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ORIGINAL ARTICLE

Benign Mechanical Transport of Breast Epithelial Cells to Sentinel Lymph Nodes

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Abstract: The evaluation of sentinel lymph nodes (SLNs) for the presence of malignant epithelial cells is essential to the staging of breast cancer patients. Recently, increased attention has focused on the possibility that epithelial cells may reach SLNs by benign mechanical means, rather than by metastasis. The purpose of this study was to test the hypothesis that pre-SLN biopsy breast massage, which we currently use to facilitate the localization of SLNs, might represent a mode of benign mechanical transport. We studied 56 patients with invasive and/or in situ ductal carcinoma and axillary SLNs with only epithelial cells and/or cell clusters (≤0.2 mm in diameter and not associated with features of established metastases) detected predominantly in subcapsular sinuses of SLNs on hematoxylin and eosin- and/or anti-cytokeratin-stained sections. No patient had an SLN involved by either micro- or macro-metastatic carcinoma. Epithelial cells and cell clusters, ≤0.2 mm in size and without features of established metastases, occurred more frequently in the SLNs of patients who underwent pre-SLN biopsy breast massage ($P < 0.001$, χ^2 test). The latter finding supports the hypothesis that pre-SLN biopsy breast massage is a mode of benign mechanical transport of epithelial cells to SLNs.

Key Words: breast cancer, sentinel lymph node, benign mechanical transport, occult micrometastasis

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Pathologic evaluation of axillary sentinel lymph nodes (SLNs) is used to stage breast cancer patients.[5] Recently, experts have focused attention on the possibility that epithelial cells may be transported by mechanical means, such as displacement of epithelial cells by surgical biopsy into lymphatic vessels, to axillary lymph nodes, rather than exclusively by metastasis, in some patients with breast cancer.[2,11,12] Because immunohistochemistry is commonly used to detect micrometastases in SLNs,[15] the previously rare detection of small clusters of epithelial cells has become almost commonplace. The prognostic significance of such findings is unknown[8] and under study. We investigated whether pre-SLN biopsy breast massage (Pre-SLNBxM), used to facilitate the intraoperative localization of SLNs for biopsy,[1,12] may represent a mode of benign mechanical transport of epithelial cells and cell clusters to lymph nodes. Specifically, we retrospectively analyzed whether epithelial cells and/or cell clusters, ≤0.2 mm in diameter and without features of established metastases, occurred more frequently in the SLNs of patients who underwent Pre-SLNBxM than in those who did not undergo such massage. The 0.2-mm size limit corresponds to the threshold above which a micrometastasis is currently diagnosed.[6]

MATERIALS AND METHODS

The Breast Cancer Database of the Comprehensive Breast Cancer Program at the H. Lee Moffitt Cancer Center and Research Institute was searched for SLN biopsies of patients with invasive and/or in situ ductal carcinoma coded as "cytokeratin-positive only cells" from March 1997 through January 2001. Patients in the database with a diagnosis of invasive lobular carcinoma or mixed invasive ductal and lobular carcinoma and SLNs with "cytokeratin-positive only cells" were excluded from the study, given the patterns that nodal metastases of metastatic lobular carcinoma may assume.[14] Specifically, because lobular carcinoma cells are not cohesive-like ductal cells, nor as frequently associated with cell proliferation or stromal reaction, scattered single cells in a lymph node may be the sole manifestation of metastatic lobular carcinoma. Such cells may be difficult to differentiate from multiple single cells transported by benign means to the subcapsular sinuses of lymph nodes. Thus, to test the hypothesis of benign mechanical transport, cases of invasive lobular carcinoma with such findings were excluded from the study. All patients with a SLN with micro- or macrometastatic carcinoma were excluded from the study. These dates were chosen because Pre-SLNBxM became routine at the approximate midpoint of this interval, allowing for a comparison of large number of patients who were staged by SLN biopsy, either without Pre-SLNBxM or following such massage.

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Pre-SLNBxM was conducted in the following manner[1]: The lymphatic mapping procedure was performed using a combination technique of blue dye and radiocolloid. The Tc^{99m}-labeled sulfur colloid was injected into either the periphery of the lesion or biopsy cavity 1 to 6 hours before the patient was to undergo SLN biopsy. A vigorous 5-minute manual massage was administered immediately after injection. With the breast compressed, massage was focused over the breast lesion. The isosulfan blue dye was injected 5 minutes before the SLN biopsy because the dye reaches a peak staining of the SLNs at 5 minutes postinjection. An identical second 5-minute massage was administered immediately after injection. Careful dissection was taken to prevent any staining of the surgical site by disruption of the lymphatics. A gamma-detector probe was used to locate the SLN, which has been defined as any blue or hot node with an ex vivo radioactivity count ratio of SLN to non-SLN of 10:1.[1]

Sentinel lymph nodes were sectioned every 2 mm and processed for routine histopathologic examination. One hematoxylin and eosin-stained section and one anti-cytokeratin-immunostained (CK-IHC) section of each formalin-fixed, paraffin-embedded tissue block were analyzed. Hematoxylin and eosin sections of the SLNs were analyzed without prior knowledge of the interpretation of CK-IHC sections. Similarly, routine CK-IHC sections were analyzed without knowledge of the analysis of hematoxylin and eosin sections. The latter were stained using an antibody directed at low molecular weight cytokeratin (Clone NCL-5D3, prediluted; Ventana, Tuscon, AZ). The hematoxylin and eosin sections were then re-reviewed to determine whether epithelial cells could be detected with the CK-IHC sections serving as a guide/aide. Immunostaining of sections had been completed using the avidin-biotin-peroxidase complex method and an automated immunostainer (Ventana). Diaminobenzidine served as the chromogen and hematoxylin as the counterstain. Positive and negative controls were also confirmed as staining appropriately. Hematoxylin and eosin and CK-IHC sections were analyzed without knowledge of whether the patient had Pre-SLNBxM or residual tumor in the biopsy site of their definitive therapeutic breast specimen.

Patients with epithelial cells and/or cell clusters, $\leq$0.2 mm in diameter and not associated with features of established metastases (in accord with the 6th edition of the AJCC Cancer Staging Manual[6]), which were detected predominantly in SLN subcapsular sinuses on hematoxylin and eosin and/or CK-IHC sections, were included in the study. Features regarded as indicative of an established metastasis included cell proliferation and stromal reaction. Fifty-six such patients were identified. The SLNs of all 56 were located in the axilla ipsilateral to the breast carcinoma. Seventeen of the 56 (30.4%) patients' breast carcinomas were diagnosed by a fine needle aspiration procedure or core biopsy; and 39 of 56 (69.6%) patients were diagnosed by excisional biopsy. The average time from the diagnostic procedure to SLN biopsy was 28 days in the 56 patients included in the study. Forty-five of the 56 (80.4%) patients were diagnosed with invasive ductal carcinoma with or without ductal carcinoma in situ, whereas 11 (19.6%) revealed ductal carcinoma in situ only. None of the 56 patients had a local or distant recurrence in an average follow-up of 3 years.

Several histopathologic features in the SLNs and definitive therapeutic breast specimen of the patients, included for study, were analyzed (without knowledge of whether the patients underwent Pre-SLNBxM) and tested for differences in occurrence rates among patients who did or did not undergo Pre-SLNBxM. The numbers of epithelial cells in the SLNs were counted from the microscopic slides and were classified as either less than or equal to 20 cells or greater than 20 cells. The cell counts do not represent a volumetric calculation. The histologic pattern of epithelial cell involvement in SLNs was classified into either one of two groups: single cell or cell cluster or multiple single cells and/or multiple cell clusters (Fig. 1). The presence of hemosiderin-laden macrophages in SLNs was also assayed. The presence of epithelial cells in granulation tissue of the breast therapeutic surgical specimen was analyzed.

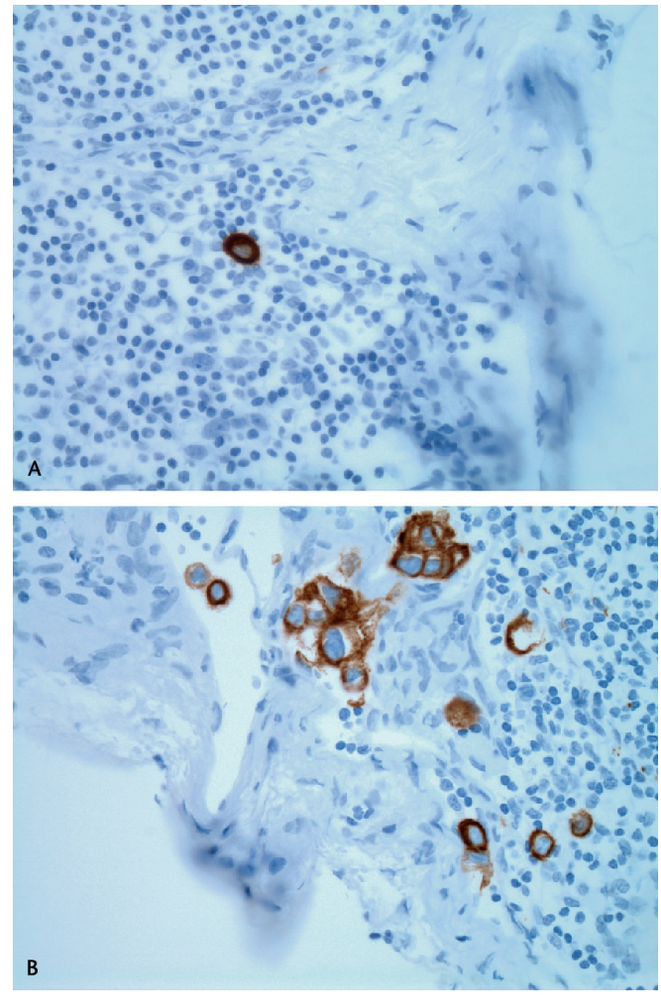

FIGURE 1. Epithelial cell pattern involvement of sentinel lymph nodes (SLNs). A, A single epithelial cell is highlighted by an anti-cytokeratin immunohistochemistry stain and hematoxylin counterstain in the subcapsular sinus of a SLN (original magnification ×400). B, Multiple single epithelial cells and cell clusters are similarly highlighted in a subcapsular sinus (original magnification ×400).

Fig. 1 live 4/C

RESULTS

Between March 1997 and May 1998, 320 patients underwent lymphatic mapping and SLN biopsy without Pre-SLNBxM. Between June 1998 and January 2001, 456 patients underwent lymphatic mapping and SLN biopsy with Pre-SLNBxM. Fifty-six of the 776 patients (7.6%) had epithelial cells and/or cell clusters, ≤0.2 mm in diameter that were not associated with features of established metastases, which were detected on hematoxylin and eosin and/or CK-IHC sections of SLN biopsy specimens. The epithelial cells were located predominantly in SLN subcapsular sinuses of patients with invasive ductal carcinoma and/or ductal carcinoma in situ. Table 1 specifies the occurrence of epithelial cells and cell clusters in SLNs based on how the epithelial cells were detected. Only 8 of the 56 cases were detected by our initial analysis of hematoxylin and eosin sections. Of the 48 cases, in which epithelial cells were initially detected on CK-IHC sections, 3 were noted to have epithelial cells on re-review of the hematoxylin and eosin-stained sections, using the CK-IHC section as a guide/aide, ie, 45 of the 56 patients had epithelial cells detected exclusively by examination of CK-IHC sections.

Epithelial cells and/or cell clusters, ≤0.2 mm in diameter that were not associated with features of established metastases, were detected in the SLNs of 3.4% (11 of 320) of patients that did not undergo Pre-SLNBxM and in 9.9% (45 of 456) of patients that did undergo massage, a statistically significant difference between the two groups ($P < 0.001$, χ^2 test). By odds ratio, such cells occur more frequently in patients with Pre-SLNBxM (3.1; confidence interval, 1.6–6.0). Table 2 summarizes how frequently these epithelial cells and cell clusters occurred in SLNs, according to whether a patient had undergone Pre-SLNBxM and whether residual ductal carcinoma was present in the definitive therapeutic breast specimen at the time of the SLN biopsy.

The occurrence of other histologic features in the SLNs and definitive therapeutic breast specimens of patients with epithelial cells and/or cell clusters (≤0.2 mm) in SLNs are listed in Table 3 according to whether the patient underwent Pre-SLNBxM. Four of the 56 patients in the study had epithelial cells and/or cell clusters ≤0.2 mm in two SLNs. In each case, the index SLN, included for the analysis detailed in Table 3, was the first SLN biopsied. In 3 cases, the number of epithelial cells in the second involved SLN was nearly identical to or less than the first. In the fourth case, the epithelial cell involvement of the second SLN was detected only on the CK-IHC section, according to the diagnostic pathology report. The hematoxylin and eosin section was negative on study analysis; however, neither the corresponding CK-IHC section nor the paraffin-embedded block was available for study .

TABLE 1. Detection of Epithelial Cells/Cell Clusters (≤0.2 mm) in Sentinel Lymph Nodes (SLNs) With/Without Pre-SLN Biopsy Breast Massage (Pre-SLNBxM)

	SLN Biopsy Without Pre-SLNBxM	SLN Biopsy With Pre-SLNBxM
No. H&E-positive (epithelial cells) on initial review or cytokeratin (CK)-positive	11	45
No. H&E-negative on initial review/CK-positive epithelial cells/H&E positive on re-review	10	38
No. H&E-negative on initial review/CK-positive/H&E-negative on re-review	10	35

TABLE 2. Frequency of Epithelial Cells/Cell Clusters (≤0.2 mm) in Sentinel Lymph Nodes (SLNs) According to Massage and Tumor Status in Breast Therapeutic Specimens

	No Pre-SLN Biopsy Massage (n = 320)		Pre-SLN Biopsy Massage (n = 456)		
	Residual Tumor Absent	Residual Tumor Present	Residual Tumor Absent	Residual Tumor Present	Total
No. of patients	150	170	181	275	776
No. with epithelial cells/cell clusters in SLNs	6	5*	14†	31*†	56

*Significant difference between groups with residual tumor depending on massage status ($P = 0.002$; χ^2 test).
†No significant difference between groups who underwent pre-SLN biopsy massage depending on presence of residual tumor ($P = 0.222$; χ^2 test).

DISCUSSION

The mechanical transport of breast epithelium to axillary lymph nodes, via epithelial displacement into intramammary lymphatic spaces, was reported in a series of 15 breast carcinoma patients by Carter et al.[2] They hypothesized that the epithelial displacement was a result of prior surgical manipulation. The displacement of breast epithelium into breast stroma and/or lymphatic spaces by core biopsy of the breast is a well-described phenomenon.[3,4,9,16]

The postulate of biopsy-associated benign mechanical transport of epithelial cells to SLNs is supported by the

TABLE 3. Occurrence of Histologic Features in Patients With Epithelial Cells/Cell Clusters (≤0.2 mm) in Sentinel Lymph Nodes (SLNs) Depending on Prior Massage

Histologic Feature	Incidence of Histologic Feature		Statistical Significance (P, χ^2 test)
	No Prebiopsy Massage	Prebiopsy Massage	
Up to 20 epithelial cells in SLNs (≤ 20)	8/11	36/45	0.60
Pattern of epithelial cells in SLNs (scattered single cells and/or multiple cell clusters)	5/11*	31/45†	0.15
Hemosiderin-laden macrophages present in SLNs	9/11	32/45	0.47
Epithelial cells present in granulation tissue of definitive therapeutic breast specimen	1/11	9/45	0.40

*Six of 11 had a single cell or single cluster.
†Fourteen of 45 had a single cell or single cell cluster.

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Moore et al study of the SLN findings in 4016 consecutive patients with breast carcinoma.[10] Epithelial cells, identified in SLNs only on examination of cytokeratin-immunostained sections, were statistically associated with the type of breast biopsy. The incidence of immunohistochemistry-only positive SLNs following no breast biopsy prior to SLN biopsy, fine needle aspiration, core biopsy, and open biopsy were 1.2%, 3.0%, 3.8%, and 4.6%, respectively.[10]

Hansen et al[7] reported perplexing results of a study of 676 patients with invasive breast cancer. They found that different techniques, used to biopsy the breast tumors, were associated with different frequencies of SLN metastases.[7] SLN metastases occurred more frequently in patients whose breast cancer was diagnosed by either fine needle aspiration (P = 0.07) or large gauge needle core biopsy (P = 0.04) than by excisional biopsy. A confounding result of the study, however, was that macrometastases occurred significantly more frequently than micrometastases and immunohistochemically detected metastases.[7] The size of epithelial fragments that may be transported through angiolymphatic spaces is presently unknown.

Rosser hypothesized that surgical compression of a breast tumor might embolize a so-called occult micrometastasis to an axillary lymph node.[11] Occult micrometastases are identified by means other than examination of one routinely stained slide and are present in approximately 20% of axillary lymph nodes of breast cancer patients, when tested by CK-IHC.[13] Rosser also hypothesized that breast massage, following the injection of a substance, such as a radioisotope, to facilitate the localization of SLNs, might also cause such occult micrometastases.[11]

Our findings support the long discussed possibility for the transport of epithelial cells to axillary lymph nodes in breast carcinoma patients by means other than metastasis. We tested whether the occurrence of a defined population of epithelial cells in SLNs was linked to Pre-SLNBxM. Specifically, epithelial cells and cell clusters, ≤0.2 mm in diameter and without features of established metastases, were found to occur more frequently in the SLNs of patients who underwent Pre-SLNBxM. These findings strongly suggest that Pre-SLNBxM represents a mode of benign mechanical transport.

The findings also suggest that the source of Pre-SLNBxM-associated epithelial cells in SLNs is epithelial cells from the breast itself. Specifically, we found that a significant difference in the occurrence of epithelial cells and/or cell clusters (≤0.2 mm in diameter) in SLNs existed between patients who had residual ductal carcinoma in the definitive therapeutic breast specimen and who underwent Pre-SLNBxM and those who did not undergo such massage. In addition, we found that such epithelial cells occurred more frequently in the SLNs of patients who had Pre-SLNBxM and who had residual tumor than those who did not have residual tumor; however, the latter difference was not statistically significant. It is worth noting that massage may not be displacing cells directly from traumatized tumor into lymphatic channels; the possibility exists that epithelial cells and/or cell clusters (≤0.2 mm in diameter) detected in SLNs were transported, after prior biopsy-related displacement to and from the lymphatic channels of the breast or axillary soft tissue, at the time of Pre-SLNBxM.

No other histologic feature in the SLN or therapeutic breast specimen assayed occurred more frequently in association with Pre-SLNBxM than without such massage. Thus, presently no histologic feature should be used to distinguish the epithelial cells associated with benign mechanical transport from those associated with a metastatic process. Nonetheless, it should be noted that currently epithelial cells and/or clusters ≤0.2 mm in diameter in lymph nodes are termed "isolated tumor cells" (when not associated with features of established metastases) and are not staged as micrometastatic disease.[6]

The prognosis of "isolated tumor cells" in SLNs is not known. Our findings provide evidence why, in some cases, such findings may eventually prove not to be associated with an adverse prognosis. More importantly, to date no evidence exists to support a contention that epithelial cells transported by mechanical means have malignant potential.[11] It is important to note the significant difference in time intervals between an event associated with benign mechanical transport and sentinel lymph node biopsy. The time interval is key because small epithelial cell aggregates transported in association with breast biopsy to a sentinel lymph node may have time to grow into a micrometastasis, whereas such aggregates associated with breast massage would not likely have time to grow into micrometastases. However, the clinical significance of the massage-associated small epithelial cell clusters of this study could only truly be tested by comparing the long-term survival of patients with such epithelial clusters who underwent breast massage with those who did not.

In summary, statistical evidence is provided that supports the hypothesis that Pre-SLNBxM is a mode of benign mechanical transport of breast epithelial cells to SLNs.

REFERENCES

1. Bass SS, Cox CE, Salud CJ, et al. The effects of postinjection massage on the sensitivity of lymphatic mapping in breast cancer. *J Am Coll Surg.* 2001;192:9–16.
2. Carter BA, Jensen RA, Simpson JF, et al. Benign transport of breast epithelium into axillary lymp nodes after biopsy. *Am J Clin Pathol.* 2000;113:259–265.
3. Diaz LK, Wiley EL, Venta LA. Are malignant cells displaced by large-guage needle core biopsy of the breast? *AJR Am J Roentgenol.* 1999; 173:1303–1313.
4. Douglas-Jones AG, Verghese A. Diagnostic difficulty arising from displaced epithelium after core biopsy in intracystic papillary lesions of the breast. *J Clin Pathol.* 2002;55:780–783.
5. Fitzgibbons PL, Page DL, Weaver D, et al. Prognostic factors in breast cancer: College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med.* 2000;124:966–978.
6. Greene F, Page DL, Fleming ID, et al, eds. AJCC Cancer Staging Manual. New York: Springer-Verlag, 2002:223–240.
7. Hansen NM, Ye X, Grube BJ, et al. Manipulation of the primary breast tumor and the incidence of sentinel node metastases from invasive breast cancer. *Arch Surg.* 2004;139:634–640.
8. Hermanek P, Hutter RV, Sobin LH, et al. International Union Against Cancer: classification of isolated tumor cells and micrometastasis. *Cancer.* 1999;86:2668–2673.
9. Liberman L, Vuolo M, Dershaw DD, et al. Epithelial displacement after stereotactic 11-guage directional vacuum-assisted breast biopsy. *AJR Am J Roentgenol.* 1999;172:677–681.
10. Moore KH, Thaler HT, Tan LK, et al. Immunohistochemically detected tumor cells in the sentinel lymph nodes of patients with breast carcinoma. *Cancer.* 2004;100:929–934.

11. Rosser RJ. A point of view: trauma is the cause of occult micrometastatic breast cancer in sentinel axillary lymph nodes. *Breast J.* 2000;6:209–212.
12. Schwartz GF, Giuliano AE, Veronesi U. Proceedings of the consensus conference on the role of sentinel lymph node biopsy in carcinoma of the breast, April 19–22, 2001, Philadelphia, Pennsylvania. *Cancer*. 2002; 94:2542–2551.
13. Siziopikou KP, Schnitt SJ, Connolly JL, et al. Detection and significance of occult axillary metastatic disease in breast cancer patients. *Breast J.* 1999;5:221–229.
14. Trojani M, de Mascarel I, Coindre JM, et al. Micrometastases to axillary lymph nodes from invasive lobular carcinoma of breast: detection by immunohistochemistry and prognostic significance. *Br J Cancer.* 1987; 56:838–839.
15. Weaver DL, Krag DN, Ashikaga T, et al. Pathologic analysis of sentinel and nonsentinel lymph nodes in breast carcinoma: a multicenter study. *Cancer.* 2000;88:1099–1107.
16. Youngson BJ, Cranor M, Rosen PP. Epithelial displacement in surgical breast specimens following needling procedures. *Am J Surg Pathol.* 1994;18:896–903.

