

# HISTOPATHOLOGICAL ASSESSMENT OF AXILLARY LYMPH NODES WITH MULTIPLE LEVEL SECTIONS IN BREAST CARCINOMA

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## MEME KANSERİNDE AKSİLLER LENF BEZLERİNİN ÇOK SEVİYELİ KESİTLER İLE HİSTOPATOLOJİK DEĞERLENDİRİLMESİ

### Özet

**Amaç:** Bu çalışma, aksiller lenf bezlerinde 25µm aralıklarla yapılan beş histolojik kesitin incelenmesi ile önemli ölçüde daha fazla tümör metastazı saptanıp saptanamayacağını belirlemek için planlandı.

**Gereç ve yöntem:** Elli altı mastektomi spesmenine ait retrospektif bir çalışma yapıldı. Her bir olgunun metastatik olmayan lenf bezlerini içeren tüm bloklarından fazladan dört farklı seviyede kesit yapıldı. Orijinal kesitler birinci seviye olarak sayıldı. Tüm beş seviyedeki lenf bezleri tümör metastazının varlığı yönünden incelendi.

**Bulgular:** Yirmi yedi N0, 10 N1, 10 N2 ve 9 N3 olgu incelendi. Metastatik olmayan lenf bezlerini içeren 312 parafin blokta toplam 1248 yeni kesit yapıldı ve 842 lenf bezi incelendi. Yeni seviyelerdeki lenf bezlerinde fazladan 13 adet metastaz saptandı. Bu metastazlardan biri birinci seviyede olmayan ve yeni ortaya çıkan lenf bezlerinden birindeydi. Fazladan lenf bezi metastazları saptanan olgulardan hiç birisinde tümör evresinde bir deđişiklik olmadı.

**Sonuç:** Bu çalışmada saptanan fazladan metastazlar evre yükseltmese de, çok seviyeli kesitlerde histopatolojik deđerlendirme yapılmasının bir seviyeli kesit ile deđerlendirmeye göre daha önemli prognostik bilgi verebileceđi düşünölmekle birlikte; laboratuvarın iş yükü ve maliyet analizi göz önünde tutulduğunda bu uygulamanın getireceđi yarar tartışmalı kalmaktadır.

**Anahtar sözcükler:** aksiller lenf bezleri, çok seviyeli kesit, histopatolojik deđerlendirme, meme kanseri.

## ABSTRACT

**Purpose:** This study was planned to evaluate whether assessing five histological sections separated by 25µm detects significantly more tumor metastases in axillary lymph nodes.

**Methods:** The study was a retrospective study composed of 56 mastectomy specimens. An extra four levels were cut from all blocks containing non-metastatic lymph nodes of each individual case. The original section was then counted as the first level. All the sections were then examined for the presence of tumor metastases within lymph nodes at all five levels.

**Results:** Twenty seven N0, 10 N1, 10 N2 and 9 N3 cases were examined. Three hundred and twelve paraffin blocks containing nonmetastatic lymph nodes were cut, resulting in an extra 1248 sections, and eight hundred and forty two lymph nodes were examined. Thirteen extra metastases were discovered in nodes at new levels. One of these extra metastases occurred in newly appearing lymph nodes that had not been present in the level 1. None of the cases in which extra lymph node metastases were changed in respect to the tumor stage.

**Conclusion:** Although extra metastases detected in this study did not increase the stages, histopathologic evaluation with multiple level sections may provide more significant prognostic knowledge compared to evaluation with one level section. But concerning the labour intensity and cost effectivity, the benefit that would be obtained from that procedure may be controversial.

**Key words:** axillary lymph nodes, multiple level sections, histopathological evaluation, breast carcinoma.

## Introduction

Breast carcinoma is the most common malignancy among female population and is second only to lung cancer as a cause of cancer death in females (1). The oldest and most reliable prognostic indicator in breast carcinoma is axillary lymph node (ALN) metastasis. In pTNM classification system, according to the number of pathologically metastatic ALNs, patients are classified as having N1 disease (1-3 metastatic lymph nodes), N2 disease (4-9 metastatic lymph nodes), or N3 disease ( $\geq 10$  metastatic lymph nodes), regardless of whether the lymph nodes are movable or fixed. In patients with N1 breast carcinoma, the average annual mortality

rate is twice that of the patients without metastatic ALN. For patients with N2, or N3, the average annual mortality rates are 4 and 5 times greater, respectively. Patients with  $\geq 10$  metastatic ALNs have a particularly poor prognosis, with the majority developing recurrent disease within 5 years (2).

Routine and specialized techniques available to the pathologist for evaluating ALNs for evidence of metastatic malignancy have been presented. A variety of methods are available for increasing the number of lymph nodes identified, more thoroughly sampling the identified nodes either by gross or microscopic serial sectioning,

and applying immunohistochemistry and other techniques to improve the sensitivity of detecting malignant cells. In the end, standard practice regarding the application of these techniques must be obtained, and tempered by the realities of cost and workload constraints on diagnostic pathology laboratories (3).

In the subspecialty area of breast pathology, there are specific evidence based guidelines issued by The National Health Service Breast Screening Programme on the assessment of lymph nodes retrieved from axillary tissue. These guidelines recommend that lymph nodes smaller than 5 mm should be embedded in entirety and examined at two levels and lymph nodes larger than this should be cut into three slices and one node processed for each cassette (4). This is based on publications of some groups such as the international (Ludwig) breast cancer study group, which found that 9% of ALNs judged to be negative on one routine histological section were subsequently found to contain metastases if they were subjected to leveling (5). Usual practice in mastectomy specimens is for several small lymph nodes to be placed into each cassette and then one H&E section to be cut from it for microscopic assessment.

We hypothesized that examining five histological sections separated by 25µm intervals would detect significantly more tumor metastases in ALNs from mastectomy specimens than the standard practice of examining.

### Methods

Fifty six consecutive patients who underwent modified radical mastectomy for primary breast carcinoma between January 2000 and August 2005 were included in the study. All the specimens were sent to and diagnosed at Cumhuriyet University Hospital Pathology Department in Turkey. The original slides and paraffin blocks were retrieved from the archives of Cumhuriyet University Hospital Pathology Department. An extra four levels (separated by 25 µm) were cut from all blocks containing nonmetastatic lymph nodes of each individual case. The original H&E section (from slide archives) was then counted as the first level. All the sections were then examined for the presence of tumor metastases within lymph nodes at all five levels. The presence of any extra metastases detected in the second, third, fourth and fifth levels that were not present in the first level were noted. These were counted as metastases that would have remained undetected if only the first level had been examined. We determined whether the discovery of extra metastases in the second to fifth levels led to the upstaging of the specimen in the pTNM classification systems. Any tumor cells visible on H&E stained sections were counted as a metastasis; no attempt was made to distinguish between metastases and micrometastases. The changes in the lymph node numbers of each individual case were recorded after first and fifth level, as well.

### Results

Fifty six cases with primary breast carcinoma were examined: 27 N0, 10 N1, 10 N2 and 9 N3 of cases. Three hundred and twelve

**Table 1.** Comparison between level I and other levels in respect to metastatic extra lymph nodes.

Cases	Previous MLN(n)/pN	Level	Extra MLN(n)	Recent pN
1	2 / N1	II	1	N1
2	8 / N2	II	1	N2
3	1 / N1	II	1	N1
4	12 / N3	II	1	N3
5	24 / N3	II	3	N3
6	5 / N2	III	1	N2
7	1 / N1	II	1	N1
8	2 / N2	II	2	N2
9	3 / N3	II	1	N3
10	5 / N2	II	1	N2

MLN: Metastatic lymph node

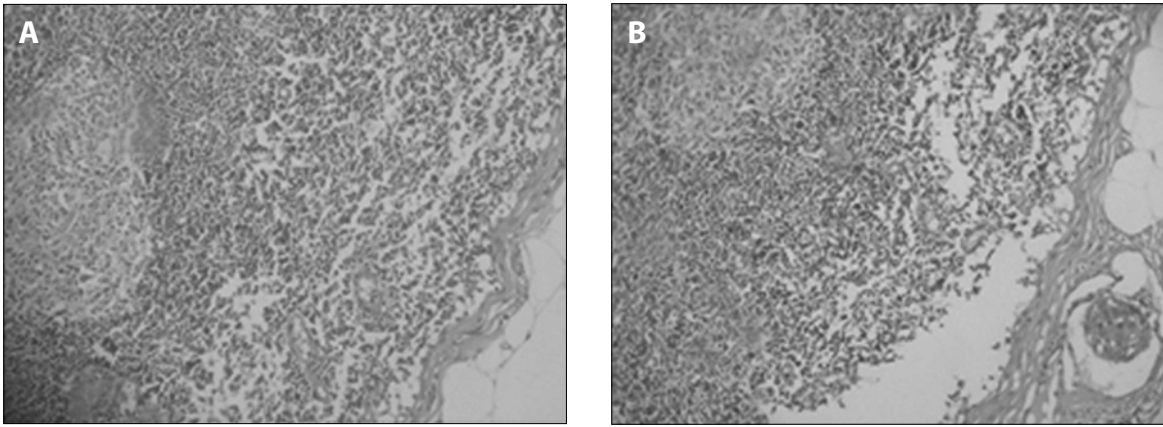
paraffin blocks containing nonmetastatic lymph nodes were cut, resulting in an extra 1248 sections, and eight hundred and forty two lymph nodes were examined. The mean average number of lymph nodes for each patient was 15.03 (range, 1- 33).

Ten of 56 cases had extra tumor metastases detected on levels 2 to 5 that were undetected on level 1 (Totally, there were 13 lymph nodes; two of them in one patient, three of them in one patient and one each in 8 patients). In these cases, the lymph node status was N1 in 3 patients, N2 in 4 patients and N3 in 3 patients. Extra lymph nodes which were established didn't lead to upstage, in none of the cases. Twelve of the 13 lymph nodes having extra metastases detected were found on level 2, whereas remaining one was on level 3 (Table 1). Two of these were observed as intracapsular deposits (Figure 1), and the others were within the parenchyma of the lymph node and subcapsular sinus deposits (Figure 2). One of these extra metastases occurred in newly appearing lymph nodes that had not been present in the first level and twelve occurred in lymph nodes that were present in level 1, but had no visible tumor deposits.

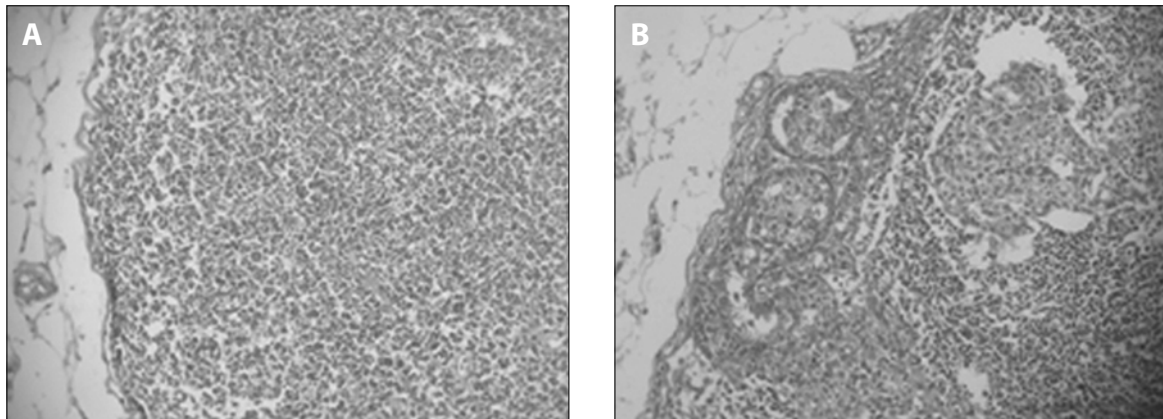
In 25 cases (%44.6), the number of the lymph nodes examined didn't change eventhough the level of sections increased. Meanwhile, the number of the lymph nodes were decreased in 15 cases (26.7%) and increased in 16 cases (28.5%). The average of the increasing lymph nodes was 3.12 (Range 1-10).

### Discussion

Presence of ALN metastases is considered as the most important prognostic factor in breast carcinoma survival. There is a clear increase of mortality associated with a node positive status. Besides the importance of axillary nodal staging, ALN dissection provides excellent local disease control, and clearly, the accuracy of staging increases with the number of lymph nodes resected. It is also used for planning further adjuvant treatment. Appropriate treatment



**Figure 1.** (A) At level 1, metastatic tumor deposit was not present, (B) At level 2, there is a small intracapsular deposit of metastatic tumor visible. (H&E;X100)



**Figure 2.** (A) At level 1, there are no visible tumor deposits, (B) At level 2, there are subcapsular sinus and intraparenchymal deposits of metastatic tumor visible. (H&E;X100)

may not be given if axillary staging is inadequate and tumor stage is underestimated (6). Therefore pathologic staging and histopathologic evaluation of the ALNs are quite important.

Because of work overload, insufficient economical status and time shortage, only one routine histological section is made from each cassette which contains lymph nodes. In this study, extra four histological sections by 25  $\mu$ m intervals were obtained from the every cassette and only the lymph nodes which were reactive in the original first level sections were evaluated.

In literature, there are several studies related in this issue, were made in colorectal cancers. Verrill et al reported that with 2 extra sections total three level (with an interval of 100 $\mu$ m) evaluations were made and extra tumor metastases were found in 11 of the 100 patients and only in one patient did the detection of extra metastases in levels 2 and 3 result in upstaging from N1 to N2 (7). In a similar study, van Wyk et al examined 72 cases of colorectal carcinoma prospectively (with the lymph nodes being sectioned at three levels with an interval of 100  $\mu$ m) and discovered four cases containing extra tumor metastases in levels 2 and 3 (8). Three of these were Duke's C and this did not change the staging. In one case, a single lymph node metastasis was discovered on levels 2 and 3, which

upstaged the tumor from Duke's B to Duke's C. Other publications include a study of triple levelling on 33 cases of Duke's A and B carcinoma, which detected no extra metastasis (9), and a study of 11 cases of colon carcinoma, which detected one further positive case when a more detailed examination with three additional sections was undertaken (10). In our study, 10 of 56 cases examined had extra tumor metastases discovered in levels 2, 3, 4 and 5 of lymph nodes that would have gone undetected if only one level had been examined, as in the current practice. None of ten patients did the detection of extra metastasis in levels 2, 3, 4 and 5 results in upstaging. In these patients, the detection of extra metastases is probably academic, with little importance on prognosis or further treatment.

In the present study, excluding extra metastatic lymph nodes, it was found that, the number of the lymph node were increased in 16 cases consequence of 2-5 levels sections. In one of these cases, there was a metastatic lymph node which was not shown in the original level 1 section, was appeared in the second.

The role of the percentage of positive lymph nodes in predicting distant metastasis and survival was highlighted recently in several institutional series. Although those studies differed in patient selection, follow-up, and types of surgery and adjuvant therapies,

they showed consistently that the percentage of positive lymph nodes was a significant independent prognostic indicator of survival in women with lymph node-positive breast carcinoma.

Truong et al examined 542 women with pathologic T1-T2 breast carcinoma who had 1-3 positive lymph nodes and who undergone mastectomy and received adjuvant systemic therapy without radiotherapy. In that study, the number of positive lymph nodes, the number of dissected lymph nodes, and the percentage of positive/dissected lymph nodes were examined using different cut-off levels (11). The study showed that the presence of > 25% positive lymph nodes was an adverse prognostic factor in patients with 1-3 positive nodes and may be used to identify patients at high risks of postmastectomy locoregional and distant recurrence who may benefit with adjuvant radiotherapy and more aggressive systemic therapy regimens (11).

Voordeckers et al found that, in 741 patients with positive lymph nodes, in multivariate analyses, percentage of positive lymph nodes appeared to improve the survival models more significantly than conventional involved ALNs for assessment of prognosis and the percentage of positive lymph nodes was the most significant prognostic factor for survival (12).

In an analysis of 453 patients with Stage I and II breast carcinoma who underwent either breast-conserving therapy or mastectomy, van der Wal et al reported that, in node-positive patients, age, the number of dissected lymph nodes, and the percentage of positive lymph nodes were significant prognostic factors for survival. Also,

a favorable prognosis was found for node-negative patients with  $\geq 14$  removed lymph nodes (6). In our study, in two of 16 cases in which lymph node number were increased, the number of lymph node that were newly found were raised to 14 and more (from 13 to 15 in a case with N2 and from 12 to 16 in another case with N0).

Camp et al reported on 290 cases of lymph node negative breast carcinoma, and their study indicated that a high number of ALNs in patients with lymph node negative was of significant predictive value in determining patient survival: the number of tumor free lymph nodes was a novel, independent predictor of aggressive disease. The authors suggested that this finding might be a biologic function of host-derived, and possibly tumor-derived, lymphangiogenic cytokines (13).

We did not make a specific prognostic evaluation related with neither the positive lymph node ratio nor the total lymph node number in the present study. However, we showed that both positive and negative lymph node numbers may be increased after five levels evaluation.

In conclusion, although extra metastases detected in this study did not increase the stages, recent studies including mentioned ones above pointed that histopathologic evaluation with multiple level section provides more significant prognostic knowledge compared to evaluation with one level section. But with regard to labour intensity and cost effectivity, further studies with large series and longer follow up of the patients are necessary to draw an exact conclusion on this subject.

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