

## Review Article

# To Level or Not: Histological Examination of Sentinel Lymph Nodes in Patients with Breast Carcinoma

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## Abstract

Management of the axilla in patients with breast carcinoma has changed rapidly in recent years due to development of an increasingly conservative approach to axillary staging. Sentinel node examination plays a pivotal role in subsequent decision for axillary lymph node dissection. There is still no consensus on how best to examine SLNs histologically. The examination of sentinel node should be thorough enough whilst not missing clinically significant information. The main goal of sentinel lymph node examination is to detect all macrometastasis. In this study we have reviewed an extensive histological protocol to identify whether it resulted in detection of clinically significant metastatic deposits. Our study identified that one hematoxylin-eosin-stained section along with immunostaining for pancytokeratin from each block results in the detection of clinically significant metastases and hence recommended as the preferred method of pathologic evaluation.

**Keywords:** Axillary node examination; Breast carcinoma; Sentinel node

**Abbreviations:** ALND: Axillary Lymph Node Dissection; MiM: Micrometastasis; SLN: Sentinel Lymph Node

## Introduction

Breast carcinoma is the most frequently diagnosed carcinoma in women in nearly all regions of the world [1]. In recent years, management of breast carcinoma has been revolutionized with the advent of new treatment methods. The decision as to which management pathway to choose depends on a number of patient factors, including age, tumour type, grade and the stage of carcinoma, hormonal and HER2 status as well as axillary lymph node metastases. An increasingly conservative approach to axillary staging has been developed. Therefore, management of the axilla in patients with breast carcinoma has changed rapidly in recent

years. Sentinel Lymph Node (SLN) assessment is a recognised technique used to assess the axillary lymph node status. It is based upon the observation that carcinoma cells migrate first to one or few lymph nodes (i.e., the sentinel node/nodes) before involving other axillary lymph nodes. SLN biopsy was implemented as an alternative procedure to axillary dissection, in order to minimize the negative impact of axillary surgery [1].

Data from the American College of Surgeons Oncology Group (ACOSOG) Z0011 trial suggest that Axillary Lymph Node Dissection (ALND) may be omitted in selected patients with 1 or 2 positive SLNs [2]. In addition, the findings of the multicentre, randomised controlled, phase 3 trial (IBCSG 23-0) after a median follow-up of 9.7 years, support the current practice of not doing an axillary dissection when the tumour burden in the sentinel nodes is Micrometastasis (MiM). This trial compared axillary dissection versus no axillary dissection in patients with breast cancer and MiM in sentinel node [3]. Meticulous attention to and reporting

of microscopic amounts of nodal disease results in over treatment which can be potentially harmful to the patient due to treatment side effects and an unnecessary psychological burden [2]. The histological assessment protocols regarding SLN examination for breast cancer are remarkably variable in different institutions both within Australia and worldwide, and there is still no consensus on how best to examine SLNs histologically. Until recently at our own institution, the SLNs were examined by slicing the lymph node at 2mm intervals and performing 6 H&E levels 200 microns apart along with one AE1/3 immunohistochemical stain on each node block. This method of multiple sectioning and a keratin staining was aimed to detect all possible MiM in the lymph node (0.2mm to 2mm) and was one of the most intensive protocols used among the major laboratories within the Sydney metropolitan area.

This study was conducted to determine whether the intensive examination protocol used resulted in detection of clinically significant metastatic deposits.

## Methods

A search was conducted using key words ‘isolated tumour cells and or ‘micrometastasis’ from our database between 2010 and 2015. The inclusion criteria included cases with available histology slides of breast cancer SLNs containing metastatic carcinoma in the form of either MiM or Isolated Tumour Cells (ITCs). Each identified lymph node had been measured in three dimensions and serially sliced at 2mm intervals perpendicular to the long axis of the lymph node, so that the macrometastasis were not missed. These slices were embedded in paraffin blocks and sectioned at 200 micron intervals into 6 levels, 4 microns thick, stained with H&E (L1 to L6). Between levels 2 and 4, a section was taken to be stained with a pancytokeratin marker AE1/3 (keratin). This method of levelling the slices at 200 micron ensured that the MiM was not left undetected. A data sheet was generated to de-identify the cases. Information in the data sheet was collected using the medical records, microscopic reports and reviewing all the histology slides of SLNs. The information included the presence or absence of tumour cells in each of the 6 H&E levels and AE1/3 sections, imprint cytology diagnosis (if available), whether ALND was performed subsequently, whether positive lymph node(s) was/were identified in ALND and the breast tumour characteristics including tumour size, grade, type and presence of lymphovascular invasion. Size of metastatic carcinoma was recorded on L1, L6 and keratin to identify if there was a significant increase in size

from ITC to MiM on deeper levels in all nodes with MiM. The analysis was performed to detect any difference in detection of MiM between doing 6 levels with 200 microns apart and doing one level (L1-L3), and whether there was an increase in detection of clinically significant metastasis by either method.

## Results

There were 119 lymph nodes identified in 110 patients with either MiM or ITC (some patients had more than one SLN with MiM or ITC). Seven cases were excluded, who also had an additional SLN containing macrometastasis. Thus, 112 SLN harboured either ITC/MiM only, of which 54 had MiM ranging from 0.2-1.8mm and the remaining 58 has ITC. Pathologic data for 112 patients studied are shown in Table 1. The size of ITC varied from 0.05mm to just less than 0.2mm. Excluding the findings from H&E levels 4-6, if only first 3 levels (L1-L3) were examined, ITC/MiM were detected in 58/112 nodes on H&E only. Of the remaining 54 nodes, ITC/MiM were identifiable in 48 of the nodes (43%) on keratin immunostain (see Table 2). Thus, there were only six false negative cases if an abbreviated protocol of H&E levels 1-3 and anti-cytokeratin IHC was performed. Only one of these false negative cases was found to have MiM on deeper levels; the remaining 5 nodes had ITCs only. This patient with MiM had a 0.4mm deposit of metastatic carcinoma (detected on L5) and a 12mm grade 3 NST carcinoma in breast. An axillary dissection was not performed.

Tumour characteristic	No. (%)
Grade 1	12 (10.7)
Grade 2	52 (46.4)
Grade 3	48 (42.8)
Invasive Lobular Carcinoma (ILC)	16 (14.3)
ILC + No Special Type (NST)	2 (1.8)
NST, micropapillary & mucinous carcinoma	94 (83.9)
T1	53 (47.3)
T2	51 (45.5)
T3	8 (7.1)

**Table 1:** Pathologic data for patients with sentinel lymph node biopsy.

	L1	L2	L3	L1-L3
Positive H&E (of the total 112 nodes)	31 (27.7%)	40 (35.7%)	46 (41.1%)	58 (51.8%)
Positive H&E and pancytokeratin	103 (92%)	103 (92%)	104 (92.9%)	106 (94.6%)

**Table 2:** Number of positive nodes on levels 1-3 with and without pancytokeratin immunohistochemistry; H&E (hematoxylin and eosin).

Similarly, there was no significant difference in detection rate of ITC/MiM between any of the initial levels 1-3 (either L1, L2 or L3) or combined levels 1-3 along with a keratin immunostain (see Table 2). By doing one level on H&E (either L1, L2 or L3) plus CK only, there was marginally higher false negative rate of 8-9 cases out of 112 (see Table 2). Again, only 1% of MiM was missed (1 out of 112). By doing anti-cytokeratin IHC only (excluding the findings from H&E), ITC/MiM were identified in 99 of 112 nodes (88%); of these 29 (26%) were detected on anti-cytokeratin IHC only (27 ITC and 2 MiM).

The size of the metastatic carcinoma was compared between L1, anti-cytokeratin IHC and L6 to determine if by doing deeper levels the size of the metastatic carcinoma was increased from ITC to MiM, leading to an increased detection of MiM. In just one node, a MiM was identified on L6 with no metastasis detected on initial levels or keratin. This patient also had a positive intraoperative imprint diagnoses at the time of surgery and had no further nodal involvement on axillary dissection. In total, there were 18 patients with MiM who underwent Axillary Lymph Node Dissection (ALND); 4 of these had macrometastasis and one had ITC in a non-SLN. In addition, five patients with ITC also underwent ALND; one of these patients was found to have macrometastasis in 3 axillaries non-SLN. The decision for ALND in this patient was based on the presence of florid lymphovascular invasion, and clinically suspicious nodes with a highly proliferative (ki-67 >90%) grade 3 NST carcinoma.

## Discussion

Identification of metastatic deposits larger than 2mm in the axilla is a significant adverse prognostic factor in women with breast carcinoma. This becomes increasingly important in early stage breast carcinoma [2]. Due to the limited sensitivity in imaging techniques, pathological examination of the lymph nodes is essential for an accurate assessment. The significance of ITC and MiM is still disputed in the literature [2,3], therefore identification of macrometastasis remains the minimum standard required from a SLN protocol. This is achieved by sectioning of each lymph node into 2mm slices along its long axis.

Although the AJCC 8th edition mentions that more comprehensive histologic evaluation of lymph nodes is not required for categorization, there is no consensus protocol nationally or internationally. Whilst some laboratories are still performing more than one level on sentinel nodes, other laboratories have moved to do just one H&E section and no immunohistochemistry to detect macrometastasis only because of the studies concluding that detecting MiM has little clinical significance [1-3]. Our study demonstrated that 88% of ITC/MiM could have been detected by doing an anti-cytokeratin IHC alone, including 26% where the metastases were solely detected by anti-cytokeratin IHC and

were not present in H&E levels. When a single H&E stain was performed along with the immunostain, detection of ITC/MiM increased up to 92-93%. By increasing the number of deeper levels beyond this point, there was minor increase in detection of ITCs and did not show an increase in clinically significant detection rate of identifying MiM.

By doing just one level (any of the first 3 H&E levels) and a keratin immunostain on lymph nodes, up to 8 ITC and only one MiM were left undetected. Only one of these exhibited MiM in deeper levels, but an axillary dissection was not performed. One of the patients with ITC in SLN went on to have ALND, based on widespread lymphovascular invasion and clinically suspicious nodes. It was inferred that by doing only one H&E and keratin on lymph node sections, only 1% of MiM was missed, and clinical significance of these was negligible, in lieu of changed clinical practices [4]. Furthermore, a significant change of size of metastatic deposit from ITC to MiM was not seen when size of metastatic deposit was compared between level 1, level 6, and keratin.

ALND, as a means for achieving local disease control, is associated with a significant risk of complications such as seroma, infection, and lymphedema [2]. In current practice, SLN status is an important factor that influences the decision to perform subsequent ALND, however numerous other factors influence the decision. In current study, there were 23 cases which underwent an ALND subsequent to the detection of ITC/MiM only. This decision was based on correlation with other clinicopathological factors including, positive intraoperative imprint diagnoses at the time of mastectomy and SLN excision, clinically suspicious nodes, extensive lymphovascular invasion and young age (less than 40 years) with large tumour. Thus, although SLN status is important, in some cases other biological factors can influence the decision to perform ALND. There needs to be a histology protocol for assessment of SLN which should be thorough enough whilst not missing clinically significant information. According to the updated AJCC-TNM edition for breast cancer, patients with pN0 (i+) only disease (ITCs only) are considered clinically node-negative. In addition, data from two randomized controlled trials provided high-level evidence that ALND following MiM carcinoma in SLN does not confer clinical benefit [3,4]. On the other hand, in certain high risk biological situations, finding of ITC/MiM may have impact on treatment recommendations [5]. For example, clinicians may make the choice of ALND or to use systemic chemotherapy for triple negative and Her2 over expressing tumors in patients presenting with ITC or MiM, as the only poor prognostic feature [5]. Thus requiring a protocol for assessment of SLN, which confers a little expected detrimental impact.

This study has demonstrated, where the lymph node mapping is successful, the histological examination of lymph nodes with one H&E level and a pan cytokeratin IHC would provide sufficient

information without compromising further clinical decision making and patient outcomes. This would further mean that a revised less intensive protocol will reduce the material cost and labour work in dealing with breast sentinel lymph nodes as well as the amount of time spent on looking at the slides by pathologists and therefore an improved productivity.

This study highlights that by following a minimal protocol, there is clinically insignificant chance of missing residual micrometastatic disease.

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