The value of cytokeratin immunohistochemistry in the evaluation of axillary sentinel lymph nodes in patients with lobular breast carcinoma


BACKGROUND: Cytokeratin immunohistochemistry (IHC) reveals a higher rate of occult lymph node metastases among lobular carcinomas than among ductal breast cancers. IHC is widely used but is seldom recommended for the evaluation of sentinel lymph nodes in breast cancer patients.

OBJECTIVE: To assess the value of cytokeratin IHC for the detection of metastases in sentinel lymph nodes of patients with invasive lobular carcinoma.

METHODS: The value of IHC, the types of metastasis found by this method, and the involvement of non-sentinel lymph nodes were analysed in a multi-institutional cohort of 449 patients with lobular breast carcinoma, staged by sentinel lymph node biopsy and routine assessment of the sentinel lymph nodes by IHC when multilevel haematoxylin and eosin staining revealed no metastasis.

RESULTS: 189 patients (42%) had some type of sentinel node involvement, the frequency of this increasing with increasing tumour size. IHC was required for identification of 65 of these cases: 17 of 19 isolated tumour cells, 40 of 64 micrometastases, and 8 of 106 larger metastases were detected by this means. Non-sentinel-node involvement was noted in 66 of 161 cases undergoing axillary dissection. Although isolated tumour cells were not associated with further lymph node involvement, sentinel node positivity detected by IHC was associated with further nodal metastases in 12 of 50 cases (0.24), a proportion that is higher than previously reported for breast cancer in general.

CONCLUSIONS: IHC is recommended for the evaluation of sentinel nodes from patients with lobular breast carcinoma, as the micrometastases or larger metastases demonstrated by this method are often associated with a further metastatic nodal load.
node biopsy. The details reported included the metastatic status of the sentinel lymph nodes, the method of detection of sentinel node involvement (HE versus IHC), and further nodal involvement. Patients were not identified during this retrospective data collection and analysis, and therefore no ethics approval was necessary.

For the purpose of this study, any tumour cell in a sentinel lymph node was considered a positive finding. Nodal involvement was then categorised as isolated tumour cells, micrometastases, or macrometastases according to the definitions of these categories within the EWGBSP guidelines. 4-5,26

The laboratories contributing cases to the current study had different histological protocols relating to the work-up of the sentinel lymph nodes, but all departments embedded the whole node if it was considered negative on macroscopy. Nodes greater than 5 mm diameter were sliced into pieces and all departments used the approach of multilevel HE staining and routine cytokeratin immunostains if the HE slides were negative. Further details are given in table 1.

RESULTS

Data on sentinel lymph node biopsies from 449 ILCs were collected and analysed. Altogether 189 patients (42%) had sentinel node involvement of any type. This rate varied from institution to institution (range 22% to 50% (SD 10%), the latter being an outlier with small amounts of data (table 1)). Although the category of nodal involvement where IHC resulted in the greatest increase in the rate of detection was isolated tumour cells (17/19; 0.89), a significant proportion of the micrometastases (40/64; 0.63) were also detected by IHC, and some larger metastases were also demonstrated by this method, and a few isolated tumour cells were also picked up on HE stained slides (table 4). IHC alone identified 34% of all cases of nodal involvement. This rate also varied from institution to institution (range 22% to 50% (SD 10%), the latter being an outlier with small amounts of data (table 1)).

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Data on non-sentinel lymph nodes were analysed only in cases with sentinel node involvement. The database included one case of a false negative sentinel node biopsy, where axillary dissection revealed a single metastatic non-sentinel node out of 11 nodes recovered, and this was associated with a negative examination of the sentinel node. Axillary dissection was carried out in 44 patients without sentinel node involvement as part of the validation phase of sentinel node biopsy. By contrast, axillary clearance was omitted in 28 cases with an involved sentinel node (tables 3 and 4). This was either because of sentinel node involvement by isolated tumour cells or micrometastases only, or because of the participation of several patients in the European Organisation for Research and Treatment of Cancer (EORTC) trial,” “After Mapping of the Axilla: Radiotherapy Or Surgery” (AMAROS), randomising patients with positive sentinel lymph nodes between axillary dissection or radiation therapy. In all, 161 cases with involved sentinel lymph nodes underwent axillary dissection. Isolated tumour cells and some micrometastases (24 of 64; 0.38) were also identified by this method, and a few isolated tumour cells were also picked up on HE stained slides (table 4). IHC alone identified 34% of all cases of nodal involvement. This rate also varied from institution to institution (range 22% to 50% (SD 10%), the latter being an outlier with small amounts of data (table 1)).

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micrometastases in the sentinel lymph nodes were associated with a low rate of non-sentinel node involvement (0 and 0.14, respectively; 0.11 overall), whereas, as expected, the rate of macrometastases associated with non-sentinel node metastases was higher (0.60). Sentinel lymph node involvement first detected by IHC was associated with a considerable rate of non-sentinel node involvement (0.24), as some of the involvement detected by IHC was due to micrometastases, five of eight of these cases being associated with non-sentinel node metastases (table 5).

**DISCUSSION**

Cytokeratin IHC of unselected axillary lymph nodes from patients with ILC has been shown to upstage these patients more often than those with ductal carcinoma, and some laboratories have therefore introduced this method as a routine means of evaluating all lymph nodes removed in this subset of patients. As sentinel lymph node biopsy selects the nodes which are the most likely sites of regional metastases, it would be wiser to limit the use of IHC to these nodes. Most guidelines do not recommend IHC for the evaluation of sentinel lymph nodes in general practice, but it may be used in special cases, such as cases of ILC. As sentinel node biopsy is as reliable in ILC as in ductal cancers, we evaluated the role of cytokeratin IHC in a multi-institutional cohort of ILC patients who underwent sentinel lymph node biopsy.

Our results show that tumour size influences the nodal involvement in lobular carcinoma. This finding is consistent with nodal involvement being more common in larger tumours of any histological type. Although no comparison was made between the rate of nodal involvement in different types of tumour, our results are in agreement with data from the era before sentinel node biopsy. We found that 34% of all cases of nodal involvement were detected by IHC, which is higher than the rate reported for breast carcinoma in general or of ductal carcinoma, and is in the rate range reported for ILC. Although there were institutional methodological variations in this retrospective study, and also differences in the rate of nodal involvement and IHC positive cases, these latter differences can only partly be accounted for by the methodology. Mean tumour sizes were also different (table 1), and this may have altered the metastatic rates mentioned above. Obviously, the more detailed the histological protocol, the more positive cases will be detected.

Diversity of methods may be a problem with this study, but such variations in methodology can be found when comparing the various studies cited previously, and are encountered in nearly all reviews dealing with the upstaging role of IHC in breast cancer. Owing to the large number of cases, it is felt that the conclusions below can be relied upon, even if there may be some variation in the pathological approach to the reported lymph nodes.

To the best of our knowledge, this is the first report on the differential rates of isolated tumour cells, micrometastases, and larger metastases detected by IHC. Surprisingly, despite the fact that the isolated tumour cell category of nodal involvement was the one in which IHC produced the greatest increase in detection rate (17/19; 0.89), the largest category of IHC-detected nodal involvement (n = 40) was the micrometastases. This is probably because ILC often produces involvement of the nodal parenchyma, and we considered this to represent micrometastasis. A smaller number of macrometastases was also first detected by IHC.

The displacement and passive transport of tumour cells after needling procedures and excision biopsy have been proposed as a mechanism for the lodging of tumour cells in regional lymph nodes, especially in sentinel lymph nodes from breast cancer patients. The phenomenon of artefactual tumour cell seeding has been seen in cases of ductal carcinoma in situ, and, although this event may be rarer in the less cellular but less cohesive lobular carcinomas, isolated intrasinusoidal epithelial cells may well be of this origin. It has been postulated that these cells are detectable mainly by IHC only, and therefore that the IHC detected cells are

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**Table 3** Differential rate of nodal involvement according to tumour size

<table>
<thead>
<tr>
<th>Tumour size (cm)</th>
<th>ITCs</th>
<th>Micrometastases</th>
<th>Macrometastases</th>
<th>IHC</th>
<th>HE</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 1.0</td>
<td>4 (0.33)</td>
<td>6 (0.5)</td>
<td>2 (0.17)</td>
<td>6 (0.5)</td>
<td>6 (0.5)</td>
<td>12</td>
</tr>
<tr>
<td>1.1 to 2.0</td>
<td>7 (0.10)</td>
<td>24 (0.34)</td>
<td>40 (0.56)</td>
<td>23 (0.32)</td>
<td>48 (0.68)</td>
<td>71</td>
</tr>
<tr>
<td>2.1 to 3.0</td>
<td>6 (0.09)</td>
<td>22 (0.34)</td>
<td>36 (0.56)</td>
<td>23 (0.36)</td>
<td>41 (0.64)</td>
<td>64</td>
</tr>
<tr>
<td>3.1 to 4.0</td>
<td>1 (0.04)</td>
<td>9 (0.36)</td>
<td>42 (0.64)</td>
<td>16 (0.44)</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>&gt;4.0</td>
<td>1 (0.06)</td>
<td>3 (0.18)</td>
<td>13 (0.76)</td>
<td>4 (0.24)</td>
<td>13 (0.76)</td>
<td>17</td>
</tr>
<tr>
<td>All</td>
<td>19 (0.10)</td>
<td>64 (0.34)</td>
<td>106 (0.56)</td>
<td>65 (0.34)</td>
<td>124 (0.66)</td>
<td>189</td>
</tr>
</tbody>
</table>

HE, haematoxylin and eosin; IHC, immunohistochemistry; ITC, isolated tumour cells.

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**Figure 1** IHC detected metastasis measuring 2.5 mm in its greatest dimension (Cytokeratin AE1/AE3; original magnification ×400).

**Figure 2** HE stained slide from the same case as shown in fig 1. Tumour cells could be verified with this stain, but were not as obvious (original magnification ×400).
irrelevant. Patients undergoing prophylactic mastectomies after biopsies were only rarely found to have IHC detected epithelial cells in their sentinel nodes, however.16 This suggests that cancer needs to be present for there to be an increased rate of IHC positive sentinel nodes after biopsy or other means of physical manipulation of the tumour. These diagnostic or therapeutic procedures alone cannot be responsible for all cases of IHC detected nodal involvement.

A word of caution is required, as cytokeratin positive nodal structures cannot always be equated with metastatic nodal involvement. Besides the artefactually displaced tumour cells discussed above, normal constituents of the lymph nodes may also stain with anti-cytokeratin antibodies. Interstitial reticulum cells have been reported to be cytokeratin positive, especially when stained by CAM5.2 or an in-house cytokeratin cocktail, whereas this was much rarer or absent with AE1/AE3.33, 40 Plasma cells have also been reported to stain with CAM5.2 and pan-cytokeratin.41 Rarely, occasional cells compatible with histiocyte morphology also stain weakly with cytokeratin antibodies. Obviously, rare epithelial inclusions of the lymph nodes are also cytokeratin positive.64 Morphology should therefore never be neglected in the face of positive cytokeratin staining, and this will usually help to discriminate cancer cells from the others. Whenever there is doubt as to the nature of cytokeratin positive cells, these should not be called metastases, in line with the general rules of the TNM staging of cancers.64 None of the cytokeratin positive cells in this study was considered to represent inclusions or non-epithelial cells.

Although the rate of nodal involvement increased with increasing tumour size, and this was also true for the macrometastases, the rate of nodal involvement, isolated tumour cells, and micrometastases detected by HE tended to decrease with increasing tumour size (tables 2 and 3). The lack of an association between IHC detected sentinel node involvement and predictors of HE detected sentinel node involvement was reported earlier.77 Although this may be because isolated tumour cells are commonly (although certainly not always) the result not of a true metastatic process but rather of previous procedures and manipulations,77 another possible explanation could be that IHC tends to detect less obvious nodal involvement (generally falling into the category of isolated tumour cells or micrometastases), which is more common with smaller tumours, whereas larger tumours have already established larger metastases that are more likely to be detected by HE staining even in ILC.

Although we were unable to analyse the prognostic significance of these metastases in terms of relapse or survival, we did analyse the status of further lymph nodes in the axilla in the 161 patients who had an axillary dissection after the diagnosis of sentinel node involvement of any type. Twelve of 50 IHC detected sentinel lymph node metastases from ILC, but none belonging in the isolated tumour cell category, were associated with non-sentinel node involvement, which was higher than our previous meta-analysis (around 9%) would have suggested for IHC detected sentinel node involvement in breast cancers in general.52 None of the studies included in that meta-analysis drew conclusions in relation to the histological type of tumour. Our findings suggest that sentinel lymph nodes should be investigated by IHC if the primary tumour is of lobular type, because this approach may often detect micrometastases and even larger metastases, requiring further axillary treatment by current standards.

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