e-ISSN: 0976-822X, p-ISSN:2861-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2023; 15(6); 116-121

Original Research Article

# A Hospital Based Comparative Assessment of Immunohistochemistry with Conventional Histopathology for Evaluation of Lymph Nodes

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**Received: 14-03-2023/ Revised: 05-04-2023 / Accepted: 03-05-2023**
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**Conflict of interest: Nil**

**Abstract**

**Aim:** The aim of the present study was to assess the comparison of immunohistochemistry with conventional histopathology for evaluation of lymph nodes.

**Methods:** The present study was conducted at Department of Pathology, IGIMS, Patna, Bihar, India from January 2016 to July 2017. 100 women with breast cancer were included in the study.

**Results:** All 100 breast cancer women recruited in this study had a clinically N0 axilla. The average age was 50.5 years (range 33–70 years) with 40 women (40%) being premenopausal and 60 (60%) postmenopausal. As per size the tumors were classified as T1=23 (23%), T2=57 (57%) and T3=17 (20%). On conventional histopathology, 40/100 (40%) of the sentinel nodes were positive for malignant deposit while 60/100 (60%) was negative. On IHC for EMA, 41/100 (41%) were positive for malignant deposit while 59/100 (59%) were negative. Histopathological evaluation of the remaining nonsentinel nodes dissected out of the mastectomy specimen was also done. Out of 45 sentinel node positive cases on histology, additional metastatic non-sentinel nodes were found in 30 patients while in 15 patients, the sentinel node was the only positive node.

**Conclusion:** 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.

**Keywords:** immunohistochemistry, conventional histopathology, lymph nodes



## Introduction

Despite the exceptional utility of genomics methods in the discovery phase of experimentation, these technologies require validation due to problems including misidentification of nucleic acid probes on gene expression microarrays[1,2], non-specificity of probes[3] and the essentially unavoidable false discovery rates associated with massive multiple hypothesis testing.[4] Appropriately powered studies to validate initial results of genomics studies often are lacking[5] or fail to confirm initial discovery-phase results[6] limiting clinical implementation of new disease biomarkers.

Immunohistochemistry (IHC) is an important technique for biomarker validation for several reasons. First, it allows direct visualization of biomarker expression in histologically relevant regions of the examined tissue. This is an important advantage over "grind and bind" assays in which tissue is solubilized for biochemical analysis, which may lead to false negative results if few biomarker-positive cells are present in a background of biomarker-negative tissue elements.[7] Second, clinical laboratories typically perform IHC on FFPE tissue sections processed by standard methods, making potentially available hundreds of millions of specimens for study.[8] Third, validated IHC assays may be implemented readily into clinical practice. For example, genomics methods were used to discover mRNA biomarkers capable of subclassifying diffuse large B cell lymphoma (DLBCL) into prognostically discrete subtypes.[9] Relevant subsets of these gene products were validated at the protein level using IHC on large numbers of DLBCL specimens[10,11] and validated IHC panels are now used clinically.

Traditionally, pathologists have visually scored IHC data. For example, in the calculation of an HSCORE, a summation of the percentage of area stained at each intensity level multiplied by the weighted intensity (e.g., 1, 2, or 3; where 0 is no staining, 1 is weak staining, 2 is moderate staining and 3 is strong staining) of staining is generated.[12] These analyses are frequently performed on specimens arrayed on stained TMA sections allowing representation of a sufficiently large number of specimens to for statistically rigorous testing.[13,14] Pathologist visual scoring is fraught with problems due to subjectivity in interpretation. Automated IHC measurements promise to overcome these limitations. Whole-slide imaging systems are widely available to convert glass slides into diagnostic quality digital images.[15] Automated IHC measurements are precise in ranges of staining that appear weak to the eye[16] and produce continuous data.[17] Moreover, when automated IHC measurements are provided to a pathologist during visual scoring, computer aided IHC analysis substantially improves both intra- and inter-observer agreement.[18]

The aim of the present study was to assess the comparison of immunohistochemistry with conventional histopathology for evaluation of lymph nodes.

## Materials And Methods

The present study was conducted at Department of Pathology, IGIMS, Patna, Bihar, India from January 2016 to July 2017. 100 women with breast cancer were included in 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 the 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.

**Results**

**Table 1: Demographic details**

| | |
|---|---|
| Premenopausal | 40 (40) |
| Postmenopausal | 60 (60) |
| **Size of tumors** | |
| T1 | 23 (23) |
| T2 | 57 (57) |
| T3 | 20 (20) |

All 100 breast cancer women recruited in this study had a clinically N0 axilla. The average age was 50.5 years (range 33–70 years) with 40 women (40%) being premenopausal and 60 (60%) postmenopausal. As per size the tumors were classified as T1=23 (23%), T2=57 (57%) and T3=17 (20%).

**Table 2: Correlation of histopathology with IHC for EMA on sentinel node**

| | | Histopathology | |
|---|---|---|---|
| | | +ve N=40 | -ve N=60 |
| IHC | +ve (41) | 36 | 5 |
| | -ve (59) | 4 | 55 |

On conventional histopathology, 40/100 (40%) of the sentinel nodes were positive for malignant deposit while 60/100 (60%) was negative. On IHC for EMA, 41/100 (41%) were positive for malignant deposit while 59/100 (59%) were negative.

**Table 3: Correlation of histopathology status of sentinel node with histopathology of non-sentinel axillary nodes**

| | | **Histopathology** | |
|---|---|---|---|
| | | +ve N=30 | -ve N=70 |
| Histopathology of sentinel nodes | +ve (45) | 30 | 15 |
| | -ve (55) | 0 | 55 |

Histopathological evaluation of the remaining nonsentinel nodes dissected out of the mastectomy specimen was also done. Out of 45 sentinel node positive cases on histology, additional metastatic non-sentinel nodes were found in 30 patients while in 15 patients, the sentinel node was the only positive node.

**Discussion**

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 micro metastasis in the axillary lymph node is also not clear. It is generally accepted that immunohistochemical (IHC) methods are much more sensitive for picking up micro metastasis 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.[19] However he also found that once a micro metastasis (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 micro metastasis in the sentinel node.

All 100 breast cancer women recruited in this study had a clinically N0 axilla. The average age was 50.5 years (range 33–70 years) with 40 women (40%) being premenopausal and 60 (60%) postmenopausal. As per size the tumors were classified as T1=23 (23%), T2=57 (57%) and T3=17 (20%). On conventional histopathology, 40/100 (40%) of the sentinel nodes were positive for malignant deposit while 60/100 (60%) was negative. On IHC for EMA, 41/100 (41%) were positive for malignant deposit while 59/100 (59%) were negative. 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.[20]

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.[21] 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 min are staged as pN1mi and metastasis less than 0.2 mm are called isolated tumor cells and staged as pN0(i+).[22] Most surgeons treat pN1mi 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.

Histopathological evaluation of the remaining nonsentinel nodes dissected out of the mastectomy specimen was also done. Out of 45 sentinel node positive cases on histology, additional metastatic non-sentinel nodes were found in 30 patients while in 15 patients, the sentinel node was the only positive node. 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.[23]

## Conclusion

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 100 patients, IHC for Epithelial Membrane Antigen (EMA) could detect micrometastasis (<2.0 mm) in sentinel lymph nodes. 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.

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