Comparison of Immunohistochemistry with Conventional Histopathology for Evaluation of Sentinel Lymph Node in Breast Cancer

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Abstract The best method of pathological evaluation of sentinel lymph node in breast cancer has not been agreed upon. Immunohistochemical (IHC) techniques have shown a greater sensitivity over conventional histology for the detection of micrometastasis. The aim of the study was to determine whether IHC for Epithelial Membrane Antigen (EMA) on the sentinel node could be more sensitive than conventional histology for diagnosing micrometastasis in sentinel lymph nodes. Eighty-four clinically node negative breast cancer patients underwent sentinel node biopsy at time of surgery for breast cancer. The node was subjected to conventional histopathology as well as IHC for EMA. The sensitivity of histology viz a viz IHC for EMA for detection of sentinel node metastasis was 88% and the specificity was 96%. The overall diagnostic accuracy of histology viz a viz IHC was 93%. There were 4 patients with micrometastasis (<2.0 mm), which were positive on IHC but negative on histology. Two patients with poorly differentiated breast cancer had a false negative IHC for

EMA result as compared to histology. Immunohistochemistry for Epithelial Membrane Antigen can increase the detection rate of micrometastasis in sentinel lymph node. This can have important bearing on deciding the need of adjuvant systemic therapy. A false negative result for EMA may be seen in patients with poorly differentiated cancer. Therefore the best policy seems to employ both histopathology and IHC for EMA for the comprehensive evaluation of sentinel lymph node in breast cancer.

Keywords Sentinel node · Breast cancer · Immunohistochemistry

Introduction

Even several years after the introduction of sentinel lymph node biopsy to reduce the morbidity of axillary lymph node dissection, the best method of pathological examination of the sentinel lymph node has not been agreed upon. The relevance of detection of micrometastasis in the axillary lymph node is also not clear. It is generally accepted that immunohistochemical (IHC) methods are much more sensitive for picking up micrometastasis as compared to conventional hematoxylin and eosin (H & E) staining only. Rutgers in his study concluded that omitting IHC for cytokeratin staining was not a capital offence when the pathologist examined a sentinel lymph node [1]. However he also found that once a micrometastasis (i.e. metastasis 0.2–2.0 mm) is found in the sentinel node, there is a 10–20% chance of involvement of non-sentinel node. Additionally most surgeons advocate axillary lymph node dissection even in the presence of micrometastasis in the sentinel node.

There are very few studies comparing single section H & E staining with IHC evaluation of the sentinel node for detection of micrometastasis. It is quite likely that the

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choice of technique could change the fraction of tumor positive lymph nodes, need for adjuvant therapy and ultimately patient prognosis. The aim of our study was to determine whether IHC for Epithelial Membrane Antigen (EMA) on the sentinel node could be more sensitive than conventional H & E stain for picking up micrometastasis.

Material & Methods

Over a 3 years period from January 2007 to January 2010, 84 women with breast cancer visiting the Department of Surgery were recruited for the study. The inclusion criteria were (a) Clinically node negative axilla (b) Not have received pre-operative chemotherapy or radiotherapy (c) Not have undergone a previous breast biopsy (d) Neither pregnant or lactating (e) Intraoperative identification of methylene blue dye stained sentinel node possible.

All patients were subjected to modified radical mastectomy (Patey's variety) as part of our standard management protocol for these patients. Just prior to surgery 5 ml of methylene blue dye was injected peritumorally for staining of the sentinel node. After surgery, the blue stained sentinel node was harvested from the mastectomy specimen. The sentinel node was sent for conventional histopathological examination as well as IHC for EMA. A complete histopathological analysis of all remaining axillary nodes harvested from the mastectomy specimen was also carried out for correlation.

Method of IHC for EMA

This was carried out on the paraffin blocks of the sentinel node using standard immunohistochemistry methods. Prediluted mouse monoclonal antibodies against EMA were used. The primary antibody clone used was E29. The reagents were procured from Biogenex Life Sciences Pvt Ltd. (Secunderabad, India).

Observations

All 84 breast cancer women recruited in this study had a clinically N0 axilla. The average age was 50.4 years (range 33–69 years) with 32 women (38%) being premenopausal and 52 (62%) postmenopausal. As per size the tumors were classified as T₁=18 (22%), T₂=49 (58%) and T₃=17 (20%).

Intraoperative identification of blue node was possible in all 84 patients recruited in this study. Of these 69 patients (82%) had one stained node and 15 patients (18%) had 2 stained nodes. The sentinel node was noted to be level 1 (inferior to pectoralis minor tendon) in 72 patients (85%) and level 2 (behind the pectoralis minor tendon) in 12 patients

(15%). On an average an additional 8.4 non-stained nodes (range 5–14) were dissected out from the axillary specimen.

The results of histopathology and immunohistochemistry for Epithelial Membrane antigen of the blue stained nodes were obtained 9–10 days after surgery. On conventional histopathology, 34/84 (40%) of the sentinel nodes were positive for malignant deposit while 50/84 (60%) was negative. On IHC for EMA, 36/84 (43%) were positive for malignant deposit while 48/84 (57%) were negative. There was discordance in the histopathology and IHC report of sentinel node in 6 patients. Two of the histologically positive sentinel nodes were negative on IHC while 4 of histologically negative sentinel nodes were positive on IHC (Table 1). The 2 patients who had a positive SLN on histology but were negative on IHC for EMA were found to have poorly differentiated breast cancer. Therefore out of 84 sentinel lymph nodes, 32 were positive for secondary deposit on both histopathology and IHC while 46 were negative in both. Thus viz a viz histopathology the false negative rate of IHC was 6% (2/34). The 4 sentinel nodes, which were negative on histology but positive on IHC, were labeled by our pathologist to harbour micrometastasis (less than 2.0 mm). Considering these 4 patients to be true positives for metastasis the sensitivity of histology viz a viz IHC was found to be 88% (32/36). The specificity of histology viz a viz IHC was 96% (46/48). The overall diagnostic accuracy of histology as compared to IHC was 93% (78/84).

Histopathological evaluation of the remaining non-sentinel nodes dissected out of the mastectomy specimen was also done. Out of 34 sentinel node positive cases on histology, additional metastatic non-sentinel nodes were found in 21 patients (62%) while in 13 patients (38%), the sentinel node was the only positive node (Table 2). Out of 50 cases in which the sentinel node was negative on histopathology, histopathology of the non-sentinel axillary node did not yield metastasis in any of the specimens. Significantly of the 4 patients labeled as harboring micrometastasis (positive on IHC, negative on histology), non-sentinel nodes on histology were negative in all cases.

Discussion

Epithelial membrane antigen (EMA), also known as MUC 1, is a mucinous glycoprotein fixed to the luminal domain

Table 1 Correlation of histopathology with IHC for EMA on sentinel node in breast cancer

		Histopathology (n=84)	
		+ve (n=34)	-ve (n=50)
IHC (n=84)	+ve (n=36)	32	4
	-ve (n=48)	2	46

Table 2 Correlation of histopathology status of sentinel node with histopathology of non-sentinel axillary nodes

		Histopathology of non-sentinel axillary nodes (n=84)	
		+ve (n=21)	-ve (n=63)
Histopathology of sentinel nodes (n=84)	+ve (n=34)	21	13
	-ve (n=50)	Nil	50

of epithelial cell membrane of normal breast ducts [2]. In breast cancer, EMA expression is upregulated and it is an indicator of poor prognosis [3]. The almost constant presence of EMA in breast cancer cells facilitates the detection of micrometastasis in lymph nodes and bone marrow. EMA staining patterns have been related to histological grade of cancer, estrogen and progesterone receptor positivity and showed the capacity for predicting nodal dissemination of breast cancer.

In a study of 961 patients of node negative breast cancer, it was found that IHC for cytokeratin picked up an additional 38 cases (3.9%) that were missed on a single H & E stain [4]. Significantly 6 of these 38 patients also had non-SLN metastasis. Conversely Smidt et al found that axillary recurrence after negative SLN biopsy on multilevel sectioning and IHC for cytokeratin was only 0.25%. They suggested that a substantial increase in axillary relapse would not occur if SLN pathological examination were based on a single H & E section alone [5].

In our own study we found that conventional H & E was able to diagnose metastasis in 34 out of 84 (40%) SLN evaluated. When the same samples were subjected to IHC for EMA, 36 out of 84 (43%) were positive for tumor deposit. There was discordance in the histopathology and IHC report of sentinel node in 6 patients. Two of the histologically positive SLN was negative on IHC while 4 of histologically negative SLN were positive on IHC. The 4 SLN, which were negative on histology but positive on IHC, were labeled by our pathologist to harbour micrometastasis (less than 2.0 mm). The two patients who had a SLN, which was positive on histology but negative on IHC for EMA, were diagnosed to have poorly differentiated breast cancer. The remaining non sentinel lymph nodes examined in these 4 cases (HPE negative, IHC positive) were free of metastasis. Given the small numbers in our study, the clinical implications of our findings should be interpreted considering the reported 10–20% tumor positive non-SLN in patients with micrometastasis in SLN [6].

Sentinel lymph node biopsy itself has led to a more detailed histopathological scrutiny of the identified node instead of a more cursory examination of several nodes. This has resulted in a significant increase in identification of low volume metastasis and consequent stage migration as many of the former node negative cases containing occult metastases are now placed into the node positive micrometastasis group [7]. The American

Joint Committee on Cancer (AJCC) has modified the pTNM classification by splitting the micrometastasis category into two. Micrometastasis between 0.2–2.0 mm are staged as pN₁m_i and metastasis less than 0.2 mm are called isolated tumor cells and staged as pN0(i+) [8]. Most surgeons treat pN₁m_i lesions as true metastasis and recommend axillary dissection and systemic therapy. Isolated tumor cells or pN0(i+) are considered as node negative for further treatment decisions.

One important clinical implication of the detection of micrometastasis is the need for systemic therapy. Groen et al (2007) found that by including IHC for cytokeratin in the evaluation of SLN increased the indication for systemic therapy by 8.4% based on current Dutch Treatment Standards [4]. They concluded that restricting pathologic examination of the SLN to a single H & E section results in erroneously omitting additional axillary treatment and systemic therapy.

Epithelial membrane antigen (EMA) or MUC 1 expression has been found to occur in 93.2% of breast cancer patients [9]. It is significantly associated with smaller tumors, lower tumor grade, ER and PR positivity. Negativity for EMA was found in less than 7% of cases and is associated with ER negativity, PR negativity and cyclin D₁ negativity. Thus considering the fact that node negative patients are likely to be associated with smaller tumors with lower histological grades, EMA expression is a good indicator for micrometastasis in SLN. Significantly EMA expressing tumors also show an increased relapse free survival. These observations are supported by the findings of Luna-More et al who reported that EMA negative tumors are high grade, ER and PR negative and are more frequently associated with positive axillary lymph nodes [10].

The primary aim of sentinel node biopsy is to enhance the detection of minimal lymph nodal involvement such as micrometastasis. However it was found that 25% of axillary node negative patients diagnosed by single section H & E stain suffered a relapse within 10 years [11]. Thus it was suspected that this method of evaluation led to understaging of the disease due to missing of micrometastasis. Literature and our own study suggest that Immuno Histo Chemistry (IHC) of the node can increase the detection of micrometastasis. IHC for EMA is extremely sensitive for detection of SLN micrometastasis. However it may show a false negative result in patients with poorly differentiated breast cancer as happened in 2 of our patients (6% false negative rate). There is also concern

that routine use of IHC may increase the detection of false positive tumor deposits and the detection of micrometastasis of no biological relevance [12].

Conclusions

The best method for the pathological assessment of the sentinel node in breast cancer has not been agreed upon. Immunohistochemical (IHC) techniques are generally thought to be more sensitive as compared to conventional histopathology. In our study on 84 patients, IHC for Epithelial Membrane Antigen (EMA) could detect micrometastasis (<2.0 mm) in sentinel lymph nodes in 4 additional patients, which were missed on conventional histopathology. Additionally 2 patients had a positive histology report but were negative on IHC for EMA. Detection of micrometastasis can have an important bearing on deciding the need for axillary dissection and adjuvant systemic therapy. However poorly differentiated breast cancer can have a false negative report on IHC for EMA. The best policy seems to employ both histopathology and IHC for EMA for the comprehensive evaluation of sentinel node in node negative breast cancer.

Immunohistochemistry for Epithelial membrane antigen (EMA) on the sentinel lymph node (brown pigment shows EMA positivity)

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