ORIGINAL ARTICLE

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

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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 needed 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.

Occult lymph node metastases that are not detected by the commonly used method of assessing a single haematoxylin and eosin (HE) stained level per lymph node have been known for decades,^{1,2} but their prognostic significance has been debated since their description.³ Such occult metastases can be demonstrated either by examining deeper levels of the tissue blocks, or by using a more sensitive method of detecting cancer cells. Immunohistochemistry (IHC) has been used for this latter purpose, with the aim of demonstrating epithelial markers. Retrospective studies, generally with small case numbers, have proved inconclusive for evaluating the prognostic significance of such occult metastases,³ which generally fall into the category of micrometastasis or isolated tumour cells^{4,5} (the latter also referred to as submicrometastases⁶). Examination of step sections is impractical when even moderate numbers of lymph nodes have been removed, and would mean a huge workload.⁷ The same applies to the use of cytokeratin IHC. In contrast, it is entirely feasible to use step sections and cytokeratin IHC when sentinel lymph node biopsy is undertaken, as these are the lymph nodes that are most likely to harbour metastases.⁸ If pathological efforts are concentrated on these selectively recovered nodes, an improved staging can be achieved.^{3,9} Although recommendations vary on the use of IHC for sentinel lymph nodes found to be uninvolved on HE staining,^{6,10–16} and most do not recommend its routine use,^{6,10,13,14,16} application of this technique is widespread both in the USA and in Europe.^{6,17}

Invasive lobular carcinoma (ILC) is known to produce nodal metastasis that can be difficult to detect on HE stained

slides, as it is composed of non-cohesive cells of a similar size to lymphocytes. Occult metastases of this tumour type detected by IHC have been reported to be more common than occult metastases from ductal carcinomas.^{18–22} Unfortunately the small number of cases of ILC investigated in these papers does not give an indication of the effect of occult metastases on recurrence and survival in this histological tumour type.²⁰ Nevertheless, two later studies with a longer follow up from the same institution suggested that the presence of IHC-detected occult micrometastases in either ILCs²³ or breast cancers in general²⁴ was not of prognostic significance. However, these two follow up studies did not distinguish between isolated tumour cells and micrometastases as currently defined.^{4,16,25}

Our present study was undertaken to assess the value of cytokeratin IHC for the detection of metastases in sentinel lymph nodes from patients with ILC.

METHODS

A previous study by the European Working Group for Breast Screening Pathology (EWGBSP) evaluated current practice concerning sentinel lymph nodes in European pathology laboratories.¹⁷ EWGBSP members working in laboratories where cytokeratin IHC is used routinely for sentinel nodes found negative on multilevel HE assessment were asked to collect data on cases diagnosed as ILC and staged on sentinel

Abbreviations: EWGBSP, European Working Group for Breast Screening Pathology; IHC, immunohistochemistry; ILC, invasive lobular carcinoma

Table 1 Methods used for the evaluation of grossly negative sentinel lymph nodes and basic characteristics of contributed cases

Department, identified by principal investigator	No of HE levels assessed per SLN (distance between levels)	No of IHC levels (antibody)	No of cases entered in this analysis (IHC positive/all positive)	Mean (SD) tumour size (mm)
Bianchi	Multilevel until extinction of the blocks* (0.1 mm)	Multiple (AE1/AE3)	146 (25/62)	17 (7)
Peterse	≥6 (distance not specified)	1 (CAM 5.2)	77 (14/42)	26 (15)
Sapino	4 (0.01 mm)	Multiple (KL1; EMA)	54 (4/18)	16 (9)
Arisio	Multilevel until extinction of the blocks* (0.15)	Multiple (mean 8) (AE1/AE3)	38 (3/13)	17 (9)
Reiner-Concin	Multilevel until extinction of the blocks* (0.25 mm)	Multiple (AE1/AE3)	33 (5/12)	21 (15)
Regitnig	Multilevel until extinction of the blocks* (0.125 mm)	Multiple (MNF-116)	33 (5/12)	21 (15)
Belloccq	Multilevel until extinction of the blocks* (0.5 mm)	Multiple (KL1)	32 (4/17)	27 (18)
Cserni	Multilevel until extinction of the blocks* (0.25 mm)	Multiple (PanCK or MNF-116 or AE1/AE3)	29 (4/11)	19 (11)
Martinez Penuela	10 (0.04 mm)	Multiple (AE1/AE3)	7 (1/2)	13 (4)

*For lymph nodes negative on macroscopy and/or intraoperative assessment only.
HE, haematoxylin and eosin; IHC, immunohistochemistry; SLN, sentinel lymph node.

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 29% to 55% (SD 9%); table 1). The rate of nodal involvement increased with increasing tumour size as expected (table 2). A similar relation with tumour size was seen with HE detected nodal involvement and macrometastases, but not with IHC detected nodal involvement, isolated tumour cells, or micrometastases (table 3).

HE staining detected most of the metastases. Although most of these metastases were classified as macrometastases,

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)). 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 means (8/106; 0.08). The median size of the macrometastases was 2.75 mm (range 2.1 to 7.0 mm), and all could be verified on HE in retrospect, after the IHC results were available (figs 1 and 2).

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

Table 2 Rate of nodal involvement according to tumour size

Tumour size (cm)	Number with negative SLNs	Number with positive SLNs	All	Proportion of SLN involvement
0.1 to 1.0	60	12	72	0.17
1.1 to 2.0	142	71	213	0.33
2.1 to 3.0	43	64	107	0.60
3.1 to 4.0	9	25	34	0.74
>4.0	6	17	23	0.74
All	260	189	449	0.42

SLN, sentinel lymph node.

Table 3 Differential rate of nodal involvement according to tumour size

Tumour size (cm)	ITCs	Micrometastases	Macrometastases	IHC	HE	All
0 to 1.0	4 (0.33)	6 (0.5)	2 (0.17)	6 (0.5)	6 (0.5)	12
1.1 to 2.0	7 (0.10)	24 (0.34)	40 (0.56)	23 (0.32)	48 (0.68)	71
2.1 to 3.0	6 (0.09)	22 (0.34)	36 (0.56)	23 (0.36)	41 (0.64)	64
3.1 to 4.0	1 (0.04)	9 (0.36)	15 (0.6)	9 (0.36)	16 (0.64)	25
>4.0	1 (0.06)	3 (0.18)	13 (0.76)	4 (0.24)	13 (0.76)	17
All	19 (0.10)	64 (0.34)	106 (0.56)	65 (0.34)	124 (0.66)	189

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

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 macrometastases, 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,^{20–23} and some laboratories have therefore introduced this method as a routine means of evaluating all lymph nodes removed in this subset of patients.²⁷ 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,^{6–10–13–14–16} but it may be used in special cases, such as cases of ILC.⁶ As sentinel node biopsy is as reliable in ILC as in ductal cancers,^{28–29} 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.^{18–22–29} 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.^{30–31} 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,^{18–22} and are encountered in nearly all reviews dealing with the upstaging role of IHC in breast cancer.³² 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,³³ and we considered this to represent micrometastasis.^{25–26} Obviously, 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^{34–36} 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*³⁷ 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

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).

Table 4 Method of detection and size of nodal involvement

	HE	IHC	All
Macrometastases	98	8	106
Micrometastases	24	40	64
ITCs	2	17	19
All	124	65	189

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

irrelevant. Patients undergoing prophylactic mastectomies after biopsies were only rarely found to have IHC detected epithelial cells in their sentinel nodes, however.³⁸ 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.^{39–40} Plasma cells have also been reported to stain with CAM5.2 and pan-cytokeratin.⁴⁰ 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.⁴¹ 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.⁴² 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 IHC 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.³⁷ 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,³⁷ 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

Table 5 Metastases in non-sentinel nodes v sentinel nodes, and the method of detection

	Non-SLN–	Non-SLN+	All
ITCs	13	0	13
Micrometastases	43	7	50
Macrometastases	39	59	98
IHC	38	12	50
HE	57	54	111

HE, haematoxylin and eosin; IHC, immunohistochemistry; ITCs, isolated tumour cells; SLN–, sentinel lymph node negative; SLN+, sentinel lymph node positive.

Take home message

Immunohistochemistry 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.

sentinel node involvement in breast cancers in general.³² 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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