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Biology of Lymphatic Metastases in Breast Cancer: Lessons Learned From Sentinel Node Biopsy

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Background: The evolution of sentinel node biopsy has placed new emphasis on the biology of lymphatic metastases in breast cancer. If radiocolloid mimics the migration of tumor cells, the nodes with the most uptake should also be the most likely to harbor metastatic cells. We attempted to correlate the frequency of metastatic disease to the greatest gamma uptake and to clarify the physiology of breast lymphatic drainage.

Methods: Data were collected from 152 patients undergoing sentinel node biopsy from January 1997 to June 1999. Localization was by injection of unfiltered ^{99m}Tc-labeled sulfur colloid. Sentinel nodes were identified with an intraoperative gamma counter and the 10% rule. A completion level I/II axillary dissection was performed in all patients.

Results: Fifty-four of 152 patients were positive for metastatic disease. There were no false-negative sentinel nodes. In 46 (85%) of 54 cases, the node with the highest uptake was positive for metastatic disease. In the remaining eight (15%) cases, another node with a lower gamma count was positive.

Conclusions: The sentinel node with the highest uptake is not the one that contains metastatic disease in 15% of cases. This may reflect variations in lymphatic channels or technical variations in colloid properties and injection technique.

Key Words: Breast cancer—Lymphatic metastases—Sentinel node biopsy—Radiocolloid localization.

The evolution of sentinel node biopsy (SNB) in the management breast cancer has made it apparent that our understanding of the biology of lymphatic metastases is limited. The myriad of different methods used in SNB and the lack of standardized techniques emphasize the need to better understand lymphatic drainage patterns in the breast. Localization of the sentinel lymph node with intraoperative gamma counters after preoperative injection of radioisotope often results in the identification of multiple nodes. The average number collected ranges from 1.4 to 2.9 with various localization techniques.[1] This suggests that the concept of the first-draining node may not be physiologically correct.

Little is published with regard to the actual method by which radioisotope migrates through the lymphatic system and becomes trapped within the sentinel node or nodes (SN). Does the radioisotope mimic the biologic migration of tumor cells to the draining basin? If so, it follows that the node with the greatest radiocolloid uptake would also be the most likely to harbor tumor cells. The objectives of this study were to determine the frequency with which the presence of metastatic disease correlated with the greatest radiocolloid uptake and to shed light on the physiology of breast lymphatic drainage.

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METHODS

Patients

The study group included patients with breast cancer who underwent SNB by three surgeons at the Princess

Margaret Hospital, Toronto, Ontario, and the Foothills Medical Center, Calgary, Alberta, Canada, from January 1997 to June 1999. All patients had invasive breast cancer documented on fine-needle aspiration or core or excisional biopsy. Patients with clinically involved axillae were excluded. A total of 152 patients were studied: 151 women and 1 man.

All patients either were scheduled for segmental or total mastectomy with axillary dissection or had a previous excisional biopsy and were presenting for axillary dissection with or without re-excision. Patients underwent SNB with ^{99m}Tc-labeled sulfur colloid radioisotope localization, followed by a completion level I/II axillary dissection. All SNs and nodes from the completion axillary dissection were sent for standard hematoxylin and eosin evaluation on blocks of 2- to 3-mm thickness. One hundred four patients (69%) had serial sections and immunohistochemistry as well.

Intraoperative Mapping

One millicurie of unfiltered ^{99m}Tc-labeled sulfur colloid in a volume of 8 mL was injected into the breast parenchyma surrounding the tumor or into the wall of the previous biopsy cavity from 2 to 24 hours before surgery. In some cases ultrasound was used to guide the injection to ensure accurate placement. A lymphoscintigram was taken, and the skin was marked only over the site of the highest uptake in the axilla. Any internal mammary nodes were excluded from analysis. After induction of general anesthesia, localization of SNs was then performed with a handheld gamma counter (C-trak; Carewise Medical, Morgan Hill, CA) covered with a sterile sheath. A separate axillary incision over the area with the greatest radioactivity was performed, and the SN was dissected and removed. Node counts were recorded. The axillary bed was then re-examined for residual radioactivity, and any node with a count >10% of the hottest node was also removed and counts recorded. Grossly positive nodes were excluded from analysis. Each SN was labeled and sent for evaluation. A completion level I/II axillary dissection was then performed and the specimen sent for pathologic evaluation. Blue dye was used in some cases according to surgeon preference.

RESULTS

Localization by radioisotope was successful in identifying an SN in 141 of 152 patients, for a 93% localization rate. The false-negative rate was 0%; that is, no patient with a negative SNB was subsequently found to have positive nodes in the completion axillary dissection. Fifty-four (38%) of 141 patients had a positive SNB. Patient and tumor characteristics are listed in Table 1. The mean age of subjects with a positive SNB was 53 years; the mean tumor size was 2.5 cm. The mean number of SNs identified and removed was 3 (range, 1–12), and the mean number of axillary nodes was 14.6 (range, 1–26). In 46 of 54 cases, the node with the highest count was found to be positive on pathology, giving a sensitivity of 85%. In the remaining 8 of 54 SNBs, the hottest node was negative, whereas another node with a lower count was found to be positive for metastatic disease (Table 2). Thus, failure to remove the second- or third-hottest nodes would have resulted in a false-negative rate of 15%. Details of relative radioactivity counts are listed in Table 3. Of the eight patients whose positive node was not the hottest, the radioactivity of the positive nodes ranged from 16% to 81% of the hottest node.

DISCUSSION

The concept of SNB exploits the ability to map the lymphatic drainage system of the breast. Various methods of localization based on studies of the lymphatic system have been used in an attempt to optimize identification rates. Intradermal, intraparenchymal, and subareolar injections of radiocolloid have all been used with success.[2–7] However, little has been published on the actual mechanism by which various mapping agents become sequestered within the SN. Does the migration of radiocolloid mimic the true pathway of the metastatic tumor cells? We report a 15% false-negative rate associated with removing the node with the highest gamma count alone; that is, tumor was not present in the node with the most radiocolloid uptake but was present in a subsequent node with a lesser colloid uptake. Our findings are consistent with reports from the Louisville Breast Cancer Study Group[5] and the Memorial Sloan-

TABLE 1. *Sentinel node–positive patient and tumor characteristics*

Variable	Data
Patient	
Age (y)	mean, 52; range, 35–75
Sex	151 women, 1 man
	2.5 cm; range, .7–7.0 cm
T stage	
I	24/54; 44%
II	25/54; 46%
III	4/54; 8%
Unknown	1/54; 2%
No. SLNs	mean, 3; range, 1–12
No. Axillary nodes	mean, 14.6; range 1–26

SLN, sentinel lymph node.

TABLE 2. *Frequency, number, and positivity of multiple SLNs*

Variable	n	%
No. Positive SNBs	54/141	38%
Highest uptake node positive	46/54	85%
Highest uptake node negative, another SLN positive	8/54	15%

SLN, sentinel lymph node; SNB, sentinel node biopsy.

Kettering group[8] by Martin et al.,[8] who also found a potential false-negative rate of 13% by Louisville and 20% by MSKCC, respectively, if only the hottest node were removed.

The widely used 10% rule states that few or no positive nodes will be missed if all nodes with >10% of the radioactivity of the hottest node are removed. The Louisville Study Group supports the 10% rule for removing SNs.[5] This was not found to be true at Memorial Sloan-Kettering Cancer Center, where Martin et al. reported that a 31% miss rate would have resulted had the 10% rule been adhered to.[8] In fact, they were unable to conclude that any ratio level was an effective cutoff point, in that a 2% rule would have missed 20% of positive nodes. It is not clear from the data, however, how the missed nodes were identified—by blue dye or the 4:1 threshold value. Subset analysis of injection site (intraparenchymal vs. intradermal) was not performed in their series, and this may have accounted for the differences, because we used intraparenchymal injection only. In our study, by evaluating the radioactivity of the positive node counts relative to that of the benign higher uptake node, the 10% rule was effective in identifying all but one node, which was grossly positive and adjacent to a node emitting 16% of the highest uptake node (Table 3). These collective findings highlight our incomplete understanding of the physiology of the metastatic process from the breast to the axilla and suggest that the spread of malignant cells is more complex than simple migration along lymphatics, a phenomenon that should be mimicked by the radiocolloid. The question remains: is this a technical/methodological difference or a true reflection of the physiology of the lymphatic system?

A recent article by Tanis et al.[9] elegantly reviews the physiology of lymph vessels and lymph nodes. Absorption of particles from the interstitium occurs via gap junctions in the lymphatic capillary membrane varying in size from 10 to 25 nm. The ability of larger particles to migrate is less clear. This may explain the variance in results when isotopes of varying sizes are used. Colloid particle size ranges from 3 nm for technetium antimony trisulfide up to 1000 nm for technetium sulfur colloid thiosulfate and even larger for human albumin.[10] The choice of radioisotope, therefore, may play an important role, not only in successful localization, but also in the timing and method of injection.[11] The flow of lymph is determined by pressure gradient as well as by active peristalsis, a point that can be exploited by intermittent external pressure to the breast after injection.[12] It follows that the volume of injectate and the force with which it is administered may influence the volume and rate of radioisotope migration.

Once the tracer enters the lymphatic channels, its path to the lymph nodes becomes paramount in our ability to localize the node most likely to sequester metastatic cells. The idea that there is a direct route from the breast to a complex network of lymph nodes via a solitary SN does not hold up when radioisotopes are used for identification. The localization of multiple nodes suggests that the drainage pattern is not serial or to a single SN; in fact, the average number of nodes identified has been reported[1] as being between 1.4 and 2.9; similarly, in our series, there were an average of 3. The finding of metastatic disease in nodes that do not harbor the highest amount of radioisotope could suggest an alternative drainage pattern. It is possible that multiple lymphatic channels and nodes from the breast parenchyma may merge and subsequently drain into one dominant node. This node would sequester the majority of radioisotope

TABLE 3. *Relative radioactivity levels for "non-hottest" sentinel nodes (SNs)*

Operation on tumor	Tumor size (cm)	Counts SN			% Hottest
		1	2	3	
Segmental	3.2	13,255	3,029[a]		22%
Segmental	2.8	445	239[a]	240[a]	53%, 53%
Re-excision	1.0 previously	1,855	297[a]		16%
Segmental	2.0	254	50[a]		19%
Segmental	1.0	364	199[a]		55%
Segmental	0.7	4,816	1,893[a]		39%
Segmental	2.7	695	567[a]	293[a]	81%, 42%
Segmental	1.0	99	76[a]		77%

[a]Denotes node with metastatic disease on pathology.

and thereby emit the highest gamma count. The parallel lymph nodes that precede the dominant lymph node may harbor migrating tumor cells that are en route to the dominant node. These secondary lymph channels and nodes, therefore, must be evaluated to ensure that accurate staging is performed. Alternatively, there may simply be a parallel drainage system in which more than one SN is fed by multiple lymphatic channels draining the breast. In this series, we report a 15% rate of pathologic positivity when a different node emits higher gamma counts.

It is postulated that grossly positive nodes have blocked lymphatic channels that thereby prevent the passage of mapping agents. If the lymphatic channels are partially occluded by tumor load, is it possible that the migration of radiocolloid is hindered en route to the lymph node with tumor burden and preferentially channeled along a clearer lymphatic pathway to a pathologically negative node? Of the positive SNs in our series, only one patient had a node that appeared grossly positive on inspection, and it was adjacent to two SNs as defined by the 10% rule. The second node, which was positive for metastases, had counts that were 16% of the counts emitted from the highest count node that did not harbor metastases. Radioactive emissions were recorded from the grossly positive node and found to be 3% of the hottest. Albeit limited to a single patient, the results are supportive of a possible tumor burden effect. Ludwig[13] describes two pathways by which lymphatic afferent channels drain directly into the sinuses, as well as an alternate channel that drapes over the node surface but may or may not have draining branches. This configuration could hold significance in the face of tumor burden or varying pressure heads with injectate agents.

The injection technique itself may account for the variation in localization of the SN. The optimal method of injection remains a topic of controversy, with various groups reporting success in each.[2–7] The volume of injectate, the area of injection, the time to the operating room, or previous biopsy site may all affect migration of the colloid. The amount of volume and the rate of injection may influence the migration through the gap junctions in the lymphatic vessel wall. A greater pressure head or a greater volume may alter flow patterns down channels that under normal circumstances would remain unused. Although other groups have reported success with blue dye, our localization rate of 93% with isotope alone is comparable to that of series that used both methods or blue dye only. Our use of blue dye was limited to early experiences and later used variably according to surgeon preference. Patients where blue dye was used were excluded from this series because the focus was isotope localization only. A similar finding was reported by Derossis et al.,[14] who concluded that as their experience with radioisotope increased, the benefit from added use of blue dye was marginal. Our series used intraparenchymal injection of radioisotope, which may have influenced our findings. The recent article from Memorial Sloan-Kettering Cancer Center, by Martin et al.,[8] of 2285 SNBs used both intraparenchymal and intradermal injection. However, analysis did not include these as separate categories.

In addition to the variable dynamics of lymph flow, the factors leading to the implantation and growth of a clone of malignant cells in a lymph node are poorly understood. Although it seems intuitively reasonable that the likelihood of developing a nodal metastasis is directly proportional to the number of malignant cells carried into its sinuses by lymph flow, this explanation may be entirely too simplistic. Even assuming that radiocolloid exactly mimics flow dynamics of tumor cells, it does not necessarily follow that the appropriate "seed and soil" also exist in the high-flow node—at least in 15% of cases.

The physiology of tumor cell migration in breast cancer as highlighted by SNB remains elusive. The question of whether radioisotope localization techniques truly mimic metastatic cells, as opposed to merely mapping a potential route, also remains incompletely understood. The challenge, therefore, is to develop a localization technique that consistently provides acceptable results in the face of biologic and technical variation. As the optimal technique to identify the clinically relevant SN continues to evolve, we support removing any node with a count of 10% or greater than the highest uptake node. Failure to do so would be associated with a false-negative rate of 15%.

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