

PREVALENCE OF OCCULT METASTASES IN AXILLARY SENTINEL LYMPH NODES OF BREAST CARCINOMA

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Recently, sentinel lymph node biopsy (SLNB) has been accepted as a standard method of assessment of axillary lymph nodes in breast cancer patients with no clinical lymphadenopathy. There is no standard pathologic method to evaluate sentinel lymph nodes. The purpose of this study is to evaluate the frequency of occult lymph node metastasis in sentinel lymph nodes via serial sectioning and immunohistochemical study with cytokeratin and its relationship with other clinicopathologic factors. Paraffin-embedded blocks of axillary sentinel lymph nodes of breast cancer patients, biopsied in 2005-2009 and reported as negative, were reviewed with 3 μ m sections, H&E staining and immunohistochemical study with an epithelial cytokeratin marker. Clinicopathologic data and relapse, if occurred was recorded and its relationship with occult metastasis was statistically analyzed. Sixty-eight sentinel pathology blocks of 66 patients (65 women and one man, median age 49 years) were investigated. Four cases (5.8%) of occult metastases were found, one by HE staining, and three cases with IHC (1 micrometastasis, 2 isolated tumor cells). Accuracy of reported cases was 94.1% upon re-examination. Sixty-four patients were followed after surgery and adjuvant therapy (range: 6-38 months, median: 21 months). No relapse was reported. There was no significant statistical relationship between occult metastasis and disease-free survival. Although 4 cases (5.8%) of sentinel lymph nodes were positive in the complementary study, with a median follow-up of 21 months, we found no difference in disease-free survival between these patients and others. To show a significant, however small, difference, one needs further research with a greater number of patients and longer follow-up.

Key words: breast carcinoma, sentinel lymph node, occult metastasis, immunohistochemistry.

Introduction

Breast cancer is one of the most important issues in health care, the most common cancer in women and the second cause of cancer deaths, after lung cancer [1].

Lymph nodes metastasis is a major factor in prognosis and also treatment strategy among these patients [2]. Dissection of the first and second levels of axillary

lymph nodes is a standard method of lymphatic staging in resectable breast cancers [3], but it increases surgery complications, so there have been efforts to use less invasive techniques in operations of patients without clinical axillary lymph node involvement. A novel technique for evaluating axillary lymph nodes in these patients is a sentinel lymph node biopsy, which carries far less complications [4].

Sentinel lymph nodes are supposed to be the first regional lymph node accessible to metastatic cells and can predict axillary stage in up to 95% [5], so SLNB has become the standard method for staging breast cancer, showing low morbidity, in patients who lack clinical lymphadenopathy.

Intraoperative frozen sections of the axillary SLNs are evaluated, in the case of overt lymph node metastasis, axillary dissection is done during the same operative procedure, otherwise, further dissection is cancelled. Later on, permanent HE staining of SLN is performed.

Occult lymph node metastases are not detected by this method, although their prognostic significance is still being debated. They can be found either by examining serial sections of the tissue blocks or by using a more sensitive method such as cytokeratin immunohistochemistry, which result in 35% increment in sensitivity, compared to the routine SLNB [6-9].

Although SLNB has been used for several years, there is still no accurate and standard technique for assessing axillary sentinel lymph nodes. Some researchers believe that micrometastatic involvement of SLNs does not need extra treatment of the axillary region, while others report that it may accompany more extended involvement in regional lymph nodes, which implies the need for more treatment procedures [10-12].

This study was undertaken to assess the value of cytokeratin IHC for the detection of occult metastasis in negative-reported sentinel lymph nodes from patients with breast carcinoma. The association of these occult metastases with some clinicopathologic factors and disease-free survival was evaluated as well.

Material and methods

All patients with breast cancer diagnosed either by a core needle biopsy or excisional biopsy in 2005-2009 and who had a sentinel lymph node biopsy for axillary staging were included in the study. Patients with the following conditions were excluded from the study: a history of ipsilateral breast or axillary surgery or radiotherapy that could intervene with regional lymphatic drainage, multifocal or locally advanced carcinoma, pregnancy or breast feeding, and a history of neoadjuvant chemotherapy for the present disease. Sampling was done by a non-randomized, easy method, based on samples of eligible cases during the research period. According to AJCC definition, metastasis > 2 mm was defined as macrometastasis, 0.2-2 mm – as micrometastasis, and the tumoral involvement < 0.2 mm was regarded as isolated tumor cells.

For this purpose, 70 pathology blocks of sentinel nodes which had been reported as negative in both frozen section and permanent study by light microscope, were taken from archives of Omid and Razavi hospitals. Sixty eight blocks of 66 patients (65 women and

one man) were amenable to IHC staining. We used the Daku test kit for IHC staining for cytokeratin (CK). CK-IHC results were classified into two groups as positive and negative by the pathologists. Existence of any cluster of malignant cells in the lymph node stained by H&E and/or CK-IHC was considered as a positive sentinel lymph node. Cells stained for CK but of non-malignant morphology (dendritic cells or macrophages) were removed.

Clinicopathologic parameters including age, sex, tumor size, grade, ER, PR, HER2 and lymphovascular space invasion, were extracted from patients' medical records, and relapse, if any, was registered. An interval between the surgery and the last visit was recorded by the researcher.

At the end, immunohistochemical findings and patient information, data tables, graphs and statistical parameters were presented. The study was performed with help of Statistical Package for Social Sciences (SPSS).

Results

Seventy paraffin-embedded pathology blocks were re-sectioned for revision and CK-IHC. Sixty eight blocks of 66 patients (65 women and one man) were amenable to staining. Their age ranged from 28 to 74 years (median: 49 years). In 4 (5.8%) cases, SLN was metastatic: one 1 cm \times 1 cm macrometastatic ductal carcinoma was found with HE staining, the other three were detected by CK-IHC. One micrometastasis from a lobular carcinoma and two cases of isolated tumor cells originated from ductal carcinoma tumors (Table 1, Fig. 1). Other samples contained no metastasis either in HE staining or in CK-IHC, so accuracy of reported cases was 94.1% upon re-assessment. Except for two patients who inhabited in Iraq and Shiraz, all other 64 patients were followed-up regularly. Median follow-up duration from surgery was 21 months, ranged from 6 to 38 months. No recurrence was reported during follow-up. Only one patient passed away for an unknown rea-

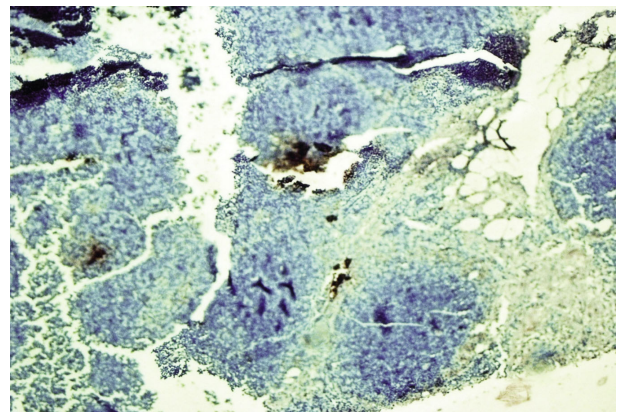


Fig. 1. Isolated tumor cells originated from ductal carcinoma in CK-IHC. Magnification 40 \times

Table I. Clinical characteristics of patients with breast carcinoma with positive sentinel nodes

PARAMETER	ISOLATED TUMOR CELLS	ISOLATED TUMOR CELLS	MICROMETASTASIS	MACROMETASTASIS
Age [years]	53	49	63	67
Pathology	Invasive ductal carcinoma	Invasive ductal carcinoma	Invasive lobular carcinoma	Invasive ductal carcinoma
Primary tumor size [cm]	4 × 3.5	1.8 × 2	2 × 3.5	1 × 1
Grade	2	2	2	2
Type of surgery	MRM	MRM	MRM	MRM
ER	-	+	-	+
PR	-	+	+	+
Her 2	-	-	-	-
LVS I	-	-	-	-

The ANOVA test showed a significant statistic association between tumor size and grade ($p = 0.021$); MRM – modified radical mastectomy

son one month after surgery and she did not receive any kind of adjuvant treatment. Pathology of other patients' tumors contained four subtypes: the most common was invasive ductal carcinoma (48 cases, 75.2%), then invasive lobular carcinoma (6 cases, 9.3%), ductal carcinoma in situ (6 cases, 9.3%), and medullary carcinoma was the least common (4 cases, 6.2%). The median number of detected sentinel nodes during surgery was 1 (range: 1-3).

Discussion

The main goal of our study was to assess the frequency of occult metastases in sentinel lymph nodes of breast cancer and its relationship to other clinicopathologic factors. We found 4 cases of occult metastasis out of 68 blocks (5.8%), one macrometastasis was found in revision by HE staining, one micrometastasis and two isolated tumor cells (detected by CK-IHC).

Since none of the patients had any kind of relapse during follow-up, a complementary study with CK-IHC could not prove to be a predictor of recurrence.

Due to the long course of breast cancer disease, and the less probability of relapse in the early stage disease, it is suggested that we need longer follow-up with a greater number of patients to find any significant difference in survival of these patients.

Since the SLNB technique has been used in this center since 2005, our study is the first research of this kind in the north-eastern Iran. All the samples were taken by one surgeon and reviewed by an academic pathologist in the Omid Hospital, Mashhad University of Medical Sciences. We found only 5.8% of occult metastasis (accuracy = 94.1% and overall 5% false negatives in SLNB, which is acceptable) [13].

In 2002, Weaver *et al.* used CK-IHC in 157 negative sentinel lymph nodes of 70 patients and found 6.4% of occult metastasis [14], which is not much more than our results.

In a review performed by Dowlatshahi in 1997, serial sections and CK-IHC showed a 9-33% increment in the detection of occult metastasis. The micrometastatic cases that were subject to adjuvant treatment proved to have better outcomes than the control group who suffered a 15-25% failure [13].

Since this was a study performed in only 2 centers and SLNB was done by only one surgeon, during a 5 years' period we could not find more than 68 cases. In a multi-center study in 2000 Donald reviewed SLN paraffin-embedded blocks of 431 patients and found 15.9% of occult metastasis. Invasive ductal carcinoma was the dominant pathologic subtype [15]. The dominant pathologic subtype in our study was invasive ductal carcinoma, too. Weinberg in 2004 showed that CK-IHC increased detection of SLN metastasis in 59 patients with invasive lobular carcinoma by as much as 12.9% [14], but we had only 6 cases (9.1%) of invasive lobular carcinoma in our study. We had also 6 cases of DCIS (9.1%) which carry a very low risk of axillary metastasis (1-2%) and they had better not be taken into account [16, 17].

McCready in 2004 and Arisio in 2000 reported the average number of SLNs as 2.8 and 2.4 and the mean size of tumor as 1.83 cm and 1-2 cm, respectively [18-20], compared to 1.55 SLNs and 3-4 cm, respectively, in our study.

Our patients were one decade younger than those in similar studies, with a median age of 49 years compared to 58 years in Viale's study and 59.4 years in Cserni's study [21, 22]. The ER, PR and HER2 positivity in the tumors of our patients did not differ much from the patients in similar studies such as a study of Gobardhan, Holland [23]. The prognosis and treatment outcomes in these occult metastasis is still under debate [24-26]. In our study, almost all of patients presented stages 1 or 2 and mostly received adjuvant systemic treatment (chemotherapy, hormone therapy, and radiation) in the case of breast conserving surgery. So,

regarding the indolent process of breast cancer metastasis and a rather short follow-up of our study (median: 21 months), an accurate and strict judgment is impossible, and of course, due to the recent use of the SLNB technique in our center, a longer follow-up was impossible, either.

In a study in Holland in 2009, Gobardhan *et al.* compared overall survival of pathologic N0 patients to PN1 micrometastatic in 703 clinical T1-2 breast cancer patients who had a surgery and SLNB. After matching confounding factors like systemic chemotherapy, they found no significant difference in overall survival of two groups [23]. However, in a study by Chen *et al.* on the prognostic value of SLN micrometastasis in invasive lobular breast cancers in 1997-2003, the prognosis of micrometastatic patients was clearly worse, even after matching patient/ tumor factors [27]. Tan *et al.* also investigated SLNs of 368 patients with serial sectioning and IHC in a 20-year interval. The DFS in the occult-metastasis group was significantly worse [28]. Both of these results may originate from a pretty long follow-up in these studies. Cserni *et al.* in 2007 and Tan *et al.* in 2008 found that micrometastases mostly originated from invasive lobular carcinomas [22, 28], but in our study there were only 6 ILCs and one of them (16.6%) led to the only micrometastasis of our study.

It seems that micrometastasis in SLN can worsen the prognosis, but the findings are still controversial.

Conclusions

Although 4 cases (5.8%) of sentinel lymph nodes were positive in our complementary study, with a median follow-up of 21 months, we found no difference in recurrence and disease-free survival between these patients and others. To show a significant difference in outcome, no matter how small it would be, we may need further research with a greater number of patients, longer follow-up time and probably, more cases of lobular carcinomas in the samples.

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