

Longer Life Foundation Final Report
Project Title: Minimally Invasive Staging of the Axilla in Breast Cancer
Investigator: Julie A. Margenthaler, M.D.

Abstract

Sentinel lymph node biopsy (SLNB) has emerged as a less invasive alternative to axillary lymph node dissection (ALND) in the treatment of breast cancer. However, SLNB has a number of limitations, and we believe that alternative strategies for staging of the axilla should be explored. The hypothesis of this proposal was that the combination of preoperative high-resolution axillary ultrasound (AUS), fine needle aspiration biopsy (FNAB), and molecular analysis using real-time reverse transcription-polymerase chain reaction (RT-PCR) represents a viable, minimally invasive alternative to SLNB.

A prospective cohort study was used to assess the diagnostic accuracy of molecular analysis of AUS-FNAB specimens. Eighty female patients with pathologically confirmed, clinically node-negative invasive breast cancer who were considered to be candidates for SLNB were eligible for enrollment. The primary endpoint of this study was to determine the feasibility of AUS-FNAB and real-time RT-PCR to predict the pathologic status of the axilla in a proof-of-principle study.

Lay Summary

The most important prognostic factor for a patient with breast cancer is the absence or presence of metastasis to the axillary lymph nodes. Survival correlates directly with the number of positive lymph nodes. Historically, all women with breast cancer underwent surgery to their breast (mastectomy or lumpectomy) plus removal of all of the axillary (i.e., armpit) lymph nodes on the side of their breast cancer. While this technique is very effective in determining the number of lymph nodes involved with cancer, it also subjects the patient to significant morbidity, including bleeding, infection, nerve injury, and permanent swelling of the arm (lymphedema).

Currently, this procedure is still performed in women who have enlarged, suspicious lymph nodes by physical exam (approximately 10-20% of all patients) and the complication rates are reported to be 10-30%. More recently, a less invasive technology has emerged to examine the axillary lymph nodes in women who do not have enlarged lymph nodes on physical exam (approximately 80-90% of all patients). This is termed a sentinel lymph node biopsy and has been rigorously tested and proved effective in patients with breast cancer.

The sentinel lymph node concept supports the notion that breast cancer cells spread in an orderly fashion from the primary tumor in the breast to a few lymph nodes in the axilla (i.e., the “sentinel” lymph nodes). By injecting blue dye and radioactive particles near the tumor, we can follow the lymph vessels to the

sentinel lymph nodes and only these lymph nodes are removed. On average, one to three sentinel lymph nodes are removed.

There are several disadvantages to these techniques. Both procedures are invasive and subject the patient to additional surgical morbidity. Although the sentinel lymph node procedure results in fewer complications, the rate of bleeding, infection, nerve injury, or lymphedema remains at 5-10%. For patients undergoing sentinel lymph node biopsy, about 30% will have positive nodes on the final pathology and will require a second procedure to remove the remainder of the lymph nodes. Finally, the current methods of identifying and examining the lymph nodes results in false results approximately 5-15% of the time.

The goal of the current project was to study an advanced method of detecting metastasis in the axillary lymph nodes of breast cancer patients using molecular genetic testing called polymerase chain reaction or PCR. PCR has been shown to be superior to our current techniques and can pick up one tumor cell in the background of a million normal cells. Using ultrasound, we can identify the lymph node and obtain a small amount of tissue using a needle placed through the skin. This tissue is tested for an array of genes specific to breast cancer and allows us to construct a “fingerprint” of the cancer, providing the exact nature of the cancer and the way that it is likely to respond to various treatments. This procedure could be performed on all patients with breast cancer in lieu of removing the lymph nodes during surgery; only those patients with a positive genetic marker would need to have their lymph nodes removed. Therefore, 70% of patients would avoid surgery on their lymph nodes. In patients who have a positive lymph node, we can tailor their surgery and treatment to their specific cancer. The results of the current study demonstrate that this technique is feasible and reliable in predicting the status of the axillary lymph nodes in patients with breast cancer. This technology is very promising and represents a significant potential advancement in the care of patients with breast cancer.

Background and Significance

The primary goal of breast cancer staging is to classify patients by the extent of disease into groups with similar outcomes. The presence of metastatic disease in the axillary lymph nodes (ALN) is considered the single most important prognostic factor for patients with breast cancer, whereby patients have a poorer prognosis with increasing numbers of metastatic lymph nodes (1). As a result, surgical management and staging of breast cancer has traditionally included an ALND. SLNB has recently emerged as an accurate, less invasive alternative to ALND, and it has rapidly become the standard of care in patients with clinically node-negative breast cancer (2, 3).

However, there are a number of limitations to SLNB. (a) Although the sentinel lymph node hypothesis is elegant (lymphatic mapping with radiolabeled sulfur colloid and/or lymphazurin blue dye is used to identify the lymph node that is

most likely to contain metastatic breast cancer, thereby accurately predicting the pathologic status of the axilla), there are practical limitations. Published SLNB identification rates are between 90-95%, and the sensitivity of SLNB is between 88-95% in experienced hands (2-4). This results in persistent axillary disease in about 5-10% of breast cancer patients, representing the false negative rate.

(b) Often, SLNB is performed as a staged procedure, requiring that breast cancer patients undergo two or more operations for definitive staging and treatment of the axilla. Such patients include those who have node-positive disease by SLNB and require completion ALND, those who require axillary staging prior to breast reconstruction, and those undergoing neoadjuvant chemotherapy. These clinical scenarios represent up to 40-50% of patients treated for breast cancer.

(c) Although SLNB is clearly less invasive than ALND, SLNB is not without morbidity. A recent randomized prospective trial of SLNB versus ALND confirms that complications of SLNB include seroma formation, lymphedema, sensory nerve injury, and limitation in range of motion (5).

(d) Finally, and perhaps most importantly, the relevance of SLNB is becoming increasingly less clear. In terms of staging, the importance of axillary staging is becoming less important as increasingly tumor size and biology (histologic grade, receptor status, and genetic profile) are driving the decision making for systemic therapy. The current recommendation is that all patients with T1c breast cancer (primary tumor > 1.0 cm) be considered for systemic therapy. In terms of therapy, there is no Level I evidence that axillary node dissection improves survival in breast cancer patients. Many surgeons now believe that ALND is not therapeutic. In fact, the American College of Surgeons Oncology Group recently closed to accrual a Phase III trial of patients with clinically negative axillae and positive SLNB randomized to completion axillary dissection versus no additional treatment (6).

Implicit in the design of this trial is the assumption that axillary dissection may have no therapeutic benefit, a concept that is strongly supported by the 25-year follow-up results of the NSABP B-04 study, which continue to demonstrate no survival advantage for patients who underwent axillary dissection versus axillary radiation (7). These limitations of SLNB strongly suggest that alternative strategies to stage the axilla should be explored.

We believe that the combination of preoperative high-resolution axillary ultrasound (AUS), fine needle aspiration biopsy (FNAB), and molecular analysis using real-time reverse transcription-polymerase chain reaction (RT-PCR) represents a viable, minimally invasive alternative to SLNB in breast cancer patients. There are now several reports in the literature that suggest that AUS is a potentially valuable technique for identifying axillary metastases (8-10). AUS permits the visualization of lymph node size, shape, contour, and changes in cortical morphology and texture that appear to be associated with the presence

of axillary metastases. Further, there are now emerging technologies that suggest that the SLN can be accurately identified by sonography (11-14). However, sonographic signs of metastatic disease sometimes overlap with those of benign reactive changes limiting the ability of this modality alone to accurately stage the axilla. We and others have begun to routinely perform FNAB of sonographically suspicious, indeterminate, or metastatic-appearing axillary lymph nodes.

One major limitation of this strategy, however, is that cytopathologic analysis has limited sensitivity and is highly dependent on the availability of a dedicated, skilled cytopathologist. The requirement for a skilled cytopathologist suggests that while AUS-FNAB may enjoy some success in academic medical centers, the universal application of this technology will be dependent on the development of more robust techniques for evaluation of cellular aspirates from ALN. Therefore, we propose molecular analysis with real-time RT-PCR as an alternative to cytopathology. Real-time RT-PCR is a robust and exquisitely quantitative technology that has been successfully used for the detection of micrometastatic breast cancer. We have considerable experience with this technology, and we have defined a very informative marker panel for the molecular detection of micrometastatic breast cancer in sentinel and axillary lymph nodes (15-18). This technology has been successfully applied in a closely related field – non-small cell lung cancer staging with endoscopic ultrasound and fine-needle aspiration (19, 20). We believe that the molecular analysis of AUS-FNAB specimens may ultimately replace SLNB as a single-stage, minimally invasive technique of axillary staging in patients with invasive breast cancer.

Methods

Study Design (Figure 1): A prospective cohort study was used to assess the combination of preoperative high-resolution AUS, FNAB, and molecular analysis using real-time RT-PCR as a minimally invasive alternative to SLNB in breast cancer patients. Eighty female patients with pathologically confirmed, clinically node-negative invasive breast cancer who are considered to be candidates for SLNB were enrolled. All patients underwent AUS by a dedicated breast radiologist in the Breast Health Center; this represents the current standard of care at our institution.

Patients were divided and enrolled in the study based on the AUS characteristics of their ALNs (n=40, positive; n=40, negative). Patients who were found to have sonographically suspicious or metastatic-appearing axillary lymph nodes underwent FNAB. FNAB specimens were sent to cytopathology and preserved for RT-PCR analysis. Patients who had positive cytopathology underwent standard ALND and those with negative cytopathology underwent SLNB using standard blue dye and radiocolloid techniques. Patients who were found to have sonographically negative axillas did not undergo AUS-FNAB; this group of

patients underwent SLNB using standard blue dye and radiocolloid techniques. A FNAB was performed on the back table on the sentinel node(s) and preserved for RT-PCR, as well as cytopathology. Aspirates were analyzed by RT-PCR with a marker panel that has been validated to detect micrometastatic breast cancer in the axilla (*mam*, *mamB*, *PIP*, *CK19*, *muc1*, *PDEF*, and *CEA*). ALND and SLNB specimens were analyzed by a pathologist in the usual fashion. The results of the final surgical pathology of the ALND or SLNB was compared to the cytopathology and RT-PCR analyses in order to define the feasibility and sensitivity of RT-PCR to predict metastatic breast cancer.

RNA isolation, cDNA synthesis, and real-time RT-PCR: Molecular analyses were performed as we have previously described (15-20). Total cellular RNA was isolated from ALN aspirates using a guanidinium thiocyanate-phenol-chloroform solution (RNA STAT-60™; TEL-TEST, Friendswood, TX). cDNA was made from 5 µg of total RNA using M-MLV reverse transcriptase (Promega, Madison, WI). The real-time RT-PCR analyses was performed on a PE Biosystems Gene Amp 5700 Sequence Detection System (Foster City, CA). All reaction components were purchased from PE Biosystems. Primers for the gene panel have been previously reported (15-20). Real-time RT-PCR data were quantified as C_t values. Results were normalized to an internal control reference gene (β2-microglobin). Threshold values for each individual marker were set at three standard deviations below the mean change in C_t value in control samples (n=51).

Statistics: The results of AUS-FNAB with cytopathology, histopathology, and molecular analysis were summarized as sensitivity, specificity, and overall accuracy.

Results

In patients with a "normal" AUS (n=39), 34 were node-negative on final pathology, while five were node-positive. All five patients with node-positive disease had a positive marker profile. Of 34 patients with node-negative disease, 30 (88%) had a negative marker profile, while four (12%) had a positive marker profile. In patients with "suspicious" AUS (n=34), 23 had positive cytopathology. Nineteen (83%) had a positive marker profile; four patients were not evaluable due to insufficient material. Eleven (17%) patients with "suspicious" AUS had negative cytopathology. Five were node-positive on final pathology and all had a positive marker profile. Six patients were node-negative on final pathology; three had a positive marker profile and three had a negative marker profile. Mean size of lymph node metastasis detected by the panel was 7.2 mm (range 3 mm – 1.7 cm). A positive marker profile was associated with traditional indicators of prognosis, such as histologic grade, estrogen and progesterone receptor and Her2neu status, and increasing tumor size (p<0.05 for each).

Discussion

This is the first report to demonstrate that real-time RT-PCR analysis of FNAB specimens is feasible in predicting the final lymph node status in patients with clinically node-negative breast cancer. Overexpression of breast-cancer associated genes correlates with traditional indicators of disease prognosis. The ability to accurately stage the axilla *in vivo* allows for further investigations. We are currently investigating the use of photoacoustic tomography (PAT) in the ability to visualize the SLNs *in vivo*.

PAT is based on the generation of photoacoustic waves by safely depositing short-pulsed optical energy into tissue. Each laser pulse causes a rapid temperature rise usually on the order of 10 millidegrees. The ultrasonic emission due to thermoelastic expansion is detected with an array of ultrasonic transducers and then used to reconstruct an image. The PAT technology is designed to overcome the poor spatial resolution of pure optical imaging yet to retain the high optical contrasts. In terms of spatial resolution, pure optical imaging suffers from strong optical scattering in tissue. By contrast, ultrasonic waves can propagate in tissue with relatively low scattering and can therefore provide good spatial resolution. Therefore, PAT integrates high optical contrast with high ultrasonic resolution in a single hybrid imaging modality.

Further studies will utilize the PAT imaging to identify SLNs for FNAB and RT-PCR analysis. The specific aims of the project are as follows:

1. Development of a laser light delivery system: Design and engineer the laser light delivery system, which includes the laser source, the light guide, the interface with the ultrasound probe, and the master control of all subsystems.
2. Adaptation of a clinical ultrasound imaging system for photoacoustic and ultrasonic imaging: A clinical Philips ultrasound imaging system will be adapted for PAT. The multi-channel ultrasound receiving system accelerates data acquisition. The adapted imaging system will then be validated with tissue phantoms. We will quantify the spatial resolution, imaging depth, signal-to-noise ratio, contrast, and frame rate.
3. Imaging of axillary lymph nodes *in vivo*: First, image a small number of human sentinel lymph nodes to fine tune the multimodal ultrasound and photoacoustic imaging system. Second, image human sentinel lymph nodes and prospectively assess the agreement between the imaging system and standard clinical practice in axillary staging for patients with breast cancer and clinically negative axillae. The hypothesis is that ultrasound and photoacoustic imaging technologies in combination provide sufficient spatial resolution and contrast to map sentinel lymph nodes with high sensitivity and specificity.

We believe that this technology represents a viable, minimally invasive alternative to SLNB and may obviate the need for axillary procedures in patients with breast cancer who have a negative RT-PCR profile.

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Figure 1

