The American Journal of Surgery 194 (2007) 426–432
Scientific Impact Recognition Award

Sentinel node staging for breast cancer: intraoperative molecular pathology overcomes conventional histologic sampling errors

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Manuscript received May 2, 2007; revised manuscript July 3, 2007

Presented at the 8th Annual Meeting of the American Society of Breast Surgeons, Phoenix, AZ, May 2–6, 2007

Abstract

Background: When sentinel node dissection reveals breast cancer metastasis, completion axillary lymph node dissection is ideally performed during the same operation. Intraoperative histologic techniques have low and variable sensitivity. A new intraoperative molecular assay (GeneSearch BLN Assay; Veridex, LLC, Warren, NJ) was evaluated to determine its efficiency in identifying significant sentinel lymph node metastases (>.2 mm).
Methods: Positive or negative BLN Assay results generated from fresh 2-mm node slabs were compared with results from conventional histologic evaluation of adjacent fixed tissue slabs.
Results: In a prospective study of 416 patients at 11 clinical sites, the assay detected 98% of metastases >2 mm and 88% of metastasis greater >.2 mm, results superior to frozen section. Micrometastases were less frequently detected (57%) and assay positive results in nodes found negative by histology were rare (4%).
Conclusions: The BLN Assay is properly calibrated for use as a stand alone intraoperative molecular test.

Keywords: Breast cancer; Molecular; Gene; Diagnostic; Axillary

For breast cancer patients, the status of the axillary sentinel lymph node(s) (SLN) provides important staging information and usually determines whether formal axillary lymph node dissection will be performed. If axillary node dissection is needed, it is ideally performed immediately after SLN dissection during the same operation. Current, widely practiced, intraoperative methods for detecting SLN metastases include frozen section and touch preparation (imprint cytology). Both of these approaches have limited sensitivity with wide performance variability compared with postoperative permanent section histology. When compared with final permanent pathology results, the reported sensitivity of frozen section SLN analysis varies from 58% to 87% [1]. Touch preparation has similar limitations in sensitivity [2].

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0002-9610/07/$ – see front matter
doi:10.1016/j.amjsurg.2007.07.008

A positive intraoperative SLN finding spares the patient a later return to surgery for completion axillary dissection. However, substantial false-negative findings with current histologic intraoperative methods cause patients and their families significant distress when they are told later that the test was incorrect. Not only must the patient and her family go through a second operation, but they also have to cope with the news that the prognosis is worse and the required treatment more extensive than they had believed. In addition, the second surgery after a false-negative frozen section incurs additional costs and potential additional anesthetic and operative morbidity.

Accurate intraoperative molecular analysis of all or part of the SLN offers the potential to significantly reduce false-negative findings that necessarily occur with the limited tissue sampling of conventional histologic methods [3–7]. Although a molecular assay can overcome the errors resulting from limited tissue sampling associated with traditional histologic evaluation, several new challenges arise. First, unless calibrated appropriately, molecular assays may detect clinically insignificant amounts of metastatic cellular material. Presently, nodal breast cancer metastatic deposits $<$.2 mm are classified as node negative (N-0) [8], with minimal or unknown clinical importance. A molecular breast SLN assay must be calibrated to routinely indicate a positive result only with quantities of disease $\geq$.2 mm to spare patients from axillary dissections or adjuvant chemotherapy, which would be considered unwarranted by current standards. Second, the molecular assay must be validated in an independent dataset to confirm proper calibration.

The validation of a molecular SLN assay with accuracy superior to conventional sampling-based histology presents an even more challenging problem. Because of the likelihood that an accurate molecular assay will be more thorough than conventional histology, the assay will likely detect disease missed by the reference histologic test to which it is compared. Traditional test validation methodology would misleadingly term such a finding "false-positive." Therefore, a modified approach is needed.

We evaluated a novel, intraoperative reverse transcriptase-polymerase chain reaction assay for SLN breast cancer metastasis (GeneSearch BLN Assay; Veridex, LLC, Warren, NJ) in a prospective, multisite trial. The molecular test was calibrated in vitro so that positive findings were only associated with significant levels of the targeted messenger RNA transcripts [9]. We have presented a comprehensive, classic sensitivity and specificity evaluation of this new test elsewhere [10]. In the current analysis, we hypothesized that agreement between the molecular test on one part of the SLN and conventional pathology from another part (alternating slices) would be greatest when metastatic involvement was most extensive, as would be expected with histologic evaluation of alternating tissue sections of the same node. We also examined assay performance related to breast tumor type and stage and compared assay performance with the performance of current intraoperative tests. In particular, we investigated if the assay could be particularly beneficial for difficult-to-detect metastases, such as those seen in stage I cancer or lobular cancer. Lobular metastases are particularly challenging for conventional, sampling-based histologic methods because they are often distributed as diminutive clusters or single cells. There is growing consensus that such metastatic deposits should be counted as node positive, even when none is $>$.2 mm, when a substantial amount of the node is so involved [11].

Methods

The calibration and validation of the molecular BLN Assay required 2 separate trials: (1) a beta (cutoff) trial of 304 patients to establish a threshold between insignificant and significant levels (corresponding with histologic metastatic deposits $>$.2 mm) of the markers mammaglobin and cytokeratin 19 (CK19) and (2) a pivotal (validation) trial of 416 patients for independent performance verification compared with permanent-section hematoxylin and eosin and immunohistochemical evaluation. Both trials were completed between July 2004 and December 2005 (Pre-IDE I040002). Patients at least 18 years of age with a diagnosis of invasive adenocarcinoma of the breast and scheduled for a SLN dissection were eligible for the calibration or validation study. Patients were excluded if they were participating in other research studies that would prevent their full study participation, if they had a prior axillary surgery on the same body side as the scheduled SLN dissection, if proper informed consent signature was not obtained, or if they did not meet inclusion criteria listed earlier. All patients in the final data analysis had written informed consent, and the study protocols were reviewed and approved by the appropriate ethics review board at each site.

Twelve clinical sites in the United States were selected based on clinical trial performance history and previous research experience with SLN studies through the American College of Surgeon's Oncology Group or The National Surgical Adjuvant Bowel and Breast Project. Site technicians were trained and qualified with the BLN Assay technology.

Node processing and comparison of BLN Assay findings with conventional histology

SLNs were identified intraoperatively and dissected according to the standard procedures of each site. Nodes were transported to the pathology testing area within 15 minutes. All nodes designated as sentinel nodes (including "grossly positive" nodes) were analyzed, except at the site pathologist's discretion (ie, node too small for adequate histological assessment for patient care if shared for molecular assay) or because of protocol deviation. Each node was sectioned along the short axis into an even number of slabs (1.5–3.0 mm thick). Alternate slabs were prepared for histologic evaluation or the BLN Assay. Histologic evaluation of each lymph node complied with or exceeded current College of

Table 1
Summary of BLN Assay results and OHR agreement

Assay−/ OHR−	Assay+/ OHR+	Assay+/ OHR−	Assay−/ OHR+	N	% Agreement
278 (67%)	106 (25%)	17 (4%)	15 (4%)	416	92.3

For OHR, + is metastases $>$.2 mm and − is metastases $\leq$.2 or no detectable metastases.