Original Articles/นิพนธ์ต้นฉบับ

# Occult Metastases in Negative Sentinel Lymph Node of Invasive Breast Cancer Discovered by Serial Section in Ramathibodi Hospital.

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## Abstract

**Background:** Sentinel lymph node (SLN) biopsy is an established method for evaluating axillary metastases in breast cancer. This study was designed to ascertain whether serial sections with haematoxylin and eosin stain could significant improve the detection of occult metastases in sentinel lymph nodes.

**Method:** Sentinel lymph nodes negative for cancer metastases by routine histology were received from 50 patients with invasive breast cancer in Ramathibodi Hospital between 2006-2007. The lymph nodes were examined by serial sections at 25 microns intervals at each level with haematoxylin and eosin stain.

**Result:** Occult metastases were detected by serial sectioning in 4 (8%) out of 50 breast cancer patients. One patient had macrometastasis, two patients had micrometastases and one patient had isolated cell metastasis. No clinical or pathological characteristics (age, mammography, tumor size, tumor grading, TNM staging, and number of SLNs) were associated with occult metastases (P- value > 0.05).

**Conclusion:** The prevalence of occult metastases in negative sentinel lymph nodes of breast cancer patients was quite low in the present study. Serial sectioning sentinel lymph nodes for all patients might not be cost-effective. However, the clinician should be aware of occult metastases in node-negative cases.

**Keyword:** Occult metastases, Breast cancer, Sentinel lymph node

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## Introduction

Axillary lymph node metastasis is still the most important prognostic factor for recurrence and survival in breast cancer patients, and forms the basis for important therapeutic decisions.[1] The information from axillary lymph node examination helps the clinician decide on the best treatment for the patient.[2]

Sentinel lymph nodes are the first group of lymph nodes to which cancer is likely to spread from the primary tumor. Cancer cells almost always appear in the sentinel node before spreading to other lymph nodes. A negative sentinel lymph node biopsy (SLNB) result suggests that cancer has not spread to the regional lymph nodes.[2,3]

SLNB is an established method for evaluating tumor involvement of the axilla in patients with breast cancer.[2] It offers the chance to gain information for staging, treatment decisions and prognostication without the need for axillary dissection in node-negative patients avoiding the morbidity of unnecessary axillary lymph node dissection.[4]

There have been several reports of a high incidence of occult nodal metastases in axillary and sentinel lymph nodes, usually on further sectioning of the histological material.[4-8] The presence of occult metastases were associated with significantly poor disease-free and overall survival.[7-10] The purpose of this study was to assess whether serial sectioning with haematoxylin and eosin stain can be used to improve the detection of tumor metastases reliably in the sentinel lymph nodes.

## Material and Method

Clinical and pathological records of patients with invasive breast cancer operated in Ramathibodi Hospital during period between June 2006 to June 2007 were reviewed. Patients were included if SLNB was performed, the pathological specimens were available for serial sectioning and if the initial pathological report based on conventional sectioning and standard haematoxylin and eosin staining did not reveal evident metastasis ("negative SLNB" on initial report). Serial sectioning of sentinel lymph nodes was performed as followed; each sentinel lymph node was examined by serial cut every 2 mm. interval and all were submitted in the paraffin blocks. Total paraffin blocks (188 blocks) containing negative sentinel lymph nodes from 50 cases were microscopic examination with serially sectioned at 25 microns intervals with 3 microns section being cut at each level and stained with haematoxylin and eosin. All paraffin blocks were examined entire levels of 25 microns intervals. One pathologist and one pathological resident examined 167 lymph nodes from 1,744 slides. Otherwise the primary tumor of each case was examined before proceeding to the serial section specimen.

## Statistical analysis

Mean and standard deviation or median and range were used to describe continuous data. Frequency and percentage were used to describe categorical data. Independent t-test (Mann-Whitney test) and Chi-square (or exact test) were used to compare characteristics between occult SLN metastases and non SLN metastasis groups for continuous and categorical data, respectively. All analyses were performed using STATA version 10.0 (StataCorp.2007. Stata Statistical Software: Release 10. College Station, TX:stataCorp LP).

## Results

During 1 year period between June 2006 to June 2007, 50 patients with negative SLNB on initial pathological report were identified. The clinical and pathological characteristics of the patients were shown in Table 1. Occult metastases were detected by serial sectioning with haematoxylin and eosin stain in 4 (8%) out of 50 patients (Table 2) of which 1/50 (2%) was macrometastasis, 2/50 (4%) were micrometa-

**Table 1.** Clinical and pathological characteristics of all invasive breast cancer patients with negative sentinel lymph node metastasis.

| Clinical Characteristics | Number (%) (n = 50) |
|---|---|
| Age (years), mean (SD) | 52.9 (9.7) |
| Mammographic BI-RADS category (n=45)** | |
| 3 | 1 (2) |
| 4 (did not subcategorize as a, b or c on initial report) | 2 (4) |
| 4a | 4 (9) |
| 4b | 6 (13) |
| 4c | 7 (16) |
| 5 | 25 (56) |
| Tumor size (cm.) (n=49)** | |
| Median (range) | 1.6 (0.1 to 5) |
| Tumor grading (Bloom-Richardson criteria) (n=46)** | |
| 1 | 9 (20) |
| 2 | 30 (65) |
| 3 | 7 (15) |
| TNM staging (n=49)* | |
| I | 21 (63) |
| II | 18 (37) |
| Number of sentinel lymph nodes examined | |
| Median (range) | 2 (1 to 9) |
| Number of paraffin sections on initial examination | |
| Median (range) | 3 (1 to 12) |

* Data were not available for all patients.

**Table 2.** Clinical and pathological characteristics of patients with occult metastases.

| Patient number | Age (Year) | Occult metastases | Number of lymph node positive | Level | Tumor size (Greatest dimension) |
|---|---|---|---|---|---|
| 1. | 61 | micrometastasis | 2 | 1 | 1.5 cm. |
| 2. | 63 | micrometastasis | 2 | 1 | 1.5 cm. |
| 3. | 60 | Isolated cell metastasis | 2 | 2 | 2 cm. |
| 4. | 46 | Macrometastasis | 5 | 1 | 2.8 cm. |

stases and 1/50 (2%) was isolated cell metastasis (Figures 1-4). No differences in clinical or pathological characteristics (age, mammography, tumor size, tumor grading, TNM staging, and number of SLNs) were found between the group of patients with and without occult metastases (P- value > 0.05). (Table 3)

## Discussion

The presence of occult axillary metastases is associated with significantly poor disease-free and overall survival in patients with breast cancer.[7-10] Methods to detect occult metastases in sentinel lymph node include additional sectioning and staining with haematoxylin and eosin, immunohistochemical stains for epithelial markers and molecular diagnostic techniques.[12] The present study used to method of additional sectioning and haematoxylin and eosin staining of all paraffin blocks to detect the occult metastases. The prevalence occult sentinel node metastases in the present study was 8% that quite low with that of other reports.[4-8] Possible explanation include the fact fewer serial sections were examined in the present

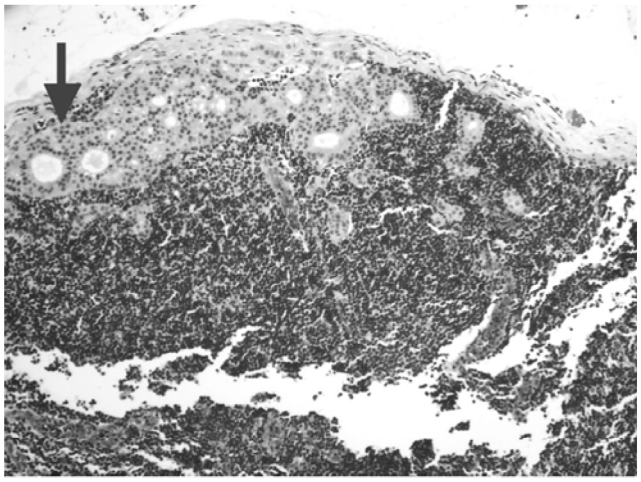

**Fig.1** *Micrometastatic tumor cells in patient number 1 (arrow). H&E x 100*

**Fig.2** *Micrometastatic tumor cells in patient number 2 (arrow). H&E x 100*

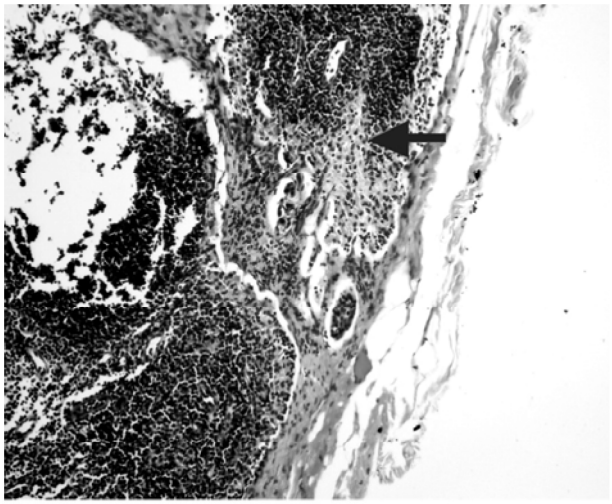

**Fig.3** *Isolate tumor cell in patient number 3 (arrow). H&E x 100*

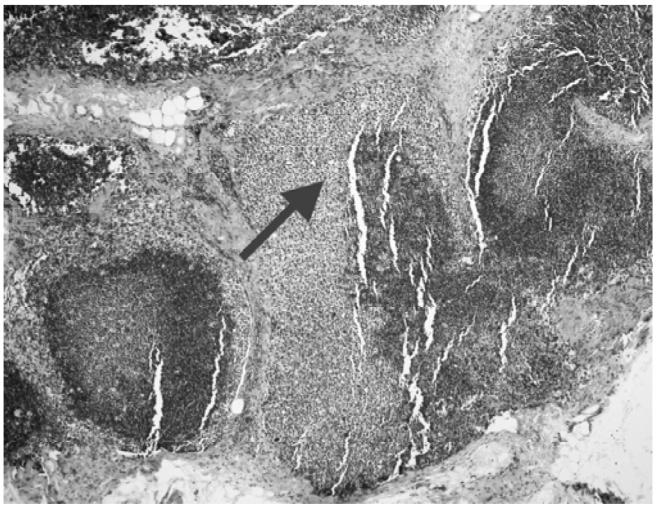

**Fig.4** *Micrometastatic tumor cells were patient number 4 (arrow). H&E x 40*

**Fig.1-4** *The pictures showed microscopic examination.*

**Table 3.** Relationship between clinical or pathological characteristics and occult SLN metastases

| Characteristics | Occult SLN metastasis (n = 4) | No SLN metastasis (n = 46) | P value |
|---|---|---|---|
| Age (years) Mean (SD) | 57.5 (7.8) | 52.5 (9.8) | 0.327 |
| Mammographic BI-RADS category | | | |
| 3 | 0 | 1 (2.44) | |
| 4 | 2 (50.00) | 17 (41.46) | 0.999 |
| 5 | 2 (50.00) | 23 (56.10) | |
| Tumor size (cm) | | | |
| Median (range) | 1.75 (1.5 to2.8) | 1.6 (0.1 to 5) | 0.660 |
| Tumor grading (Bloom-Richardson) | | | |
| 1 | 1 (25.00) | 8 (19.05) | |
| 2 | 3 (75.00) | 27 (64.29) | 0.999 |
| 3 | 0 | 7 (16.67) | |
| TNM staging | | | |
| I | 3 (75.00) | 28 (62.22) | 0.999 |
| II | 1 (25.00) | 17 (37.78) | |
| Number of SLNs | | | |
| Median (range) | 2 (2 to 3) | 2 (1 to 9) | 0.864 |

Some characteristics were not available for all patients; see table 1.

study than in the other studies; but standard pathological serial sectioning intervals for detecting occult metastases in the lymph nodes do not exist.[2] Thin serial sections with haematoxylin and eosin stain increased prevalence of axillary lymph node metastases in very few patients but the serial sectioning was time consuming and rather expensive. Further study on a long number of patients is needed to confirm these results before definite recommendations can be made regarding the true utility of serial sectioning. However, clinicians should aware that a negative sentinel lymph node result on routine sectioned with haematoxylin and eosin stain in breast cancer patient does not exclude the risk of occult metastases. The presence of occult metastases was not associated with age, mammography, tumor size, tumor grading, TNM staging, and number of SLNs accurate according to with statistically analysis.

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Original Articles/นิพนธ์ต้นฉบับ

# การตรวจพบการแพร่กระจายที่ซ่อนเร้นในต่อมน้ำเหลืองเซนติเนล ที่เคยรายงานว่าปลอดเซลล์มะเร็ง โดยการตัดซอยต่อมน้ำเหลืองของผู้ป่วยมะเร็งเต้านม ในโรงพยาบาลรามาธิบดี

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## บทคัดย่อ

การตรวจต่อมน้ำเหลืองเซนติเนล มีความสำคัญ เนื่องจากใช้ช่วยพิจารณาการรักษาด้วยวิธีผ่าตัดในผู้ป่วยมะเร็งเต้านม งานวิจัยเรื่องนี้ได้ศึกษาถึง การแพร่กระจายที่ซ่อนเร้นในต่อมน้ำเหลืองเซนติเนล ที่เคยรายงานว่าปลอดเซลล์มะเร็ง (ตรวจด้วยวิธีปกติ) โดยการตัดซอยต่อมน้ำเหลืองของผู้ป่วยมะเร็งเต้านมในโรงพยาบาลรามาธิบดี ระหว่างปี พ.ศ. 2549-2550 (50 ราย) ผลการวิจัยจากการตรวจทางพยาธิวิทยา พบว่ามีผู้ป่วย 4 ราย (ร้อยละ 8) ที่ตรวจพบการแพร่กระจายที่ซ่อนเร้นในต่อมน้ำเหลืองเซนติเนล จากการตัดซอยต่อมน้ำเหลือง ซึ่งแสดงถึงโอกาสเสี่ยงของผู้ป่วยมะเร็งเต้านมที่รักษาในโรงพยาบาลรามาธิบดี ที่เคยรายงานว่าต่อมน้ำเหลืองเซนติเนลปลอดเซลล์มะเร็ง โดยการค้นพบการแพร่กระจายที่ซ่อนเร้นในต่อมน้ำเหลืองเซนติเนลที่เคยรายงานว่าปลอดเซลล์มะเร็งนี้ใช้เวลาและเงินเป็นจำนวนมากในการตรวจผู้ป่วยแต่ละราย ดังนั้นแพทย์ผู้รักษาพึงตระหนักว่าผู้ป่วยมะเร็งเต้านมทุกรายที่เคยรายงานว่าต่อมน้ำเหลืองเซนติเนลปลอดเซลล์มะเร็งนั้นก็มีโอกาสตรวจพบการแพร่กระจายที่ซ่อนเร้นในต่อมน้ำเหลืองได้

**คำสำคัญ:** การแพร่กระจายที่ซ่อนเร้น, มะเร็งเต้านม, ต่อมน้ำเหลืองเซนติเนล