The sensitivity and specificity of sentinel lymph node biopsy for breast cancer at Baylor University Medical Center at Dallas: a retrospective review of 488 cases

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Sentinel lymph node (SLN) biopsy has become the standard of care for breast carcinoma management, as it precludes the negative morbid effects—including decreased shoulder range of motion, lymphedema, and paresthesias—of unnecessary axillary lymph node dissection. However, the method of pathologic evaluation of the lymph node has been scrutinized to obtain the greatest sensitivity, specificity, and negative predictive value, ultimately for the benefit of the patient. This retrospective study analyzed 488 biopsies completed by two surgeons and read by multiple pathologists affiliated with Pathologists Biomedical Laboratories. When metastatic disease was not grossly obvious, analysis of the SLN began with touch imprint cytology and, if necessary, a frozen section analysis. On the subsequent day, three levels of the SLN were analyzed with hematoxylin and eosin stain and immunohistochemistry with cytokeratin AE1-3 and the appropriate control. Touch imprint cytology and/or frozen section analysis (where applicable) correctly identified 78 of 89 macrometastases, with a sensitivity of 88%, specificity of 100%, and negative predictive value of 97%. Sensitivity was 72% for micrometastases and 60% for isolated tumor cells, each with 100% specificity. In conclusion, the sensitivity and specificity of SLN biopsy at our institution compares with the higher end of percentages reported in the literature.

The sentinel node is defined as the first lymph node to drain the area under investigation. Sentinel lymph node (SLN) biopsy was performed originally in the setting of penile carcinoma and melanoma and is now the standard of care in the management of melanoma (1). SLN biopsy has since been applied to the management of several malignancies, including breast carcinoma, where it has had a profound impact on decreasing postoperative morbidity. Before the adoption of SLN biopsy in breast carcinoma, widespread performance of axillary dissection carried the risk of decreased shoulder range of motion and lymphedema, with a significant impact on patients' quality of life (2–16).

Histopathologic evaluation of the SLN can be performed using touch imprint (TI) cytology, frozen section (FS) analysis, or both. The size of the SLN influences how it is sampled, with nodes measuring <0.5 cm bivalved at the hilum and their cut surfaces touched. Lymph nodes >0.5 cm are sectioned at 1- to 2-mm sections, and the cut surfaces of all sections are touched (1). Ideally a metastasis is determined at the time of primary

surgery, to avoid all of the risks, cost, and inconveniences of a second surgery (17). TI cytology has been compared head to head in numerous studies against FS, and no statistically significant difference has been found between the two approaches (3, 5, 12, 18). Hence, TI cytology is the most common method of analysis of the SLN due to its rapid results, absence of convoluting artifact imposed by FS for permanent section and immunohistochemistry postoperative analysis, and decreased cost (3, 5, 12, 18).

This study compares the performance of histopathologic evaluation of axillary SLN biopsy in breast carcinoma at Baylor University Medical Center at Dallas to that of other pathology practices.

METHODS

A total of 488 consecutive SLN biopsies performed by two surgeons at various surgical centers from January 1, 2005, through April 1, 2009, were retrospectively reviewed to determine the method of lymph node analysis (i.e., TI or FS); cases of macrometastasis, micrometastasis, and isolated tumor cells (ITCs) (*Figure*); and any false-negative or false-positive results. Among the study population, 2 patients were in their 20s, 10 in their 30s, 73 in their 40s, 157 in their 50s, 140 in their 60s, 72 in their 70s, 33 in their 80s, and 1 in their 90s.

TI cytology was performed using the standard 0.5-cm cut-off for lymph node sectioning. Once touched, the slide was fixed in ethanol and then stained with hematoxylin and eosin. Cases that had suspicious cells but were not definitive were subsequently frozen and stained, also using hematoxylin and eosin. Standard definitions were used for micrometastasis (<2 mm) and isolated tumor cells (individual tumor cells or small clusters, <0.2 mm).

Analysis of the permanent processing included reassessment of the TI cytology and FS analysis (where applicable) and evaluation of three step sections of the lymph node by hematoxylin

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Figure. (a) Macrometastasis by hematoxylin and eosin (H&E) stain at 40× and (b) at 200×; (c) micrometastasis by H&E at 40× and (d) at 200×; and (e) isolated tumor cells at 200× by H&E, (f) at 400× by H&E, and (g) at 100× by immunohistochemistry.

and eosin stain, immunohistochemical staining with a positive and negative control, and cytokeratin AE1-3 (pan-cytokeratin) on the Ventana iView system (Tucson, AZ).

RESULTS

From the 488 cases, 922 total lymph nodes were assessed by TI cytology, with an average of 1.89 lymph nodes per case. Of these, 179 lymph nodes were also submitted for FS analysis, a rate of 19.4%.

The *Table* summarizes the results. Considering only macrometastases, the sensitivity of SLN biopsy was 88%, the specificity 100%, the positive predictive value 100%, and the negative predictive value 97%. Eleven macrometastases were missed intraoperatively, a rate of 1.2%.

Table. Sensitivity, specificity, and false-negative results for 488 cases of sentinel lymph node biopsy for breast cancer at Baylor University Medical Center at Dallas

Variable	Statistic	Percentage
Macrometastasis	Sensitivity	88
	Specificity	100
	Rate of frozen section	19
	Positive predictive value	100
	Negative predictive value	97
	Rate of missed macrometastasis	1
Micrometastasis	Sensitivity	72
	Specificity	100
	Negative predictive value	97
Isolated tumor cells	Sensitivity	60
	Specificity	100
	Negative predictive value	95
Rate of change of diagnosis postoperatively		7

*N = 65: 13 (1.4% of total) involving micrometastases and 52 (5.6% of total) involving isolated tumor cells.

For ITCs or micrometastases, 65 intraoperative negative diagnoses were changed at the time of permanent section. This change occurred at an overall rate of 7.1%, with 13 consisting of micrometastasis (1.4%) and 52 consisting of ITC (5.6%). The sensitivity for detecting micrometastasis was 72.2% with 100% specificity, and the negative predictive value was 96.9%. ITC sensitivity was 60%, with 100% specificity and a 94.7% negative predictive value.

DISCUSSION

SLN biopsy continues to be an area of extensive study in the management of breast carcinoma (2–10, 12, 13, 19). Studies have considered aspects of sensitivity and specificity with respect to breast carcinoma subtype, tumor size, metastasis size, method of morphologic evaluation, method of sectioning lymph nodes, recurrence rate, rapid use of cytokeratin staining, and the utility of intraoperative molecular-based assays. Universally, SLN biopsy has provided decreased morbidity for the patient (14–16). However, recurrent disease still occurs, perpetuating research to discover the best evaluative approach in the management of the disease.

Many studies have considered the sensitivity and specificity of SLN biopsy, and a metaanalysis of 31 studies conducted by Tew et al (12) described a sensitivity range of 44% to 100% (with most studies within 50% to 70%) and a specificity of 100%, including all analytical techniques. Repeatedly, the sensitivity and specificity of TI cytology have paralleled those of FS analysis, without a statistically significant difference. Sensitivity for macrometastasis ranged from 70% to 98% with a pooled 81% average, and sensitivity for micrometastasis ranged from 5% to 57%, with a pooled 22% average. These findings coincide with those of additional relevant studies that were reviewed (4, 8, 20, 21).

We report an 88% sensitivity for macrometastasis and a 72% sensitivity for micrometastasis. Variables reported to influence the spectrum of results include the experience of the reviewing pathologist, skill of the individual submitting tissue for intraoperative evaluation, sampling error (e.g., when no tumor

cells were seen in a retrospective review of the intraoperative TI cytology, but tumor cells were present in the permanent section), and median tumor size (4, 12, 20). Specifically, as expected, sensitivity for SLN biopsy increased as tumor size increased and decreased as the proportion of micrometastasis increased (12). Other studies have shown that the size of the primary tumor and the size of the SLN metastasis were predictors of nonsentinel node metastasis (12).

Cserni and colleagues considered the number of levels examined with respect to sensitivity (1). In their study, a mean of 49 levels were examined, and it was determined that one or two sections from the central component of the node (the hilar region) would have missed 6 of 21 cases (29%). Further, despite this extensive method of sectioning, micrometastasis was detected in two lymph nodes only by immunohistochemistry. This study found that three levels should be investigated along with immunohistochemistry but that, in the event of a negative study, further levels may be necessary if clinically warranted (e.g., large tumor size/T2 or greater, unfavorable histology) (1). Another study by Turner et al (22) suggested immunohistochemistry at two levels to assist in the detection of micrometastasis and two levels with hematoxylin and eosin to increase sensitivity, control cost, and improve efficiency. The method suggested by Cserni is followed at Baylor University Medical Center at Dallas.

A change in diagnosis postoperatively occurs 9% to 30% of the time, according to a study by Sahin, Guray, and Hunt from M. D. Anderson Cancer Center (11). As reported above, at Baylor University Medical Center at Dallas, a postoperative change in diagnosis occurred less frequently: 7% of the time overall, with 1.4% occurring with micrometastases and 5.6% with ITC. This also coincides with the greater rate of detection with increasing size of metastasis.

Management of micrometastatic disease, frequently in comparison with ITC, is another component of breast carcinoma addressed by much research. In a study by Reed et al (23), which included 1259 patients, with 57 patients with micrometastasis and 25 patients with ITC, no additional positive nodes were identified in 13 patients who were diagnosed with ITC and subsequently had an axillary lymph node dissection (ALND). However, 11 of 41 patients (27%) with micrometastasis had additional positive nodes. Demographically, a greater percentage of patients with micrometastases are 50 years old or younger, with lymphatic or vascular invasion. Similarly, in Reed et al's study (23), 6 of the 8 (75%) patients with micrometastasis and distant recurrence were 50 years old or younger, and most (7 out of 8) patients with micrometastatic disease had only one focus at the time of initial surgery. However, 7 of the patients with recurrent micrometastasis underwent ALND, with recurrent disease in 5 of 7 (71%) cases. Most of the patients with micrometastatic disease received chemotherapy ($n = 45$, 79%), radiation therapy ($n = 39$, 68%), or both ($n = 31$, 53%). Of these patients, 10% who received both modalities or radiation alone had recurrence with distant metastases, as opposed to 22% of patients who received no treatment after a mean follow-up of 4.9 years. However, these differences were not statistically

significant. Two patients with ITC had recurrences, both with poorly differentiated, hormone-receptor–negative tumors, after having previously received chemotherapy. Further, both of the ITC patients with recurrence had ALND without additional positive nodes identified. Ultimately, this study determined that nodal micrometastasis is associated with more positive nonsentinel nodes and a worse prognosis.

Another study by de Boer et al (24) involved 2707 patients: 856 with node-negative disease and no adjuvant therapy, 856 with ITC or micrometastasis and no adjuvant therapy, and 995 with ITC or micrometastasis with adjuvant therapy. The median follow-up in this study was 5.1 years. For both patients with ITC and patients with micrometastasis who did not receive adjuvant therapy, the unadjusted 5-year disease-free survival rate was significantly reduced when compared with node-positive, adjuvant therapy patients (76.5% vs 86.2%, $P < 0.001$ for the groups combined; 77.2% vs 83.0%, $P < 0.04$ for the ITC group alone; 75.9% vs 87.9%, $P < 0.001$ for the micrometastasis group alone). Ultimately, a reduced risk of events was noted in the node-positive, adjuvant-therapy cohort when compared with the node-positive, no-adjuvant-therapy cohort (hazard ratio 0.57; 95% CI 0.45–0.73). As cited in other studies, other factors influencing the risk of recurrence included tumor size and tumor grade. This was one of a few studies that found that ITC influenced overall disease-free survival. Further, de Boer et al proposed that the size of tumor deposit may not have an influence on 5-year disease-free survival (24). Van Deurzen et al (25) reported second echelon lymph node metastasis in 13%, 20%, and 48% of cases for ITC, micrometastasis, and macrometastasis, respectively. The cases of ITCs in sentinel lymph nodes and second echelon metastasis involved one lymph node. The issue of the management of ITC versus micrometastasis lacks definitive resolution at present.

The differences in detecting metastatic invasive lobular versus ductal carcinoma have also been considered and debated. While consistent agreement on the issue does not exist, three important features distinguishing metastatic lobular carcinoma are agreed upon: 1) lobular carcinoma cases have a higher rate of conversion from node-negative to node-positive disease with immunohistochemistry, 2) lobular carcinoma has a higher percentage of immunohistochemistry-detected disease, and 3) lobular carcinoma has a greater proportion of single-cell metastases. Horvath et al (26) and Creager et al (5) reported no difference in sensitivity and specificity in detecting metastatic or micrometastatic lobular or ductal breast carcinoma. The study by Horvath et al considered FS analysis, and the study by Creager et al considered TI cytology. The sensitivity for TI cytology reported by Creager et al was 82%, well below the sensitivity determined in the retrospective analysis at our institution. Moreover, the same issues arise with ITCs and micrometastases as exist with ductal carcinoma.

The future direction of the management of ITC and micrometastasis lies in methods to determine this limited, though potentially significant, disease in real time. Methods investigated thus far have included intraoperative cytokeratin staining by immunohistochemistry and molecular-based assays.

A study performed by Krishnamurthy et al at M. D. Anderson in Houston, Texas, investigated the intraoperative processing of SLNs using a rapid pan-cytokeratin immunohistochemical stain (18). The procedure required an average of 25 minutes (range 8–25 minutes), not including time to retrieve and prepare the specimen. Patients included in the study had T1 to T3 tumors; those with stage IV disease were excluded. In the study, the lymph nodes were sectioned according to previously mentioned standards, with a TI cytology prepared and two sections—one FS and one slide prepared with the rapid cytokeratin analysis. Overall, of the 297 lymph nodes studied ($n = 100$), intraoperative rapid cytokeratin detected 100% of macrometastases and 50% of micrometastases, with an overall sensitivity of 80% in detecting metastatic disease. Thus, overall, the sensitivity was similar for the detection of macrometastasis and slightly improved for detecting micrometastasis, which coincided with previous studies on the topic. One case of micrometastasis was detected by rapid cytokeratin that was not detected with FS. Again, as determined previously with other TI and FS studies, this one difference could be attributed to sampling error. The advantages for rapid cytokeratin immunohistochemistry reported by Krishnamurthy et al included better detection of micrometastasis, as well as the ability to accurately measure the size of the metastasis to distinguish it from ITC. Certainly, this could be helpful if the ultimate decision is that ITCs do not present a decreased 5-year disease-free survival rate. However, the management of the finding of ITC is still debated in the literature. Further, the improvement is not statistically significant, and the practical aspects of incorporating this into the model of the intraoperative workup of SLN biopsies should be considered on an institutional basis. As of yet, this is not a widely practiced approach, and the literature on the topic has been experimental and comparative in nature.

Another area of future promise for the intraoperative analysis of SLN biopsies involves the use of molecular-based assays. To date, no effective assays have been developed. In fact, in testing solid tumors of any sort, a reasonable amount of tumor (usually no less than 20% of nucleated cells in a sample) must be present for detection. However, for hematolymphoid malignancies, assay design has allowed detection of much lower tumor burden in a sample (sometimes as low as one or two detectable cells). These methods are impractical at present for implementation in the intraoperative setting due to cost and time constraints. Of course, with the increasing sensitivity seen with these assays, some specificity is lost. Further, the hematolymphoid malignancies of this nature are usually directed at detecting minimal residual disease, which implies a known mutation upon initiation of the study. While these same principles can be applied to solid tumors, including breast cancer, this paradigm has yet to be established. Further, it is understood that while a molecular genetic event may be detected, due to the natural biology of cancer, new clones are constantly emerging and a unique clone can prevail.

In a study by Blumencranz et al (27), a molecular-based assay for detection of axillary lymph node metastasis found encouraging results for macrometastases. The main limitation of this study, as with any study considering molecular analysis of axillary lymph node status, is sampling, particularly with metastases of decreasing size. Another study by Martin-Martinez et al (9) compared the GeneSearch BLN assay to intraoperative histologic evaluation. In this study, results similar to the gold standard histologic studies were reported. Again, however, this was limited to macrometastases. Moreover, this approach requires additional staff trained in setting up such assays, which is not economically practical in all facilities. A similar study by Tsujimoto et al (28) also found sensitivity in detecting macrometastasis similar to histologic evaluation. Unlike the previous two studies mentioned, however, this study also reported potential for detection of micrometastatic disease, but the sensitivity was not provided.

Each of the above-mentioned studies considered the use of polymerase chain reaction amplification of extracted DNA with primers for cytokeratin (9, 27, 28). Veridex is the only company currently approved by the Food and Drug Administration for evaluation of the sensitivity and specificity of detection of circulating tumor cells utilizing molecular-based assays on patients. This company reports that a positive finding in the setting of breast cancer is five circulating tumor cells (29). While Veridex does not purport to resolve the ITC/micrometastasis issue, the company offers an alternative method to determine prognosis without the morbidity of ALND. Veridex has yet to be validated in clinical trials to determine relevance, but studies are in progress.

Another alternative method gaining interest in molecular diagnostics is next-generation sequencing. With this highly parallel, high-throughput evaluative method, fewer numbers of aberrant cells are required for detection. However, as a new technology, this method still requires significant improvements in the balance between speed and accuracy before it reaches clinical practice.

CONCLUSION

Since axillary lymph node status is the single most important prognostic indicator in breast carcinoma, evaluation of the sensitivity and specificity of the techniques utilized to establish this information is critical. Currently, the gold standard for intraoperative analysis is TI cytology or FS analysis. The variability in the sensitivity and specificity in these methodologies indicates that a more sensitive method is needed. Further, the management of micrometastasis and ITCs and their impact on long-term survival need to be more clearly delineated. From a pathological perspective, remaining limitations in evaluation include histopathologic guidelines to classify tumors present in the subcapsular sinus, capsule, or perinodal soft tissue. Measurement of tumor cells/clusters with a dispersed pattern in nodal sections is the other issue that remains unresolved. The pathologists at Baylor University Medical Center at Dallas perform above the national average in the intraoperative diagnosis of axillary lymph node status.

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