

## RESEARCH ARTICLE

# Improved Detection of Metastases by Step Sectioning and Immuno-Histochemical Staining of Axillary Sentinel Nodes in Patients with Breast Carcinoma

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### Abstract

**Background:** The object of this study was to examine whether a new protocol including step-sectioning and immunohistochemistry (IHC) staining of axillary sentinel nodes (SN) would lead to detection of more metastases in patients with breast cancer. **Materials and Methods:** Sixty-nine tumor free sentinel lymph nodes were examined. Step frozen sectioning was performed on formalin fixed SN and stained both by hematoxylin and eosin (H and E) and cytokeratin markers using IHC. Any tumoral cell in IHC stained slides were considered as a positive result. Metastases up to 0.2 mm were considered as isolated tumor cells and 0.2 up to 2 mm as micrometastasis. **Results:** Mean age of the patients was 48.7±12.2 years. Step sectioning of the SN revealed 11 involved by metastasis which was statistically significant (p<0.001). Furthermore, 15 (21.7%) of the patients revealed positive results in IHC staining for pan-CK marker and this was also statistically significant (p=0.001). Ten patients had tumoral involvement in lymph nodes harvested from axillary dissection and 4 out of 15 lymph nodes with positive result for CK marker were isolated tumor cells. However, 4 of 10 patients with tumor positive lymph nodes in axillary dissection were negative for CK marker and in contrast 6 of the pan-CK positive SN were in patients with tumor-free axillary lymph nodes. **Conclusions:** Both IHC and step sectioning improve the detection rate of metastases. Considering the similar power of these two methods, we recommend using either IHC staining or step sectioning for better evaluation of harvested SNs.

**Keywords:** Breast cancer - sentinel node (SN) dissection - multilayer sectioning - immunohistochemistry

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### Introduction

Breast cancer is one of the most common cancers in women. The researchers of the last two decades have seen a paradigm shift in the management of women with breast cancer. Late presentation, delayed diagnosis and radical ablative surgery to the breast and axilla have been replaced by effective screening program, prompt diagnosis, breast conservation and more targeted approach to the axilla. Among various prognostic factors, it has been shown that axillary lymph nodes status is the most important predictor of prognosis (Chagpar et al., 2005); as, the prognosis get poorer if the number of the tumor-positive lymph nodes increases (De Boer et al., 2010). Breast surgery (radical mastectomy or breast conserving therapy) with axillary lymph node dissection (ALND) is the most common method for breast cancer treatment. Although ALND is performed for local controlling the disease, it is followed by adverse sequelae such as hematoma, infection, sarcoma, etc. (Yeoh et al., 1986;

Ivens et al., 1992; Langer et al., 2005; Giuliano et al., 2011). Sentinel lymph node biopsy (SLN) is therefore developed to examine accurately the first draining lymph node for presence of tumor metastasis (Giuliano, 1996). SLN biopsy reduced the rate of axillary dissection and as a result the sequelae by sparing ALND in patients with no evidence of SLN metastasis (Veronesi et al., 1997; Lucci et al., 2007). Several studies have compared the accuracy of ALND with SLND and an excellent correlation is obtained (Rubio et al., 1998; Choi et al., 2003).

Several studies have reported similar sensitivity for both methods and some authors mentioned that SLN biopsy can be more sensitive than ALND if additional procedures such as immunohistochemical (IHC) staining be performed on the prepared slides of SLN (Giuliano et al., 1995; Dowlatshahi et al., 1999; Fréneaux et al., 2002; Stitzenberg et al., 2002). Performing IHC on all lymph nodes obtained from ALND is not cost-beneficial and labor-intensive, but can be performed on 1 or 2 lymph nodes harvested from SLN biopsy. Step sectioning is

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**Table 1. Comparing the Results of Standard Method, Multilayer Sectioning and IHC Staining in Sentinel Node Evaluation**

	Standard method	Positive lymph nodes in Serial sectioning	p value	Standard method	Positive lymph nodes in IHC staining**	p value
Detected tumoral lymph nodes	0/69	11/69	<0.001		15/69	<0.001**
Age (mean±SD) year	48.8±12.7	48.2±10	>0.05	48.8±12.7	47.7±10.3	>0.05
Axillary involvement						
Tumor diameter(mean±SD) cm	3±1.7	3.1±1.5	>0.05	3±1.7	3.5±1.8	>0.05
Presence of Vascular invasion	15/69	9/11	0.001*	14/69	10/15	<0.001*
Presence of Perineurial invasion	6-69	5/11	0.01*	6/69	5/15	0.022*

\*Immunohistochemistry staining, \*\*p value <0.05 is considered as significant

also another technique to increase the sensitivity of SLN examination in comparison to current standard evaluation with single section. Some studies have shown increased detection rate of metastasis by means of multi sectioning approach; however there is still no standard guideline for the numbers of sectioning and thickness of the sections (Ryden et al., 2007) The aim of the current study is to compare standard single section hematoxylin-eosin (H and E) SLN with step sectioning and IHC staining of the SN in metastasis detection.

## Materials and Methods

### Subjects and methods

Patients with diagnosis of breast carcinoma on their needle biopsies were consecutively enrolled in the study. In all the patients the axillary lymph nodes were clinically negative for malignancy. All patients underwent tumor resection (either breast conserving or mastectomy), SLN biopsy and frozen section examination. Axillary lymph node dissection was also performed during surgery. Patients with tumor involved SLN were excluded. Totally, 69 patients were enrolled in this study. Step sectioning was then performed on formalin fixed sentinel nodes on five levels, 200 micron apart and stained by H&E. Sections obtained from sentinel nodes were also underwent IHC staining for cytokeratin marker (multi-CK, code: AE1/AE3, Leica Biosystems, Newcastle Ltd, United Kingdom) using manufacturer's protocol. Any tumoral cell in IHC stained slides were considered as positive result. The metastasis size was measured using ocular micrometer. Metastasis up to 0.2 millimeters (mm) was considered as isolated tumor cells (Ni) and 0.2 up to 2 mm as micro-metastasis (Dowlatshahi et al., 1997). Lymph nodes harvested from Axillary dissections were also sectioned after formalin fixation and stained by H&E. all the prepared slides were examined under light microscope. Written informed consent was obtained from all patients and the study is approved by regional ethics committee.

### Statistical analysis

The results are expressed as mean±SD. Statistical analysis was performed using SPSS version 16.0.1 (SPSS Inc., Chicago, IL, U.S.A.). The statistical differences between proportions were determined by  $\chi^2$  analysis. Numerical data were evaluated using analysis of variance, followed by Tukey's post hoc test and correlation test. The p value<0.05 was considered as significant.

## Results

A Total of 69 patients were enrolled in the study with mean age of 48.7±12.2 years. The mean diameter of the breast masses was 3.0±1.6 centimeters. Fifty-two of the tumors were invasive ductal carcinoma, 12 were carcinoma in-situ (either ductal or lobular), 3 were invasive lobular carcinoma and 2 were mucinous carcinoma. Fuethger investigations showed that 15 of the tumors were classified as low grade (grade I), 16 as high grade (grade III) and 38 were classified as intermediate grade (grade II). Reviewing multiple slides prepared from step sectioning of the SN revealed 11 SLN involved by metastasis which was statistically significant (p<0.001). Furthermore, 15 (21.7%) of the patients had positive results in IHC staining for CK markers which was also statistically significant (p=0.001). ten patients had tumoral involvement in lymph nodes harvested from axillary dissection. Among 15 lymph nodes with positive result for CK marker, 4 of them were isolated tumoral cells (ITC, <0.2 mm). However, 4 of 10 patients with tumoral positive lymph nodes in axillary dissection were negative for CK marker and in contrast 6 of the CK positive SN were in patients with tumor free axillary lymph nodes. Among our 69 patients, 11 had perineurial and 24 had vascular invasions in microscopic examination who had more SN involvements than others (p<0.05, Table 1). There was no correlation between CK staining or step sectioning results with axillary dissected lymph nodes involvement (p>0.05). There was also no correlation between grade or either lymph node involvement in axillary dissections with CK staining or step sectioning results of SLN (p>0.05).

## Discussion

Accurate assessment of axillary lymph node status in patients with breast carcinoma is important for staging, prognosis and therapeutic decisions. There are lots of challenges on axillary lymph node mapping in breast cancer since its first description. Despite increasing usage of SLN biopsy in detection of metastasis, heterogeneity in approach to SLN evaluation still exists. According to American college of Pathology and American Society of Clinical Oncology thin sectioning of nodes at 2 mm intervals, correct embedding all sections and examining one section from the surface of blocks is a strategy to detect all metastasis larger than 2 mm (Czerniecki et al., 2000; Weaver et al., 2000). It is recognized that

more comprehensive sampling will identify additional micrometastasis and isolated tumor cells (ITCs). Currently, the most common method SLN examination is a single section stained with H&E. Among 69 patients with tumor free SLN in standard examination, 11 (16%) had evidence of lymph node metastasis in serial sectioning, furthermore IHC staining revealed 15 tumor positive sentinel nodes in which 11 were micrometastasis and 4 were ITC. Our results show that both serial sectioning and IHC staining significantly increase the detection rate of tumor metastasis to SLN.

As previously mentioned our results showed that step sectioning increased the number of positive SLN. Other studies have also demonstrated significant difference between single sectioning and step sectioning. However, there are other studies with similar (Ryden et al., 2007) or opposite results (Dowlathshahi et al., 1999, Fréneaux et al., 2002).

Although there is increasing data supporting the theory that additional procedures such as IHC increases sensitivity of SLN biopsy in detection of lymph node metastasis (Giuliano et al., 1995; Dowlathshahi et al., 1997; Czerniecki et al., 1999; Weaver et al., 2000; Stitzenberg et al., 2002; Ryden et al., 2007), it is still a debate regarding routine usage of these techniques. Our data also revealed that IHC staining just detected more isolated tumor cells than multilayer sectioning. The need for ALND in breast cancer patients with micrometastasis and /or ITCs in the absence of macrometastasis remains controversial. While some authors believe micrometastasis /ITCs is associated with poorer disease free and overall survival (Van Zee et al., 2003; Lyman et al., 2005; Loya et al., 2009; Krag et al., 2010) other studies support the theory that ALND might be avoided in these patients (26, 27, 28, 29). Relying upon the latter's opinion, ITC do not worsen patients' prognosis (Ryden et al., 2007). Excluding ITC from totally detected metastasis by IHC staining, shows that there is no difference between serial sectioning and IHC staining in metastasis detection. Some other studies suggest that IHC staining can be useful only in particular types of breast cancer such as lobular breast carcinoma (Pernas et al., 2010; Ozcinar et al., 2011). Several studies have also shown the false positive results of IHC staining for metastasis detection (Hansen et al., 2009).

We did not find any relation between sentinel and non sentinel node metastasis. Some studies have reported some factors as predictors for non sentinel lymph node involvement such as metastasis size in SN tumor size and number of involved SNs (Van Zee et al., 2003; Ryden et al., 2007). Presence of at least one tumor positive non sentinel node in patients with negative SN is also reported in previous studies but no negative prognostic effect has been declared in such situations (Weaver et al., 2000; Loya et al., 2009; Krag et al., 2010).

Follow up of our patients will clarify whether there is any significant difference between patients with positive axillary lymph nodes comparing those with negative axillary lymph nodes; while the SLN biopsy reveals negative result for metastasis. Moreover, the effect of ITC detection on prognosis would better distinguish the difference between step sectioning and IHC staining.

Our results revealed that perineurial and vascular invasions increase the risk of SN metastasis. Some studies have attempted to explore the factors affecting the results of SLN biopsy. Body mass index (BMI), tumor location and also histologic grade of the tumor are supposed to be risk factors which decrease predictive value of negative SLN biopsy (de Boer et al., 2009).

In conclusion, we evaluated the differences between standard examination of SLN and additional IHC staining and step sectioning in metastasis detection and revealed significant increase in detection of metastatic lymph nodes by means of additional diagnostic procedures. We showed that by comparing step sectioning, IHC staining only increases the detection of ITC. It is quite clear that routine use of these techniques is impractical. In the context of the new molecular classification of breast cancer subgroups may be identified where detection of micro-metastases has clinical significance.

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