

The value of step-sectioning in the diagnosis of lymph node micrometastasis in breast cancer

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ABSTRACT

Objectives: Routine practice is to examine one histological section for evaluating secondary tumor deposits in the axillary lymph nodes in patients with breast cancer. The aim of the present study is to evaluate whether multiple levels of histological section detect significantly more metastatic deposits in axillary lymph nodes from breast cancer resection specimen than the standard practice of examining one section.

Methods: A retrospective study of 30 patients with node negative breast cancer was performed whose specimens were received at AL-Jamhuri Teaching Hospital and private laboratories in Mosul city between the years 2008 and 2010. The original slides and the paraffin wax blocks containing the lymph nodes were retrieved from the archive, and 4 extra levels (separated by 30 µm) were cut from each block and were stained with H & E stain.

Results: Of the 30 cases with node negative breast carcinoma, 4 (13.3%) were found to contain extra tumor deposits at deeper levels that were not detected at the original sections.

Conclusion: Multiple levels of histological sections separated by relatively small intervals detect more tumor deposits in the axillary lymph nodes than the current practice of examining a single section.

الخلاصة

الأهداف: العمل الروتيني هو فحص مقطع نسيجي واحد لتقييم تراكمات الورم الثانوية في العقد اللمفاوية الإبطية لمرضى سرطان الثدي. الهدف من الدراسة الحالية هو تقييم أهمية المقاطع المتعددة في تشخيص انتشار سرطان الثدي في العقد اللمفاوية ومقارنتها مع استخدام المقطع الواحد لمرضى سرطان الثدي غير المنتشر في العقد اللمفاوية.

الطريقة: دراسة تراجعية ضمت 30 مريضة مصابة بسرطان الثدي غير المنتشر في العقد اللمفاوية والتي تم استلام عينات الفحص في مستشفى الجمهوري التعليمي والمختبرات الخاصة في مدينة الموصل بين 2008 - 2010. تم استخراج الشرائح الزجاجية الأصلية وقوالب شمع البارافين الحاوية على العقد اللمفاوية من الأرشيف وتم قطع أربع مقاطع إضافية فصلت بمقدار 30 مايكرومتر وصبغت المقاطع بصبغة الهيماتوكزولين والايوسين الروتينية.

النتائج: من ضمن 30 حالة لسرطان الثدي غير المنتشر في العقد اللمفاوية وجدت أربع حالات (3, 13%) حاوية على ورم إضافي متراكم في المقاطع الأكثر عمقاً والتي لم تكن مكتشفة في المقاطع الأصلية.

الاستنتاج: المقاطع النسيجية المتعددة والمفصولة بمسافات صغيرة نسبياً تكشف عن تراكمات ورمية في العقد اللمفاوية الإبطية أكثر من الطريقة المتداولة لفحص مقطع نسيجي واحد.

The status of the axillary lymph nodes remains one of the most important

prognostic factors in patients with invasive breast cancer⁽¹⁻³⁾. Survival rate, disease-free

interval, and treatment failure, are largely influenced by the pathological staging of these lymph nodes^(2,4).

Two pathological staging systems are commonly used, the TNM staging and the anatomical stage⁽⁵⁾. It is well known that lymph nodes can be classified as positive or negative on the basis of the presence or absence of metastatic carcinoma, which in most cases, is a straightforward diagnosis. However, in some cases there are only clusters of tumor cells and a diagnosis of metastasis is not easily established⁽¹⁾.

It is also known that different pathologists in different laboratories follow different protocols for the processing and examination of lymph node specimens⁽⁶⁾. The recommendations published by the Association of Directors of Anatomic and Surgical Pathology for the processing and reporting of lymph node biopsies being evaluated for metastatic carcinoma include the submission of the entire node for microscopical examination, slicing it into 3-4 mm slices and microscopical examination of several levels of each slice, stained with hematoxylin and eosin (H & E) only^(6,7). However, for lymph node dissection specimens being studied for metastatic carcinoma, the Association recommended submission of every node for microscopical examination with one H & E slide per cassette^(6,7).

It has long been recognized that the microscopical examination of a single H & E stained section fails to identify all metastatic foci in a lymph node, and some investigators have proposed serial subgrossing and histologic step-sectioning of each node which has been shown to up stage nodal status in a significant number of patients⁽¹⁾.

This has also been recommended by the updated protocol for the examination of specimens with invasive breast cancer⁽⁵⁾.

Additional recent techniques such as immunohistochemistry and reverse transcriptase-polymerase chain reaction have been used to detect isolated tumor cells or micrometastases^(1,5-7). However, the prognostic importance of these metastases remains unknown^(1,3,5). In addition, such

detailed histopathologic evaluation of the entire axilla is prohibitively expensive⁽¹⁾.

The aim of this retrospective study was to evaluate whether multiple levels of histological sections detect significantly more metastatic deposits in axillary lymph nodes from breast cancer resection specimens than the standard practice of examining one section.

Patients and methods

In order to achieve the aim of the present study, a retrospective study design was adopted. Thirty women with clinical stage Tis – T₃ N₀ M₀ breast cancer who underwent surgical resection with axillary clearance that were received at the histopathological laboratory of AL- Jamhuri Teaching Hospital in Mosul city, and from private laboratories of the same city, between the years 2008 and 2010 were included in the study.

After obtaining approval from Nineveh Health authorities, the original slides and paraffin wax blocks containing the lymph nodes were retrieved from the archive and reviewed at the Department of Pathology - College of Medicine, where an extra four levels (separated by 30 µm) were cut from each paraffin wax block containing lymph nodes and stained with H & E only. These multiple levels were labeled as A, B, C, and D, respectively. All those four levels were examined and carefully searched for the presence of tumor metastasis that was not detected in the original H & E sections. Any tumor deposit visible at any extra level was counted as metastasis. The number of examined lymph nodes and the histological pattern of the primary tumors were also recorded.

Basic statistical methods were used to calculate means, numbers and percentages.

Results

The mean age of the study sample was 51.4 year. Review of the slides from 30 females with breast carcinoma was done, according to pTNM staging; they were segregated into 4 groups: 3 were Tis N₀ M₀, 11 were T₁ N₀ M₀, 13 were T₂ N₀ M₀ and 3 were T₃ N₀ M₀.

The total number of examined lymph nodes was 148 with a mean of 5 lymph nodes for each case. A total of 53 paraffin wax blocks

containing lymph nodes were cut resulting in a total of 212 sections with a mean of 7 sections for each case and 1.4 sections for each lymph node.

Extra tumor deposits were detected in 4 (13.3%) of the 30 cases, 2 of them were detected at the 4th level (level D), and their corresponding initial pathological stage was T₁N₀M₀ and T₃N₀M₀. In the third case the tumor deposit was detected at the 2nd level (level B) (T₂N₀M₀). In all these cases the primary tumor type was invasive ductal carcinoma (figure 1 and 2). While in the last case, the tumor deposit was detected at the 3rd level (level C) (T₁N₀M₀) and the tumor type was invasive lobular carcinoma.

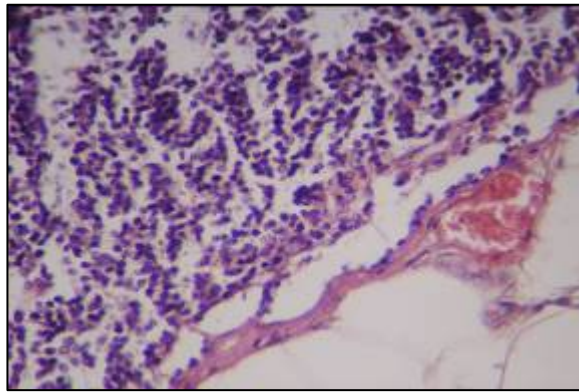


Figure (1): A lymph node with no visible metastases at the first level (level A) (H & E x 400).

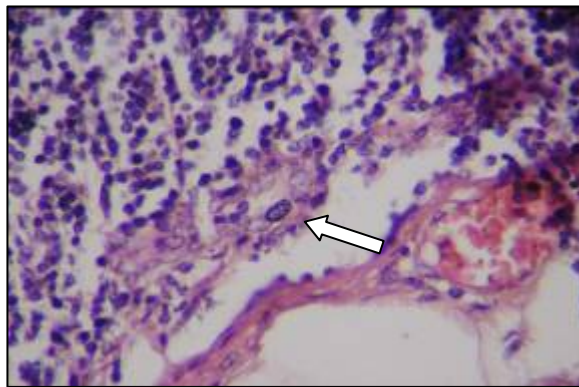


Figure (2): A lymph node with metastatic deposit at the sub capsular sinus (arrow) that is detected at the fourth level (level D) (H & E x 400).

Discussion

Detailed pathological examination of axillary lymph nodes with serial sectioning and H & E examination has resulted in an increased rate of detection of tumor deposits for breast cancer patients^(1,5,9).

The present study included 30 patients with node negative breast cancer, as detected by current practice of using single histological section. Of these, four (13.3%) patients are found to have new tumor deposits in the lymph nodes after subjecting them to serial sectioning and H & E examination of multiple levels which correlates with previous published data that found unequivocal lymph node metastases in four (12%) of 33 patients with node negative invasive breast cancer on additional H & E sections⁽⁸⁾. However in that study the addition of cytokeratin cocktail fails to show metastases over that detected by H&E apart from single strongly keratin positive sinus-based cell in one lymph node with unclear nuclear nature (benign vs malignant)⁽⁸⁾.

Previous publications from groups such as The International Breast Cancer Study Group had reported that 9% of axillary lymph nodes which are judged to be negative on one routine histological section were subsequently found to contain metastases if they were subjected to levelling⁽⁹⁾. Based on this publication, step-sectioning had been also applied on lymph nodes extracted from 100 colorectal cancer resection specimens in a previous study in whom 11 cases were found to have extra tumor deposits in level 2 and 3 that would have gone undetected when only one level had been examined. This resulted in upstaging of only one patient from N₁ to N₂ (pTNM). Despite this, the authors justified the small increase in the workload pressure at pathology laboratories to increase the accuracy in staging⁽¹⁰⁾.

In spite of the excellent prognosis for patients with node negative breast cancer, recurrence or distant metastasis within 10 years was encountered in 25% to 30%^(1,8). This was attributed in part to undetected occult metastases in the lymph nodes which are not observed during the initial routine

histopathological evaluation but become apparent at deeper levels of routine histologic sections or on immunohistochemical analysis⁽¹⁾.

The identification rate of micrometastases in sentinel lymph node biopsy depends on the number of sections evaluated from each paraffin block and the interval between each section⁽¹⁾. There is no question that the examination of more levels at smaller intervals will increase the detection rate of micrometastases⁽¹⁾. However, according to the recommendations of the Association of Directors of Anatomic and Surgical Pathology, it is not currently clear that how many sections (and from what levels of the block) are optimal^(6,7). It is also unclear whether immunohistochemical stains add clinically relevant information and are not routinely advocated in any consensus recommendations^(1,6,7). They are particularly helpful for detecting tumor cells which do not form cohesive clusters in lymph nodes such as metastatic invasive lobular carcinoma⁽¹⁾ but the results should be interpreted in the context of H & E stain^(1,6,7).

A large retrospective study done by the International Breast Cancer Study Group that reassessed the axillary nodal status (negative for metastases by routine histology) of 921 patients with serial sectioning and H & E examination showed that the presence of micrometastases was associated with an increased chance of relapse when compared with patients without micrometastases on reassessment⁽¹¹⁾. Thus far, processing of lymph nodes in serial sections and careful analysis of the H & E stained sections results in good accuracy and greater practicality in the identification of metastasis⁽⁴⁾.

The pathological stage of the axillary nodes remains a major prognostic determinant in patient with operable breast cancer^(1-4,11,12) and is crucial in the decision on the need and type of adjuvant therapy^(2,11,12). Guidelines and recommendations proposed at the 8th International Conference on Adjuvant Therapy of Primary Breast Cancer emphasized a substantial difference for treatment guidelines between patients with node negative breast cancer and patients with node positive disease

^(11,12). In general, patients without nodal involvement or with minimal involvement received significantly less chemotherapy and less anthracycline-containing chemotherapy in particular, when compared with patients with node positive breast cancer^(11,12). Furthermore, patients with hormone responsive node negative disease were prescribed significantly less chemotherapy within the adjuvant program⁽¹¹⁻¹³⁾.

In conclusion, multiple levels of histological sections separated by relatively small intervals detect hidden tumor deposits in the axillary nodes that were otherwise missed by the routine practice of evaluation of a single section and will increase the detection rate of lymph node micrometastases despite the slightly increased workload at pathology laboratories. This may influence the stage, choice of adjuvant therapy and prognosis of node negative operable breast cancer.

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