Breast Cancer Cells in the Afferent Lymphatic Tracts of Sentinel Lymph Nodes

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Background: *To identify breast cancer cells in the afferent lymphatic tracts of axillary sentinel lymph nodes (SLNs).*
Material and Method: *The authors performed a prospective study of 100 breast cancer patients who underwent SLN biopsy between June 2009 and January 2010. The afferent lymphatic tracts of SLNs were identified by isosulfan blue or radiocolloid or both and were examined histologically.*
Results: *One hundred three SLNs and afferent lymphatic tracts were examined. The mean age of the patients was 53.2 years (range, 24 to 78 years). The median number of SLNs was 2 (range, 1 to 7). Twenty-four (24%) patients had positive SLNs. Most patients had stage I breast cancer (67%). Three patients with positive SLNs (13%) and stages IIB-IIIC breast cancers had tumor cells in the afferent tract tissue. There were no tumor cells in the afferent tracts of negative SLNs.*
Conclusion: *Only a small proportion of operable breast cancer patients have tumor cells in the afferent lymphatic tract tissue of SLNs. There was a probable trend for more advanced stage breast cancer to harbor tumor cells in the afferent lymphatic tract tissue.*

Keywords: *Sentinel lymph node, Breast cancer, Afferent lymphatic tract*

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Although sentinel lymph node biopsy is an established method for determining the axillary tumor status in early breast cancer[1], its false negative rate still ranges between 2 to 10%[2,3]. Methods to increase the sensitivity of sentinel lymph node biopsy procedures might improve the accuracy of breast cancer staging[4,5]. For example, the examination of the afferent lymphatic tracts of sentinel lymph nodes might provide further information for the staging of the axilla[6].

The present study was motivated in part by previous studies in malignant melanoma, which seemed to show that the performance of sentinel lymph node biopsy might increase the chance of local recurrence because in-transit tumor cells are left in the lymphatic vessels and are not removed with the sentinel nodes[7-9]. Although there is no evidence that local recurrence is increased in breast cancer after sentinel lymph node biopsy[1,10-12], a recent study on a small number of breast cancer patients found that the afferent lymphatics of sentinel nodes also contained tumor cells not detected by the usual biopsy and examination techniques[6]. If true, then the presence of tumor cells in the afferent lymphatics of sentinel nodes, especially when the nodes themselves are free of tumor metastases, might have an impact on axillary staging and treatment.

The objectives of the present study were to determine the prevalence and nature of tumor metastasis in the afferent lymphatic tracts of sentinel lymph nodes in a group of operable breast cancer patients and to discuss the importance of these findings for prognosis and treatment.

Material and Method

Between June 2009 and January 2010, consecutive women with operable breast cancer treated at the Breast and Endocrine Surgery Unit were enrolled in a prospective study to determine the prevalence of tumor metastasis in the afferent lymphatic tract of sentinel lymph nodes. Patients were excluded from the present study if they did not give informed consent, if

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they had a previous axillary node dissection and other contraindications for sentinel lymph node biopsy[1-3], and if the afferent lymphatic tract could not be identified during surgery. The hospital's Research Ethics Committee approved the present study.

All patients underwent sentinel lymph node identification using combined blue dye and radiocolloid injection methods. In the blue dye method, 1 to 2 ml of 1% isosulfan blue was injected intradermally at the periareolar or at the subareolar area, followed by a 2 to 5-minute massage at the injection site. In the radiocolloid method, 0.4 mCi of Technetium-99m labeled dextran was injected intradermally at the periareolar or at the subareolar area, at least two hours prior to operation. Standard methods and criteria, for each detection method, were used independently to detect sentinel lymph nodes[13,14]. The afferent lymphatic tract of each node was identified intraoperatively by the presence of the blue dye in the tract, or the presence of radiocolloid as detected by the gamma probe, or both. A 10 mm-segment of the afferent tract adjacent to the hottest node or the node with most intense blue dye stain was removed and sent for histological examination.

All sentinel lymph nodes underwent sectioning at standard 2-mm. intervals and conventional hematoxylin and eosin (H&E) stain. The afferent tract was sectioned at 2-mm intervals, each section embedded in a paraffin block, and a representative slice from each was stained with H&E and immuno-histochemical techniques for cytokeratin. A pathologist specializing in breast cancer examined all the histologic sections.

Clinical and demographic data for each patient were collected, as well as the pathological staging of the breast cancer. Histological characteristics of the tumor and status of the hormone receptors, human epidermal growth factor receptor-2 (HER2/neu), and the Ki67 proliferation index were also obtained.

Results

One hundred breast cancer patients were enrolled. Three patients had bilateral breast cancer. One hundred three sets of sentinel lymph nodes and afferent lymphatic tracts were examined. Clinical and surgical characteristics of patients are presented in Table 1. The mean age of the patients was 53.2 years. Sixty one percent of the patients were post-menopausal. Sixty six percent of the patients were diagnosed preoperatively by core needle biopsy (CNB). The most common location of the tumor was in the upper outer quadrant of the breast (70%) and the most common breast operation was total mastectomy (58%). Seventy two percent used the combined technique for sentinel lymph node detection. Only 24 patients had positive sentinel nodes. The median number of sentinel nodes removed was 2 (range, 1 to 7 nodes).

Most patients had stage I breast cancer[15] (66%). The main histologic type was invasive ductal carcinoma (79%) and most had grade II nuclear morphology (45%). Eighteen percent of the patients had lymphovascular invasion. Only 4% of the patients had resection margin less than 1 mm. Over half of the patients were endocrine responsive (estrogen receptor or progesterone receptor positive). HER2/neu status was negative in 58% of patients. One third of patients had high proliferative index (Ki-67 > 30%); see Table 2.

Three patients with positive sentinel lymph nodes were found to have tumor cells in the afferent tract tissues or lymphatics. A summary of the clinical and tumor characteristics are given in Table 3. Two patients had cancer cells in the afferent tract tissues

Table 1. Patient and sentinel node characteristics and type of surgery performed (n = 103 unless stated otherwise)

Characteristic	Number (%) unless stated otherwise
Age, year (total = 100)	
Mean (standard deviation)	53.2 (11.0)
Tumor location	
Upper quadrants	72 (70)
Lower quadrants	17 (17)
Central	12 (12)
Whole breast	2 (2)
Tumor size, cm	
Mean (standard deviation)	2.6 (1.5)
Breast surgery	
Breast-conserving surgery	10 (10)
Total mastectomy	60 (58)
Total mastectomy with reconstruction	33 (32)
Sentinel lymph node detection	
Blue dye alone	29 (28)
Combined blue dye and radiocolloid	74 (72)
Sentinel lymph node metastasis	
Positive	24 (23)

Table 2. Pathological characteristics and stage of breast cancers (n = 103)

Characteristic	Number (%) unless stated otherwise
Histology type	
Invasive	81 (79)
Ductal carcinoma in situ (DCIS)	10 (10)
DCIS with microinvasion	6 (6)
Others	6 (6)
Bloom-Richardson nuclear grade	
I	21 (20)
II	46 (45)
III	31 (30)
Unknown	5 (5)
Lymphovascular invasion	
Yes	18 (18)
Estrogen receptor status	
Positive	61 (59)
Progesterone receptor status	
Positive	56 (54)
HER-2/neu status	
Negative	60 (58)
Positive 2+	20 (20)
Positive 3+	23 (22)
Ki-67	
≤ 30%	42 (41)
> 30%	34 (33)
Unknown	27 (26)
Cancer stage	
0 (DCIS)	10 (10)
I	69 (66)
II	14 (14)
III	10 (10)

only, invading the blood vessel in one, although no cells were found in the afferent lymphatics. One patient had isolated tumor cells in the afferent tract lymphatics only. All tumor deposits were less than < 0.2 mm. in size. In one patient with extensive axillary lymph node involvement (positive axillary lymph nodes in 27 of 27 nodes removed), there were extranodal tumor deposits outside the afferent lymphatic tracts as well. No afferent lymphatic tract tissue involvement was found in any patient with negative sentinel lymph nodes.

Discussion

Early experience with sentinel lymph node biopsy in patients with malignant melanoma emphasized the possibility of in-transit malignant cells left in the afferent lymphatic tracts of sentinel nodes, which could increase the risk of local recurrence[7-9]. Subsequent multicenter randomized controlled studies did not demonstrate such an increased risk[16,17]. Nonetheless, a recent study attempted to examine the frequency of the finding of in-transit tumor cells in the afferent tracts of sentinel lymph nodes in breast cancer patients[6]. The study included 17 early-stage breast cancer patients, and found tumor cells in one small lymph node situated along the afferent tract of a removed sentinel node in only one patient with otherwise negative nodes. Hence, technically speaking, this study found evidence for a missed sentinel node and illustrated a type of false negative sentinel lymph node biopsy result[18] but did not show evidence of tumor involvement of in-transit, afferent lymphatic vessels.

Although the present study was probably the first to focus on examining the afferent lymphatics of sentinel lymph nodes explicitly for tumor involvement in breast cancer patients at various stages of disease, only three patients in the present study had evidence of such involvement. Of the three patients, only one was found to have tumor cells inside the lymphatic vessels, and in all these patients, the sentinel lymph nodes were also involved. There was no evidence that patients with negative sentinel nodes had tumor cells lodged in the in-transit, afferent lymphatics of these nodes.

In addition, all three patients with afferent lymphatic tract tissue involvement had locally aggressive disease with large tumors, extensive axillary nodal involvement, and poor prognostic features such as triple negative disease (negative ER, PgR and HER2/neu status) or HER2-positive disease with high proliferation indices[19,20]. Therefore, afferent lymphatic tissue involvement in these patients might simply represent aggressive disease where perinodal and extranodal tissue involvement are well-known phenomena[15,21,22].

For patients with positive sentinel lymph nodes, the presence of afferent lymphatic tissue involvement was 3 of 24 sets of sentinel nodes, or 13%. The prognostic and therapeutic significance of extranodal involvement beyond that provided by the number of positive axillary nodes probably depended

Table 3. Clinical and tumor characteristics of patients with tumor involvement of afferent tract tissue

Characteristic	Patient 1	Patient 2	Patient 3
Age (years)	59	48	44
Tumor in the afferent lymphatic tract/tissue	Tumor cells in blood vv of afferent tissue	Tumor cells in the afferent tract tissue	Isolated tumor cells in afferent lymph vessel
Size of tumor deposit	<0.2 mm	<0.2 mm	<0.2 mm
Lymphovascular invasion	Positive	Positive	Positive
Status of SLNs	Macrometastasis	Macrometastasis	Macrometastasis
Status of ALNs	Positive 9/17	Positive 27/27	Positive 2/20
Primary tumor & size	IDC gr III; 7.2 cm	IDC gr II; 6 cm	IDC gr II; 4 cm
ER status	Negative	Negative	Negative
PgR status	Negative	Negative	Negative
HER2/neu status	Negative	Positive	Negative
Ki67 index	Not obtained	30%	45%
AJCC stage	IIIA	IIIC	IIB

SLN = sentinel lymph node; ALN = axillary lymph node; IDC = invasive ductal carcinoma; gr = nuclear grade; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor-2; AJCC = American Joint Committee on Cancer, 7th edition; vv = vessels

on the extent of involvement[21,23,24]. Since the afferent tissue metastases in the present study were all minimal[24], this finding might not affect the prognosis of any patient.

A more significant, but negative, finding was the absence of tumor cells in the afferent lymphatic tissues of patients with negative sentinel lymph nodes (all 79 sets of negative sentinel nodes). The present study supported the current practice of leaving the afferent lymphatic tissue behind after a negative sentinel lymph node biopsy. However, if the sentinel nodes were involved, the remaining axillary nodes and perilymphatic tissues should still be removed.

One limitation of the present study was a relatively small sample size, which might not favor the appearance of rare events such as the presence of afferent tract metastasis in sentinel node-negative patients. Other limitations included the small number and size lymphatic tract specimens used for histological study, as well as the rather large sectioning interval (2 mm). Future studies involving a larger number of patients, the examination of more representative lymphatic tracts, and sectioning the specimens at finer intervals might increase the sensitivity of the present study.

Conclusion

In a group of 100 women with breast cancer and 103 sentinel lymph node biopsy procedures, only three had tumor involvement of the afferent lymphatic tissue. Two of these had isolated cell clusters outside of the lymphatic vessels and one had isolated cells inside the afferent lymph vessel. In all these cases, the sentinel lymph nodes were also positive for cancer and all had poor prognostic features. No tumor cells in the afferent lymphatic tissues of negative sentinel nodes were identified.

Ethical approval

The present study was approved by the Hospital's Research Ethics Committee (protocol No. ID02-52-23; approved MURA 2009/1234, March 24, 2009).

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The authors declare no funding sources. The study was an extension of routine clinical service.

Potential conflicts of interest

None.

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การหาเซลล์มะเร็งในทางเดินน้ำเหลืองก่อนถึงต่อมน้ำเหลืองเซนติเนลในผู้ป่วยมะเร็งเต้านม

ประกาศิต จิรัปปภา, กำพล รัชวรพงศ์, ศันสนีย์ วงศ์ไวศยวรรณ, ภาณุวัฒน์ เลิศสิทธิชัย, รณรัฐ สุวิกะปกรณ์กุล, ยอดยิ่ง วาสุฤิตย์, เยาวนุช คงด่าน

วัตถุประสงค์: *เพื่อประเมินทางเดินน้ำเหลืองก่อนถึงต่อมน้ำเหลืองเซนติเนล (afferent lymphatic tract of sentinel lymph node) ว่ามีเซลล์มะเร็งหรือไม่*

วัสดุและวิธีการ: *เป็นการศึกษาวิจัยแบบไปข้างหน้า (Prospective study) ในผู้ป่วยมะเร็งเต้านม 100 ราย ที่เข้ารับการรักษาที่แผนกศัลยกรรม โรงพยาบาลรามาธิบดีระหว่าง มิถุนายน พ.ศ. 2552 ถึงมกราคม พ.ศ. 2553 โดยใช้วิธีการฉีดสี Isosulfan blue dye และ/หรือ Radioisotope เข้าไปในบริเวณเต้านม จากนั้นทำการผ่าตัดหาต่อมน้ำเหลืองเซนติเนลและทางเดินน้ำเหลืองก่อนถึงต่อมน้ำเหลืองเซนติเนลประมาณ 1 เซนติเมตร แล้วนำไปย้อมทางพยาธิวิทยาเพื่อดูว่ามีเซลล์มะเร็งหรือไม่*

ผลการศึกษา: *จำนวนทั้งหมด 103 ต่อมน้ำเหลืองเซนติเนล และทางเดินน้ำเหลืองก่อนถึงต่อมน้ำเหลืองเซนติเนลประมาณ 1 เซนติเมตร นำไปย้อมทางพยาธิวิทยาเพื่อดูว่ามีเซลล์มะเร็งหรือไม่ ค่าเฉลี่ยอายุผู้ป่วยมะเร็งเต้านมเท่ากับ 53.2 ปี (ระหว่าง 24 ถึง 78 ปี) ค่าเฉลี่ยต่อมน้ำเหลืองเซนติเนลที่ทำการผ่าตัดคือ 2 ต่อม (ระหว่าง 1 ถึง 7 ต่อม) พบว่า 24% ของผู้ป่วยมะเร็งเต้านมมีการกระจายไปต่อมน้ำเหลืองเซนติเนลและส่วนใหญ่ (67%) เป็นมะเร็งระยะที่ 1 การศึกษานี้พบผู้ป่วยมะเร็งเต้านม 3 คนในระยะ IIB-IIIC คิดเป็น 13% มีการกระจายไปต่อมน้ำเหลืองเซนติเนล และตรวจพบว่ามีการกระจายไปทางเดินน้ำเหลืองก่อนถึงต่อมน้ำเหลืองเซนติเนล จากการศึกษานี้ไม่พบการกระจายไปทางเดินน้ำเหลืองก่อนถึงต่อมน้ำเหลืองเซนติเนลในผู้ป่วยมะเร็งเต้านมที่ไม่มีการกระจายไปต่อมน้ำเหลืองเซนติเนล*

สรุป: *การศึกษานี้ตรวจพบว่ามีผู้ป่วยบางรายในกลุ่มผู้ป่วยมะเร็งเต้านมที่ผ่าตัดได้มีการกระจายไปทางเดินน้ำเหลืองก่อนถึงต่อมน้ำเหลืองเซนติเนล ดังนั้นในผู้ป่วยมะเร็งเต้านมในระยะที่สูงขึ้นมีความเป็นไปได้ที่จะพบการกระจายไปทางเดินน้ำเหลืองก่อนถึงต่อมน้ำเหลืองเซนติเนล*