrine resistance (33).

**p38 protein**

p38 is another member of the MAP kinase pathway but the presence of the active form in breast carcinomas relates to endocrine response, in contrast to ERK1/2 (34).

**Estrogen receptor β**

Estrogen receptor β is a second form of ER that has a wider tissue distribution than ER α, and in normal breast is present in stromal cells and myoepithelial cells as well as epithelium (35).

**BCL2**

Expression of the anti-apoptotic protein BCL2 has been found to relate to a favourable prognosis (36).

**P-cadherin**

P-cadherin expression has been observed in a number of carcinomas and is usually associated with advanced stage and progression (37).
References


