

**Evaluation of Pathologic Parameters and Published Nomograms in Predicting Non-Sentinel
Lymph Node Metastases in Female Breast Cancer Patients
with Positive Sentinel Lymph Nodes**

by

Lisa Elaine Murch

A Thesis submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Pathology
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA
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Of

Master of Science

Lisa Elaine Murch @ 2007

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ABSTRACT

OBJECTIVES: To define a subgroup of sentinel lymph node positive breast cancer patients who can safely decline an axillary lymph node dissection after sentinel lymph node biopsy.

PATIENTS & METHODS: Ninety-six cases from 2002-2007 were reviewed. Clinical and pathologic variables were assessed through univariate and multivariate logistic regression. The predictive accuracy of published nomograms in predicting the likelihood axillary lymph node positivity was tested through receiver operator curve analysis.

RESULTS: We found that hematoxylin and eosin sentinel lymph node detection method, extracapsular extension, sentinel lymph node metastasis size ≥ 10 mm and tumor size > 5 cm were predictors of axillary positivity. One of the nomograms was validated, with an area under the curve of 0.71198.

CONCLUSIONS: Micrometastasis in sentinel lymph nodes was not associated with axillary disease. No subgroup was identified who could safely decline an axillary lymph node dissection. Secondly, one of the three nomograms was validated.

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III. LIST OF ABBREVIATIONS:

ACOSOG.....American College of Surgeons Oncology Group

ALN(s).....Axillary lymph node(s)

+ALN(s).....Positive axillary lymph node(s)

-ALN(s).....Negative axillary lymph node(s)

ALND(s).....Axillary lymph node dissection(s)

+ALND(s).....Positive axillary lymph node dissection(s)

-ALND(s).....Negative axillary lymph node dissection(s)

ATM.....Ataxia telangiectasia mutated

AUC.....Area under the curve

BCN(s).....Breast cancer nomogram(s)

BCT.....Breast conservation therapy

BRCA1.....Breast cancer gene 1

BRCA2.....Breast cancer gene 2

CI.....Confidence interval

DCIS.....Ductal carcinoma in situ

ECE.....Extracapsular extension

+ECE.....Positive extracapsular extension

-ECE.....Negative extracapsular extension

ER.....Estrogen receptor

+ER.....Positive estrogen receptor status

FNA.....Fine needle aspiration
GH.....Grace Hospital
HSC.....Health Sciences Centre
H&E.....Hematoxylin and eosin staining of slides
IHC.....Immunohistochemistry
LCIS.....Lobular carcinoma in situ
LIQ.....Lower inner quadrant
LN.....Lymph node
LOQ.....Lower outer quadrant
LVI.....Lymphovascular invasion
MRI.....Magnetic resonance imaging
MSKCC.....Memorial Sloan-Kettering Cancer Center
N/A.....Not Applicable
OR.....Odds Ratio
PET.....Positron emission tomography
PR.....Progesterone receptor
ROC.....Receiver operating curve
SBGH.....St. Boniface General Hospital
SLN(s).....Sentinel lymph node(s)
 +SLN(s).....Positive sentinel lymph node(s)
 -SLN(s).....Negative sentinel lymph node(s)
SLNB(s).....Sentinel lymph node biopsy(s)
SCF.....Subcapsular foci

TNM.....Tumor, lymph node and metastases grading system

cTNMClinical TNM classification

pTNM.....Pathologic TNM classification

T.....Primary tumor

N.....Regional lymph nodes

M.....Distant metastasis

US.....Ultrasound

UOQ.....Upper outer quadrant

UIQ.....Upper inner quadrant

UICC.....International Union Against Cancer

WRHA.....Winnipeg Regional Health Authority

IV. INTRODUCTION

Breast cancer is currently the second most common form of malignancy in women^{15, 22, 27, 29}. It affects females more commonly than males with a female-to-male ratio of 100:1³⁵. As the second most common cause of cancer deaths in women, it is a dreaded disease that accounts for 22% of all female cancers, more than one million cases world wide annually^{15, 28, 30}. In the United States, 1 in 9 women will develop breast cancer in their lifetime. Of these women, 1/3 will succumb to the disease¹⁵. In 2001, 1 in 101 Canadian women developed breast cancer⁴. In Canada from 1993 to 1997, there were a total of 81, 861 documented cases of female breast cancer, with a frequency of 28.8% among all cancers²⁰. The estimated number of breast cancer cases in Canada for 2006 was 22 200, of which 5300 were estimated to be fatal⁴.

Breast cancer occurs predominantly in females because females have a more complex breast structure, greater breast volume and an extreme sensitivity to endocrine influences¹⁵. Each of these factors independently or in combination, predisposes the breast to a range of pathologic conditions, most notably breast cancer¹⁵. Interestingly, breast cancer is more common in the left versus the right breast with a ratio of left-to-right ratio of 110:100. In 4% of documented cases the cancer is bilateral at the time of diagnosis¹⁵.

The overall incidence of female breast cancer increased steadily over the past several decades until 1988, at which time incidence and mortality stabilized as a direct result of advances in population based mammographic screening, adjuvant chemotherapy and hormonal therapy^{15, 27, 30}. Breast cancer is influenced strongly by age, very rarely

affecting women under the age of 25 with the exception of familial cases^{15, 27}. It has been suggested that the age specific differences in the incidence may be due to hormonal influences at the time of menopause²⁷. Incidence is highest in women age 45-50, declines in women age 50-59 and peaks again in women older than 59²². In Canada, the lifetime risk is about 1 in 10²⁶. The average age at time of diagnosis is 64¹⁵. Etiologic factors, aside from age and gender, are geographical, genetic, patient, hormonal and environmental factors^{15, 30, 35}.

The incidence varies significantly when Asian countries are compared to the United States and Northern Europe¹⁵. The incidence in North America is approximately five times that in Japan²⁷. Typically, breast cancer is a disease of affluent populations and within these populations, breast cancer accounts for 26% of female cancers³⁰. The highest incidence rates are observed in North America, Europe and Australia, where 6% of the female population develop breast cancer before age 75³⁰. The proportion of women who will succumb to the disease in developed countries is 2% and in developing countries is 0.5%²⁸. This difference may be explained on the basis of genetics, lifestyle and environmental factors²⁸.

An increased risk of breast cancer has been identified in women with a strong familial history of the disease. Of all breast cancers, 5-10% are of hereditary origin²⁷. The magnitude of the risk is subject to increase if there are three or more first or second relatives on the same side of the family who are affected^{15, 27, 28}. The recently identified high penetrance genes, breast cancer gene 1 (BRCA1) and breast cancer gene 2 (BRCA2) are strongly recognized as relative risk factors for breast cancer^{15, 28}. Mutations in these genes account for approximately 5% of breast cancer cases^{15, 35}. Individuals who are

carriers of these gene mutations have an estimated 85% risk of breast cancer up to age 70²⁷. Other genetic factors linked to breast cancer are the heterozygous ATM (ataxia telangiectasia mutated) gene and germline mutations of the p53 tumor suppressor gene^{28, 35}.

Many patient factors influence the risk of breast cancer, including reproductive status. The risk is elevated in women with early menarche, those who are nulliparous, those who were older than 30 at the time of first child, those who are infertile and in women with a late age at menopause^{15, 27, 30}. The use of either high dose oral contraceptives or long term hormone replacement therapies is associated with a small increased risk of breast cancer^{30, 35}. Nutrition may be associated with a slightly decreased risk for women who have a diet high in fruits and vegetables³⁰. For obese women there is an increased risk for those who are under the age of 40 and postmenopausal¹⁵.

The World Health Organization pathologically defines breast cancer as a "group of malignant epithelial tumors, characterized by invasion of adjacent tissues and a marked tendency to metastasize to distant sites"³⁰. Typically, breast cancer arises from the terminal ductal-lobular unit of epithelial tissue which accounts for 10% of the total breast volume²⁸. Many morphologic phenotypes and specific histopathological types of breast cancer exist, each having particular prognostic and clinical characteristics³⁰.

Carcinoma of the breast can be classified as either invasive (70-85% of cases) or non-invasive (15-30% of cases)¹⁵. Invasive cancers are characterized by their penetration and involvement of adjacent tissues, often with vascular and lymphatic involvement (termed lymphovascular invasion-LVI). Of invasive breast cancers, 50-70%

are ductal carcinomas (more commonly known as infiltrating ductal carcinoma) and 5-15% are lobular carcinomas (more commonly known as infiltrating lobular carcinoma). The remainder are of so called "special type" ²². Non invasive carcinomas, more commonly referred to as *in situ* carcinomas, are those cancers not yet infiltrating adjacent tissues ²². Of non-invasive or *in-situ* cancers, 80% are ductal carcinomas (DCIS) and 20% are lobular carcinomas (LCIS) ¹⁵. DCIS is characterized by malignant epithelial cells confined to the mammary ducts and lobules ²⁸. If untreated, it has a 30% chance of progressing into invasive infiltrating ductal carcinoma ^{22, 28}. LCIS is characterized by a slow growing, solid proliferation of small cells with uniform, round nuclei ²⁷. LCIS is not frequently detected during a clinical exam or a mammogram and as such is often an incidental finding. Although not an obligate precursor lesion, it is a marker for significantly higher risk of developing an invasive cancer ^{22, 27}.

Breast cancer either presents asymptotically or symptomatically. Asymptomatic cases are usually identified during annual breast screening programs. Mammography is the standard of care for women over the age of 40, is highly sensitive detecting 85-90% of breast cancers and reduces overall mortality by 30% ^{28, 30, 35}. If there is a strong familial history, screening may begin as early as age 25 ³⁵. Screening programs allow for the early detection of cancers and consist of a clinical breast exam and mammography ³⁵. A non-palpable lesion detected via screening may require a fine needle aspiration (FNA) or core biopsy to diagnose. The results of these procedures may necessitate the excision of the lesion ²⁷.

Symptomatic cases typically present as a painless, palpable lumps. More obvious indicators include dimpling of the skin, nipple inversion, edema or peau d'orange and

abnormal nipple secretions ^{15, 27, 30, 35}. To be palpable, a breast lump is usually greater than one centimetre maximum dimension ³⁵. Of all malignant tumors, 50% are located in the upper outer quadrant (UOQ), roughly 10% in each of the remaining quadrants: upper inner quadrant (UIQ), lower outer quadrant (LOQ), lower inner quadrant (LIQ) and 20% in the sub-areolar region (beneath the nipple) ^{22, 30}.

Complete diagnosis of suspected lesions is achieved by triple assessment consisting of a clinical examination, imaging and tissue sampling ^{13, 27, 30}. The clinical examination involves careful physical evaluation of the patient's symptoms, skin changes and gynaecologic history followed by a complete evaluation of the breasts noting, size, symmetry, contour and nipple anomalies ^{22, 30}. Most importantly, the supraclavicular and infraclavicular areas along with the axillae and the breasts are examined by meticulous palpation to identify any suspicious areas ²². If a lesion is identified, its relative size, consistency and mobility are documented. The patient is then sent for breast imaging by mammography, ultrasound (US), magnetic resonance imaging (MRI) or positron emission tomography (PET) ^{22, 30, 33}. Despite the sensitivity of mammography, physical examination remains extremely important as ~10% of cancers are undetectable mammographically ³³. Diagnostically, US is recommended in women with small lesions and dense breast tissue and is useful in distinguishing cystic from solid lesions ³³. Due largely to cost and availability, MRI is only recommended when mammography and US are inconclusive ^{22, 27, 33}. PET, a recent development, is used to detect metastatic foci in other organs and to assess axillary lymph node status pre-operatively. PET is not universally accepted for this purpose because of its poor sensitivity. It is only able to detect lesions greater than 5 mm ³³. Despite these options, mammography remains the

most widely used diagnostic imaging technique for the detection of breast cancer. To improve the sensitivity of mammography, increased image quality, two-view mammography and double reading are employed ²⁷. Calcifications and soft-tissue densities are the most common findings on mammograms ^{22, 30}. The morphology and distribution of calcifications are assessed and classified as: typically benign, probably benign, suspicious or typically malignant ²². Both calcification and soft tissue densities are often indicative of a tumor and warrant further assessment through tissue sampling by FNA or needle core biopsy ^{22, 27, 30}. FNA was developed in the 1960's and is used world wide. It is advantageous because it is minimally intrusive. However because FNA yields only clusters of cells, cytologists are limited to determining if a lesion is benign or malignant; they cannot distinguish invasive from non-invasive lesions ^{22, 27, 37}. Needle core biopsies yield a larger tissue sample and can be sent for histological examination ²². Malignant versus benign lesions, invasive versus non-invasive lesions and hormone receptor status are determinable through needle core biopsies ^{22, 27}. Needle core biopsies result in a low false negative rate, 1%-7%, and very rarely report false positives ³⁷. Once a patient is accurately diagnosed with a malignancy they must discuss their treatment options with their physician.

The stage and grade of malignant breast tumors have a direct impact on treatment therapies and prognosis. The stage is defined as the anatomical extent of the disease based on three components: the extent of the primary tumor (T), the absence or presence and extent of regional lymph node metastasis (N), and the absence or presence of distant metastasis (M) ²⁵. Tumors are classified in the TNM system through the assignment of numbers to each of the components. There are two classifications described for breast

cancers, clinical and pathological. Clinical classification is based on evidence acquired pre-treatment from physical examinations and imaging (designated TNM or cTNM) ²⁵. Pathological classification is based on evidence acquired from surgery and pathologic examination (designated pTNM) ²⁵. Once assigned, the TNM stage proves to be a pivotal factor in the selection of treatment therapies and the estimation of prognosis. A summary of TNM clinical and pathological classification of breast tumors is provided in Tables 1, 2 and 3 ^{22, 25}.

There are 24 possible outcomes for TNM staging (4 T categories x 3 N categories x 2 M categories). These outcomes were grouped with respect to survival, ensuring that the survival rates among the groups remained distinctive. More specifically, stage 0 includes all Tis N0 M0 tumors. Stage I includes all T1 (including T1mic) N0 M0 tumors ²⁵. Stage IIA includes the following tumors: T0 N1 M0, T1 (including T1mic) N1 M0 and T2 N0 M0 ²⁵. Stage IIB includes T2 N1 M0 and T3 N0 M0 tumors ²⁵. Stage IIIA includes the following tumors: T0 N2 M0, T1 (including T1mic) N2 M0, T2 N2 M0 and T3 N1 or N2 M0 ²⁵. Stage IIIB includes all T4 N1, N2 or N3 M0 tumors ²⁵. Stage IIIC includes any T, N3 M0 tumors and Stage IV includes any T, any N M1 tumors ²⁵.

Tumor size, defined as the maximum single dimension of a breast tumor, is important prognostically. The most accurate estimated size is determined through complete assessment of the reported clinical estimated size, mammographic size, macroscopic size in the intact specimen, slice radiographs of the dissected specimen and microscopic size ²⁷. Controversy exists regarding the size criteria for defining a 'small' and 'large' tumor. Regardless, a small tumor carries a better prognosis than a large tumor ²⁷.

The grade of malignant breast tumors refers to the cellular architecture and cytological atypia of the tumor determined microscopically. When assessing these criteria, grade I is defined as well differentiated, grade II is defined as moderately differentiated and grade III is defined as poorly differentiated^{15,27}. The most commonly used grading system, known as Scarff-Bloom-Richardson, is based on three histological components: tumor architecture (tubule formation), nuclear pleomorphism and mitotic activity²⁷. Each factor is assessed independently and scored on a scale of 1-3. When assessing tubule formation, a score of 1 is assigned when tubule formation is identified in approximately 75% of the tumor, a score of 2 is assigned when tubule formation is identified in approximately 10-75% of the tumor and a score of 3 is assigned when tubule formation is identified in approximately 10% of the tumor²⁷. When assessing nuclear pleomorphism, a score of 1 is assigned when the nuclei are small, round and uniform, a score of 2 is assigned when the size and variability of the nuclei has moderately increased and a score of 3 is assigned when there is marked variation among the nuclei²⁷. The criteria for scoring mitotic counts are variable depending on the microscopic field area²⁷. Summation of the scores for each category assigns the score, with a maximum score of 9. Grade I is scored as 3-5/9, grade II is 6-7/9 and grade III is 8-9/9^{22, 27}. The stage and grade are extremely important prognostically²⁶.

For stage I, II and III cancers the primary goal of treatment is to locally control the cancer and prevent a recurrence at the primary tumor site or at another organ site within the body¹⁶. For stage IV cancers the treatment goal is disease control because stage IV cancers are rarely curable. For terminally ill patients, the clinical goal is minimizing the symptoms and improving the patients comfort level¹⁶.

Towards the conclusion of the 19th century, Halsted developed a technique for radical mastectomy. In 1882 this involved the removal of the entire breast, a wide skin excision, resection of the pectoralis muscle with underlying fascia and a complete axillary dissection ^{13, 22}. Since then there has been a major shift towards more conservative treatment techniques, commonly known as breast conservation therapies (BCT) ^{13, 22, 27}. The initial surgical treatment choice is dependant on the stage of the cancer. Until the 1960's radical mastectomies were the standard surgery for breast malignancies. In the 1970's there was a shift toward modified radical mastectomies where the pectoralis muscle was preserved and the skin excision was minimized ²². Currently, because of medical advancements and improvements, earlier detection through imaging is possible and therefore BCT is acceptable. BCT is defined as a local removal of the tumor with clear surgical margins, excision of the sentinel lymph node (SLN) and axillary lymph node dissection (ALND) with or without adjuvant radiotherapy ^{13, 22}. Surgical resection remains the standard of care for local control of breast malignancies and provides accurate information used to correctly stage the disease ²⁷.

The primary objective of BCT is to locally control the entire tumor and obtain negative surgical margins while preserving the breast and the patient's body self image ^{27, 37}. BCT is not specific to malignant breast lesions, it is also the primary treatment for benign lesions ²². BCT is not always curative, with local recurrence rates ranging from 1-4% per year ¹³. Patients who do not qualify for BCT include those who have a breast lesion greater than 3 cm and those who have small breasts ²². If the lesion is greater than 3 cm the patient may best benefit from a more radical surgery like a mastectomy ²². Patients who have a malignant lesion and small breasts are not favoured for BCT because

to obtain clear surgical margins a significant volume of tissue need be removed, yielding a cosmetically negative result ²². In addition to the above qualifying criteria, Borgen *et al.* report multiple absolute contraindications to BCT including: multicentric disease, pregnancy, cancer in a previously irradiated area, persistent positive margins and collagen vascular disease ^{3, 13, 27, 35}. Noteworthy contraindications are a large tumor and a tumor located below the nipple ^{3, 35}. In these cases a mastectomy may be the most appropriate treatment option ²⁸.

To localize the tumor before surgery, patients sometimes undergo image-guided needle localization ¹³. First, a hollow needle is inserted into the breast and placed in or adjacent to the lesion. This occurs under local anaesthesia using either mammographic, US or stereotactic guidance ²². A wire with a hook at the distal end is then passed through the needle and imbedded in the breast tissue ²². Optimal placement of the needle is challenging and may require the use of more than one imaging technique ¹³. For a patient to qualify for the technique, the breast must not compress to less than 2 cm when lying in the prone position ¹³. If the breast is overall too small, the needle may pass clear through to the opposite side of the breast ¹³. In addition to BCT for malignant cases, adjuvant local and systemic therapy may be needed to eradicate as many remaining undetectable malignant cells as possible ²⁸.

Adjuvant therapy may consist of radiotherapy, chemotherapy and hormonal therapy and commences 4-8 weeks post surgery, allowing the patient sufficient time to recover and heal ³⁵. Although not always curative, adjuvant therapy averts relapses in 1/3 of breast cancer patients ²⁷. Patients undergoing BCT or a mastectomy for large breast cancers will receive radiotherapy ^{27, 35}. Radiotherapy facilitates local control and is often

administered prior to chemotherapy ³⁵. BCT in conjunction with adjuvant radiation yields the same prognostic result as mastectomy ²⁷. Chemotherapy is the standard of care for all sentinel lymph node positive (SLN+) and many sentinel lymph node negative (SLN-) patients ^{13, 35}. The duration of multi-drug or single drug treatment spans 3-6 months and improves long term and overall survival ³⁵. Hormonal therapy is administered to those patients who are estrogen (ER) and/or progesterone (PR) receptor positive as determined by pathologic analysis of the tumor ³⁵. Estrogen is a known growth factor for tumors that express estrogen and/or progesterone receptors ³⁵. The rationale is that anti-estrogen therapy will prevent undetectable malignant cells from receiving estrogen stimulation ^{27, 35}. Hormonal drugs are widely used and are very effective ²⁷. Tamoxifen, a non-steroidal anti-estrogen drug, is the most commonly prescribed hormonal therapy and is administered post-chemotherapy ^{28, 35}. Tamoxifen has been recently supplanted by the newer class of drugs known as aromatase inhibitors ⁵.

Management of the axilla has also changed significantly in recent years from total axillary dissection, to excision of only levels I and II lymph nodes and most recently the sentinel lymph node biopsy (SLNB) ¹³. The SLN is the first lymph node to receive lymphatic drainage from the primary breast tumor. In theory, lymph flow is linear and as a result, tumor cells within the lymphatics are expected to infiltrate the SLN before spreading to other regional lymph nodes ^{22, 25}. The SLN is identified intra-operatively and mapped using one of three techniques: intradermal injection of technetium-labelled sulphur colloid, intradermal injection of isosulfan blue dye or intradermal injection of a combination of both agents ^{13, 22}. The agent of choice is administered pre-operatively into the subcutaneous tissue just below the dermis, close to areolar margin ^{13, 22}. Some

surgeons choose to inject the agent over the tumor site. In either instance, it takes approximately 15-30 minutes for the agent to reach the SLN^{13, 22}. The SLN is identified by a gamma probe and/or the visible blue dye¹³. Once identified, the SLN is removed and sent to the surgical pathology department. Depending on specific departmental policies, the SLN is assessed intra-operatively by cytological imprints or frozen section, or is assessed after for routine histology^{6, 22}. If the SLN contains metastatic tumor, this indicates that the cancer is not confined to the breast and that additional regional lymph nodes may contain metastatic tumor²⁵. If no metastatic tumor is identified in the SLN, this indicates that the cancer is confined to the breast and additional regional lymph nodes are not likely to contain metastatic tumor²⁵. Therefore, a +SLN diagnosis generally necessitates a completion ALND. If the SLN diagnosis is negative, a completion ALND is not necessary. If a false negative intra-operative report is determined through subsequent routine histology, serial hematoxylin and eosin stains (H&E) or immunohistochemistry (IHC), a completion ALND may be executed during a second surgery^{22, 25, 13}. Several large studies have shown success rates of 95% in detecting the SLN with a false negative rate of 5%²⁷. Furthermore, a SLNB validation study from 1993-1999 showed a SLN detection rate of 90%, an accuracy of 97% and a false negative rate of under 10%⁶.

SLNB is increasingly adopted globally as the standard of care for breast cancer patients¹². Lymph node status remains the single most important prognostic factor in patients with operable breast cancer¹². SLNB allows for staging of the disease without complete excision of the axilla²². Morbidity due to axillary dissection is well documented²⁷. Patients suffer a gamut of post-surgical complications including:

lymphedema, numbness, paresthesia, infection and loss of use of the affected shoulder²⁷,²⁸. Petrek and Heelan report 10% to 25% of all patients with breast cancer treated with axillary dissection develop lymphedema²¹. Patients suffering from lymphedema are disfigured and often experience bouts of cellulitis and lymphangitis¹³. Therefore, through SLNB, patients who are not likely to have metastatic spread to regional lymph nodes are identified and consequently, can safely decline an ALND, avoiding unnecessary discomfort and disfigurement¹³.

The current standard of care for patients with a +SLN is a completion ALND¹⁰,²⁷. Of these patients, 40% to 60% will have no metastases in the axillary lymph nodes (ALN) apart from the solitary +SLN. If these patients could be identified based on characteristics of their tumor and SLN, they may be spared the morbidity of an ALND¹⁰. Attempts have been made to predict non-sentinel node involvement when the sentinel node is positive using mathematical formulae based on patient, breast tumor and sentinel node characteristics¹². These formulae are commonly referred to as nomograms, more specifically, breast cancer nomograms (BCN). The two nomograms discussed in this study are the Memorial Sloan-Kettering Cancer Center (MSKCC) BCN and the Mayo Clinic BCN. The nomograms predict the individual patient risk of ALN positivity based on a multivariable logistic-regression analysis¹. In developing and validating BCNs, studies are conducted to determine which variables of the patient, tumor and SLN metastases are predictive of ALN metastases¹⁰. Potential variables include: patient age, breast tumor size, tumor type, tumor grade, LVI, tumor multifocality, SLN metastasis detection method, ER/PR status, number +SLN, number -SLN, size of SLN metastasis and extracapsular extension (ECE)^{1, 10, 11, 12, 17}. The variables included in the MSKCC

BCN include: whether a frozen section was performed, the pathologic size of the tumor, tumor type and grade, LVI, multifocality, ER status, method of SLN metastasis detection, number of +SLN and number of -SLN ¹⁷. The variables included in the Mayo BCN include: patient age, tumor size, size of SLN metastasis, ER positivity, ECE, number +SLN, number -SLN, age with +ER status and number of +SLN with ECE ¹⁰. Of these variables, tumor size is accepted as a significant predictive parameter as it correlates with ALN positivity ²⁷. Of all patients with a tumor less than 1 cm greatest dimension, 15% will have +ALNs ²⁷. Of all patients with a tumor larger than 5 cm greatest dimension, 60% will have +ALNs ²⁷. Although not universally accepted it is possible that, if used correctly under the guidance of a physician, the BCNs may prove to be a useful tool in determining individual risks of ALN positivity in female breast cancer cases with +SLNB.

The overall prognosis for breast cancer has improved significantly. Many factors previously discussed, have had an influence on the overall survival of breast cancer in recent decades. There is improved survival among patients age 35-49 years at the time of diagnosis ²⁷. Patients age 40-49 at the time of diagnosis have the best prognosis whereas patients age 50-59 have a poor prognosis ²⁷. Patients under the age of 35 and over the age of 75 have the worst prognosis, albeit the proportion of patients in this category is small ²⁷. High risk patients (those with a strong family history or those with BRCA1 or BRCA2) are monitored closely by their physicians. Prophylactic treatment may be selected by these patients ²⁸. With respects to ALNs, prognosis worsens as the number of +ALNs increases. More than 10 +ALN, yields a 5 year survival rate of 20-30% ²⁷. For patients with all -ALN, the 5 year survival rate is 70-80% ²⁷. The pathologic stage of the

cancer is an important indicator of overall prognosis. As the stage of the disease increases, the 5 year survival rate decreases ¹⁶. These rates are as follows: 88% for stage I, 66% for stage II, 36% for stage III and 7% for stage IV ¹⁶. In summary, the following factors are associated with a good prognosis: age 40-49 years, negative axillary lymph nodes, small tumor size, low histologic grade, no LVI and the presence of ER and/or PR receptors ²⁷. The following factors are associated with a poor prognosis: age <40, >49, positive axillary lymph nodes, large tumor size, high histologic grade, LVI and absent ER and/or PR receptors ²⁷.

Given that survival is improving in patients with breast cancer, attention is being devoted to decreasing treatment related morbidity. As ALND is one of the causes of long term morbidity, the issue of whether select patients with a +SLN may be spared a completion ALND has become a topic of interest. The two well known BCN in the published literature, the MSKCC BCN and the Mayo BCN, were derived through studies that used different histological approaches and protocols specific to their institutions ^{10, 36}. Before applying these BCN to another patient population, statistical validation is required particularly if significant methodological differences exist in pathological processing. This is one of the primary objectives of our study.

The aim of this study is to validate two published BCN, the MSKCC BCN and the Mayo BCN using retrospective data from three regional hospitals. In validating the BCNs, we will correlate clinical as well as pathological variables of the patient, SLN and primary breast tumor with the known ALN status to determine, in our population, which variables are accurately predictive of ALN positivity. We will then compare and contrast

our findings to those published in recent literature. Furthermore, we aim to identify a subgroup of +SLN patients who can safely and reasonably decline an ALND.

V. LITERATURE REVIEW

A prospective clinical trial (Z0011) conducted by the American College of Surgeons Oncology Group (ACOSOG) aimed to answer the question of whether or not completion ALND provides a survival benefit in SLN+ patients³¹. The trial aimed to quantify and compare the surgical morbidities associated with SLN dissection accompanied by ALND versus ALND independently³¹. Patients with a SLN+ were randomized with respect to the subsequent treatment of their axillae^{23, 31, 36}. One sub-group underwent a routine ALND while the other sub-group underwent axillary observation²³. Unfortunately this study was terminated due to poor recruitment²³.

The prognostic significance of micrometastases in SLNs is widely debated in the literature¹. With respect to ALND, Van Zee *et al.* indicated that the primary tumor size and the SLN metastases sizes are significantly predictive of the likelihood of additional ALN metastases³⁶. In a retrospective BCN validation, Umesh *et al.* reported 13.3% of patients with micrometastases in their SLN had ALN metastases and 34.8% of patients with macrometastases in their SLN had ALN metastases³⁴. Degnim *et al.* reported that 13-22% of patients with SLN micrometastasis had metastatic spread to ALN¹⁰. Umesh *et al.* and Rivers *et al.* concluded that SLN metastasis size is and the risk of ALN metastases are strongly correlated^{23, 34}. More specifically, Chu *et al.* and Reynolds *et al.* suggested a minimal risk of ALN metastases in patients with SLN micrometastasis provided their primary tumor was ≤ 2 cm (T1 tumor)¹⁹. A retrospective study conducted by Soni *et al.* determined SLN metastasis size to be a more consistent and possibly more valuable variable when incorporated into a BCN²⁶. Ozman *et al.* reported that ALND

could be safely avoided in patients with SLN micrometastases provided additional risk factors were absent, specifically perineural invasion and tumors >2 cm (T2 tumors)¹⁶.

Van Zee *et al.* developed the MSKCC BCN with the acknowledgement of limitations in its design, specifically, the fact that all completion ALND lymph nodes were examined by routine histology only³⁶. In other studies the ALND lymph nodes were examined by routine histology, serial sectioning and IHC. Because of the greater sensitivity of the latter methods, a greater proportion of patients with additional ALN metastases were identified in these studies³⁶. In summary, when detecting micrometastases, IHC is more sensitive than serial sections, serial sections are more sensitive than routine histology and routine histology is more sensitive than frozen section³⁶. Degnim *et al.* recognized one clear advantage of the Mayo BCN; the elimination of the histologic detection method for the SLN metastases as a variable¹⁰. This is important when standardizing the BCN because the protocols for each detection method vary greatly among different institutions and contribute to the variations in the resulting risk assessment using the MSKCC BCN^{10, 26}.

In the literature there are a multitude of studies that investigate the associations between various patient and histopathologic variables, and several are incorporated into the MSKCC BCN and/or the Mayo BCN¹. If statistically significant variables are not included in existing BCNs, then these nomograms should be modified to include such variables or a new predictive tool should be developed. Degnim *et al.* found that the proportion of the SLN replaced by tumor, metastasis size and ECE were highly predictive of ALN metastasis^{1, 10}. These variables were not included in the MSKCC BCN;

consequently Degnim *et al.* developed a new predictive model that incorporated the latter two variables. They proceeded to validate their model internally with bootstrapping ¹⁰.

The MSKCC BCN was developed through a retrospective analysis based on data from their study population ³⁶. Multivariate logistic regression was employed to analyze the associations of patient and histopathologic variables with the likelihood of ALN metastases ³⁶. Van Zee *et al.* determined that pathologic tumor size, LVI, SLN detection method, number of +SLNs and number –SLNs were all associated with ALN metastases ³⁶. Tumor multifocality was borderline significant and histological tumor type, tumor grade and ER/PR status showed no significant association with ALN metastases ³⁶. Umesh *et al.* investigated similar variables and determined ECE to be the most significant variable in predicting ALN metastases within their population ³⁴. Furthermore, Umesh *et al.* found tumor size to be predictive of ALN metastases, particularly in primary tumors <2 cm largest dimension ³⁴. In contrast to the findings of Van Zee *et al.*, Umesh *et al.* found that neither LVI nor the number of +SLNs showed a significant association with ALN metastases ³⁴. A study conducted by Ozman *et al.* investigated associations between patient and histopathologic variables with respect to ALN status and determined that tumor size >2 cm, SLN macrometastases and ECE were variables indicative of ALN metastasis ¹⁹. They also determined that age, tumor multifocality, histological tumor type, location of tumor, tumor grade, ER/PR status, method of SLN detection and number of +SLN were insignificant ¹⁹. In a study analyzing the findings of 11 published studies, Degnim *et al.* found 5 variables: SLN metastasis size >2 mm, ECE, primary tumor size >2 cm, >1 +SLN and LVI were associated with the highest likelihood of ALN metastases ⁹. This study reported that for each of the five variables, there is a >2 fold increase in the

likelihood of finding ALN metastasis ⁹. Based on the findings of the aforementioned studies, ECE is a variable that is consistently predictive of ALN metastasis. Stitzenberg *et al.* reported SLN ECE to be a strong predictor of ALN status. In their study, 78% of patients with SLN ECE had ALN metastasis while only 29% of patients without SLN ECE had ALN metastasis ²⁹. They concluded that all patients with SLN ECE “should undergo a mandatory completion ALND” ²⁹. The identification of different statistically significant variables predictive of ALN metastases may in part be attributed to variations among the different study populations with respect to clinical, surgical and pathological approaches and protocols.

Despite the recognized differences in SLN metastasis detection, controversy persists regarding the sensitivity of SLN intra-operative diagnostic methods, specifically imprint cytology and frozen section. As SLN biopsy became more widely accepted, an alternative method of intra-operative SLN diagnosis was introduced: imprint cytology. Ceserni *et al.* reported that frozen section had an accuracy of 79-98%, a sensitivity of 55-91% and a false negative rate of 9-45% (if the intra-operative SLNB diagnosis is negative and the routine histologic definitive diagnosis is positive, this constitutes a false negative) ⁸. In comparison, they reported that imprint cytology had an accuracy of 78-99%, a sensitivity of 46-96% and a false negative rate of 5-70% ⁸. Motomura *et al.* reported imprint cytology to have superior sensitivity over frozen section to the order of 96% to 52% respectively ¹⁸. In explaining the inferior sensitivity of frozen section to imprint cytology in this study, Tomiko *et al.* recognized that when diagnosing the SLN by frozen section, only one cross section of the largest lymph node was submitted. In contrast, when diagnosing the SLN by imprint cytology, a slide was prepared from each of the

submitted SLN³². Tomiko *et al.* identified that the slide staining method may also factor into the sensitivity of frozen section versus imprint cytology³². Diest *et al.* found that the sensitivity of H&E staining was superior to that of other staining methods³². A prospective study conducted in 2004 by Tomiko *et al* demonstrated the sensitivity of frozen section and imprint cytology to be comparable. They reported the sensitivity per SLN of frozen section and imprint cytology to be 89% and 86% respectively³². The sensitivity per patient was 85% for both diagnostic methods³¹. It is therefore reasonable to equate the relative sensitivity of frozen section with imprint cytology when evaluating the SLN and substitute imprint cytology for frozen section as a BCN variable^{7, 14, 32}.

A validation study conducted by Degnim *et al.* concluded that the predictive accuracy of a BCN can degrade as the model is applied to different populations¹⁰. For a BCN to be accepted universally, the predictive accuracy must be reproducible and portable among distinct populations¹⁰. Predictive models based on data from single institutions require validation using distinct populations before they may be universally accepted¹⁰. Smidt *et al.* demonstrated that the MSKCC BCN was applicable for populations that differ greatly from the population used to develop the BCN^{2, 24}.

The MSKCC BCN has been previously validated by a number of different institutions^{2, 14}. Lambert *et al.* validated the MSKCC BCN using a large, external and independent cancer care database where Smidt *et al.* used a population of Dutch patients from a Dutch teaching hospital^{14, 24}. In contrast, Soni *et al.* and Kocsis *et al.* failed to validate the MSKCC using their respective data sets and consequently cautioned its use^{14, 26}. Soni *et al.* based their conclusion on the small magnitude of their study²⁶.

Although there are many BCN validation studies spanning institutions, their validity and acceptance remain questionable.

Degnim *et al.* validated both the MSKCC BCN and their newly developed Mayo BCN using two distinct datasets, one from the University of Michigan (Michigan) and the second from the Mayo Clinic (Mayo) ¹⁰. Using the Mayo data, the MSKCC BCN reported a false negative rate of 0% and 14% for a predictive probability of 5% and 10% respectively ¹⁰. Using the Michigan data, the MSKCC BCN reported a false negative rate of 17% and 11% for a predictive probability of 5% and 10% respectively ¹⁰. It was noted that the false negative rate for the Michigan data “was not trivial in the group with a predicted probability of 5% or less, despite an overall model performance that would be considered excellent” ¹⁰. The MSKCC BCN performed well using the Michigan and Mayo datasets. However, Degnim *et al.* identified that the model may not perform consistently in clinically useful subgroups ¹⁰. Alran *et al.* found that the MSKCC BCN was not reliably predictive of ALN positivity in cases with positive micrometastasis in the SLN ¹.

An important issue, as noted previously in this literature review, is whether or not +SLNBs require routine ALND. This question is unlikely to be answered in a prospective trial given the termination of the ACOSOG trial. An old National Surgical Adjuvant Breast Project (NSABP) study from the 1970’s suggested that there is no survival advantage to axillary dissection in patients with clinically negative nodes ²³. In the adjuvant chemotherapy era, it is likely that the survival advantage would be even more questionable. In this scenario, completion ALND after +SLNB would only be considered in patients at risk of high stage (N2) nodal disease, due to significant

differences in locoregional radiotherapy ²³. Rivers *et al.* analyzed clinicopathological features associated with N2 disease and reported that several factors were associated with and increased risk of N2 disease including: tumor size >2 cm, LVI, increased number of +SLN and increasing size of the SLN metastasis ²³. Of particular interest was their finding that SLN micrometastasis is rarely associated with N2 disease (1 of 74 cases) ²³.

VI. OBJECTIVES AND HYPOTHESIS

The primary objective of this study is to define a subgroup of +SLN female breast cancer patients who can safely decline an ALND. This will be assessed in three different ways.

Firstly, we intend to test the predictive accuracy of two published BCNs, the MSKCC BCN and the Mayo BCN. We will attempt to validate these BCNs using our patient population.

Secondly, in testing the predictive accuracy of the BCNs, we will correlate clinical and pathological variables of the patient, SLN and primary breast tumor with the ALN status of each patient to determine in our population, which factors individually or in combination are predictive of ALN positivity. We then will compare and contrast our findings to similar studies published in recent literature.

Thirdly, we will test the hypothesis that SLN micrometastases are rarely associated with ALN positivity and that patients with micrometastases in only one SLN can safely decline an ALND.

Finally, we will test the hypothesis that SLN micrometastases are rarely associated with N2 (high stage) nodal disease.

VII. PATIENTS AND METHODS

The patient population for our retrospective analytical study was comprised of all available female breast cancer cases meeting inclusion criteria. A total of 96 cases were included in the study. Cases included were limited to all female patients who underwent curative surgical resection (BCT or mastectomy), successful SLNB (determined positive through routine histology) and immediate or subsequent ipsilateral ALND for primary breast cancer at one of three Winnipeg Regional Health Authority (WRHA) hospitals: Health Sciences Centre (HSC), St. Boniface General Hospital (SBGH) or Grace Hospital (GH) between January 2002 and March 2007. The 96 cases included: 69 cases from HSC, 14 cases from SBGH and 13 cases from GH.

The methodologies for obtaining pertinent cases varied among the three hospitals. These methodologies included accessing cytology, surgical pathology, frozen section and patient medical records.

A list of all breast SLN imprint reports from 2002-2006 was obtained from HSC cytology. The daily specimen lists from the gross lab were accessed to obtain a list of all SLNB cases from 2007. In order to differentiate between +SLN and -SLN cases, the surgical pathology reports were obtained for each case. The surgical pathology reports were reviewed and a list of all +SLN (on routine histology) cases was derived. Of the +SLN cases, only those including an immediate or subsequent completion ALND were included in the study.

Medical records at SBGH were contacted and specific database searches were requested. The searches were executed using keyword and coding abbreviations for

“breast cancer” and “sentinel lymph node”. A list of chart numbers for potentially relevant cases was generated, including cases from 2002-2006. The chart numbers were reviewed and surgical pathology reports obtained for cases including SLNB. The reports were reviewed to differentiate +SLN and –SLN cases as determined through routine histology. Of the +SLN cases, only those in which an immediate or subsequent completion ALND was performed were included in the study.

Intra-operative assessment audit records for GH from 2002-2007 were accessed and reviewed to generate a list of all breast SLNB cases. In order to differentiate between +SLN and false -SLN cases, the surgical pathology reports were obtained and reviewed for each case. Of the +SLN cases (determined through routine histology) only those including an immediate or subsequent completion ALND were included in the study.

The routine histopathology slides for all SLNs (submitted for SLNB and/or routine histology) for the 96 cases were obtained and reviewed by Dr. H. R. Wightman, Laboratory Director at GH. Initially 103 cases were identified in total. However, the routine histopathology slides for 7 of these cases were either missing, on loan or lost at the time of our slide review. This reduced the study population size to 96 cases.

Clinical information collected for each case included: pathology number, year, hospital, method of SLN diagnosis (imprints versus routine histology) and left versus right breast. Collected pathologic data of the primary breast tumor included: tumor greatest dimension, multifocality, LVI, tumor grade and histological type. Collected pathologic data of the SLNs included: total number of SLN submitted, number +SLN on imprint diagnosis, number +SLN on routine histology, which SLN contained the largest

metastasis, size of largest metastases, ECE and presence of subcapsular foci (SCF). Additional pathologic information included; ER/PR status, total number of ALN and number of +ALN.

SLN metastases sizes were re-evaluated by Dr. H. R. Wightman, measured in millimetres and classified as either a micrometastasis (≤ 2 mm) or a macrometastases (> 2 mm). Metastases were further subdivided into the following groups with respect to size: ≤ 2 mm, > 2 mm and ≤ 5 mm, > 5 mm and ≤ 10 mm, > 10 mm. In cases when there were multiple discrete sub-capsular metastatic foci in the SLN and if the linear distance between the two foci was less than the greatest diameter of the smaller focus, the foci were considered confluent and their greatest dimension was measured. This criterion was not limited to two discrete foci; it applied to any number of foci within a SLN.

ECE was defined as direct extension of tumor cells through the lymph node capsule into the extracapsular soft tissue.

Associated subcapsular foci (SCF) were defined as one or more foci of tumor distinct from the measured metastatic focus within any of the SLNs submitted for routine histology. SCF was further classified as either present within the SLN with the SLN with the largest metastasis, within additionally submitted SLNs or within both the SLN with the largest metastasis and additionally submitted SLNs.

If LVI was not specified on the surgical pathology report it was presumed negative. If multifocality of the primary tumor was not indicated on the surgical pathology report or the gross description, the tumor was presumed unifocal. If there was any doubt concerning the correct stage to which a particular primary tumor should be classified, the lower stage classification was chosen. This universally accepted guideline

is described by the International Union Against Cancer (UICC) TMN classification of malignant tumors.

Two variations of the MSKCC BCN were assessed, defined for the purposes of this study as MSKCC version A and MSKCC version B. A total of 76 cases were entered into the MSKCC version A BCN. In this group, imprint cytology was considered equivalent to frozen section. Ninety-six cases were entered into the MSKCC version B BCN in which the detection method was “routine H&E” by definition. A total of 96 cases were entered into the Mayo BCN.

The computerized MSKCC BCN, available from the MSKCC website, was utilized to calculate the predicted probability of ALN positivity for each case. A predicted probability was calculated for MSKCC version A and MSKCC version B BCN variations based on the variables outlined in Table 4. The Mayo BCN predicted probabilities were calculated based on the variables listed in Table 4, as described in Degnim *et al.*¹⁰.

Aforementioned clinical and pathologic variables of the patient and primary tumor were assessed through univariate and multivariate statistical analyses. The χ^2 univariate effects of the clinical and pathologic variables on ALN(s) positivity were summarized using *P* values and odds ratios including 95% confidence intervals (CI). The multivariate analysis executed through logistic regression and backwards stepwise logistic regression were summarized using *P* values and odds ratios (OR) including 95% confidence intervals (CI).

Validation of the three BCN was assessed through an area under the curve (AUC) of the receiver operating characteristic curve (ROC).

VIII. RESULTS

1. PATIENT CHARACTERISTICS

The study population was comprised of 96 cases. The average patient age was 55 (range 30-85 years). The incidence of breast cancer in the left versus right breast was 1 to 1.13 (total of 45 to 51). The average breast tumor size was 3.17 cm (range 0.7 cm to 8.5 cm), the average SLN metastasis size was 9.5 mm (range 0.010 mm to 28.00 mm), the average total number of SLN submitted per case was 2.25 (range 1 to 6) and the average total number of ALN submitted per case was 16.6 (range 4 to 44). Within the entire study population, 47% of cases had a +ALND. All descriptive clinical and pathologic variables included in the study are listed in Table 5. Table 5 lists the incidence of each variable within the study population and the proportion of cases possessing each variable that had a +ALND within the study population.

VIII. RESULTS

2. MICROMETASTATIC SUBGROUP

In the study population a subgroup of cases with micrometastatic SLNs was identified, comprised of twelve cases. Within this subgroup, three had +ALNs and the remaining nine had no additional nodal disease. Two cases demonstrated ECE, and the remaining ten did not. Of the ten cases without ECE, one demonstrated a +ALND, with N2 disease. Of the three cases with SLN micrometastasis and +ALNDs, one had no LVI and no ECE the other two had LVI coupled with ECE. Of the two latter cases, one had N1 (total of 3 +ALN, including all +SLN) and the other had N2 (total of 5 +ALN, including all +SLN) axillary disease. The case where LVI and ECE were absent had N2 disease of the axilla (total of 6 +ALN, including all +SLN).

VIII. RESULTS

3. UNIVARIATE ANALYSIS

The univariate analysis was executed on the following clinical and pathologic variables: age, breast, LVI, ER status, SLN detection method, total number SLN(s) submitted, ECE, SLN metastasis size, tumor size, tumor type, tumor grade and tumor type and grade (Table 6). The following variables were determined statistically significant: SLN detection method, SLN ECE, SLN metastasis size and breast tumor size (Table 6).

SLN detection method, specifically cases diagnosed through routine histology versus cases diagnosed through imprint cytology with subsequent routine histology (H&E), was determined statistically significant (*P* value 0.0276, OR 0.300, [95% CI] 0.0991-0.9083) (Table 6).

SLN ECE, specifically cases where ECE is present versus cases where ECE is absent, was determined statistically significant (*P* value 0.026, OR 2.5263, [95% CI] 1.1089-5.7553) (Table 6).

Breast tumor size, specifically ≤ 2 cm versus > 5 cm, was determined a significant predictor of +ALN status (*P* value 0.013, OR 6.670, [95% CI] 1.492-29.787) (Table 6).

SLN metastasis size, specifically > 5 mm versus ≤ 5 mm (*P* value 0.0255, OR 2.80, [95% CI] 1.1170-7.0188), > 10 mm versus ≤ 10 mm (*P* value 0.0095, OR 3.00, [95% CI] 1.2929-6.9612) and ≤ 2 mm versus > 10 mm (*P* value 0.0302, OR 5.00, [95% CI] 1.167-21.423), was determined statistically significant (Table 6).

The proportion of cases within each of the SLN metastasis size subgroups [≤ 2 mm, (>2 mm, ≤ 5 mm), (>5 mm, ≤ 10 mm) and >10 mm] that have +ALN(s) are listed in Table 7. Within the ≤ 2 mm subgroup comprised of 12 cases, 25% had +ALN(s). Within the >2 mm, ≤ 5 mm subgroup comprised of 18 cases, 33.3% had +ALN(s). Within the >5 mm, ≤ 10 mm subgroup comprised of 26 cases, 42.3% had +ALN(s). Finally, within the >10 mm subgroup comprised of 40 cases, 62.5% had +ALN(s) (Table 7).

VIII. RESULTS

4. MULTIVARIATE ANALYSIS

The following variables were excluded from the multivariate analysis: breast, lobular carcinoma, total number SLN submitted, tumor multifocality, SLN metastasis size ≤ 5 mm, tumor grade I and breast tumor size ≤ 2 cm. These variables were excluded based on the following: firstly their degree of statistical significance in the univariate analysis and secondly, the quantity of cases demonstrating the variable.

The multivariate analysis was executed on the following clinical and pathologic variables: age (by 10 year intervals), ductal carcinoma, LVI, SLN detection on H&E only, ECE, SLN metastasis size (>5 mm, ≤ 10 mm), SLN metastasis size ≥ 10 mm, tumor size (>2 cm, ≤ 5 cm), tumor size >5 cm, grade II, grade III, +ER status and +PR status (Table 8). Only age, (P value 0.0439, standard error 0.2375, OR 0.620, [95% CI] 0.389-0.987) and tumor size >5 cm (P value 0.0037, standard error 1.0751, OR 22.666, [95% CI] 2.756-186.407) were determined significant predictors of +ALN positivity (Table 8).

Although not determined to be significant, SLN detection on H&E only and SLN metastasis size ≥ 10 mm approached statistical significance and are worth mentioning. The P values for these variable are 0.0657 (standard error 0.7155, OR 0.268, [95% CI] 0.066-1.089) and 0.0531 (standard error 0.7334, OR 4.131, [95% CI] 0.981-17.391) respectively (Table 8).

Backward stepwise logistic regression identified the following variables as statistically significant: SLN detection on H&E only (OR 0.226, [95% CI] 0.065-0.781), ECE (OR 2.604, [95% CI] 1.01-6.721), SLN metastasis size ≥ 10 mm (OR 3.089, [95%

CI] 1.195-7.988) and tumor size >5 cm (OR 6.807, [95% CI] 1.479-31.33). These variables are listed in Table 9.

VIII. RESULTS

5. RECEIVER OPERATING CURVE ANALYSIS

Criteria for ROC validation of the MSKCC BCN version A and B are outlined in Table 4. Criteria include: pathologic size of tumor (cm), tumor type (ductal I, II, III or lobular), LVI, multifocality, ER status, method of SLN metastasis detection (touch imprints or routine H&E), number +SLN and number –SLN (Table 4) ¹⁷.

The criteria for the Mayo BCN as indicated in Degnim *et al.* were utilized for the ROC validation of the nomogram. These criteria included age, tumor size (cm), size of metastasis, +ER status, ECE, number of +SLN(s), number of –SLN(s), age coupled with +ER status and number of +SLN coupled with ECE ⁹.

Because of the procedural variations between the three hospitals (SBGH, GH and HSC) with respect to SLN procedures, the number of cases for validation of the MSKCC BCN version A (n=76) was different than for the validation of the MSKCC BCN version B (n=96) and the Mayo BCN (n=96). At SBGH the SLNs were diagnosed by H&E only, no imprint analysis was performed. At GH and HSC immediate SLN diagnosis by cytologic imprint analysis was performed with subsequent final diagnosis by H&E.

With respect to this study, the AUC is best understood as the probability of a correct diagnosis or assignment of ALN positivity in random pairs of patients, one with a +ALND and one with a –ALND. Based on random chance alone, on average, the outcome for the probability is 0.5 (50% chance of a correct assignment of ALN positivity ¹³. The AUC for the respective data indicates how our results differ from that of random chance.

The MSKCC BCN version A predicted the probability of correct assignment of ALN positivity with an AUC of 0.66597 (standard error 0.06296, [95% CI] 0.52392-0.77198) (Figure 1). This result may be interpreted as a 0.16597 increase in the probability of a correct assignment of ALN positivity by the nomogram, rather 0.16597 higher than “would be expected from random chance alone” (0.5)¹⁴. The prevalence of ALN positivity using this nomogram was 52.63% (Table 10). The lowest predicted probability of ALN positivity using the MSKCC BCN version A was 21% (Data not shown).

The MSKCC BCN version B predicted the probability of correct assignment of ALN positivity with an AUC of 0.71198 (standard error 0.05305, [95% CI] 0.59174-0.80123) (Figure 2). This result may be interpreted as a 0.21198 increase in the probability of a correct assignment of ALN positivity by the nomogram, rather 0.21198 higher than “would be expected from random chance alone” (0.5)¹⁴. The prevalence of ALN positivity using this nomogram was 46.88% (Table 10). The lowest predicted probability of ALN positivity using the MSKCC BCN version B was 17% (Data not shown).

The Mayo BCN predicted the probability of correct assignment of ALN positivity with an AUC of 0.64532 (standard error 0.05761, [95% CI] 0.51810-0.74450) (Figure 3). This result may be interpreted as a 0.14532 increase in the probability of a correct assignment of ALN positivity by the nomogram, rather 0.14532 higher than “would be expected from random chance alone” (0.5)¹⁴. The prevalence of ALN positivity using this nomogram was 46.88% (Table 10). The lowest predicted probability of ALN positivity using this BCN was 1% (Data not shown).

The predicted probability cut-off values for the ROC analysis (at 5%, 10%, 20%, 30%, 40% and 50%) using the MSKCC BCN version A, B and the Mayo BCN are listed in Table 11. Using the MSKCC BCN version A, no cases were identified with a predicted probability of a +ALND under 20% (Table 11). The lowest predicted probability of ALN positivity using this nomogram was 21%. Using the MSKCC BCN version B, no cases were identified with a predicted probability of a +ALND under 10% (Table 11). The lowest predicted probability of ALN positivity using this nomogram was 17%. Using the Mayo BCN, 15 cases were identified with a predicted probability of a +ALND under 5%, 19 cases under 10% and 20 cases under 15% (Table 11). The lowest predicted probability of ALN positivity using this nomogram was 1%.

IX. DISCUSSION

The primary goal of this study was to validate two literature derived BCN that could potentially predict ALN positivity with an accepted degree of accuracy. As indicated in Lambert *et al.*, it is generally accepted that an AUC value between 0.7 and 0.8 represents a reasonable probability of a correct assignment of ALN positivity by a nomogram¹³. Among the three BCNs tested, only the MSKCC version B achieved this minimum standard, with an AUC of 0.71198 (Table 10). The MSKCC BCN version A demonstrated an AUC of 0.66597 and the Mayo BCN demonstrated an AUC of 0.64532 (Table 10).

Despite the successful validation of the MSKCC BCN version B, it is of no usefulness in our patient population. This is due to fact that the nomogram did not identify any cases with a low enough predicted probability of +ALNs to be clinically useful. In the Degnim *et al.* paper presenting the Mayo BCN, the issue of predicted probability cut off points was discussed in some detail¹⁰. It was felt to be unlikely that a clinician would recommend against a completion ALND at a predicted value of >10%¹⁰. Ideally, the value should be <10%. In our population, as noted in Table 11, no patient had a predicted probability low enough to comfortably recommend against a completion ALND.

This issue of poor clinical utility is one that has not been significantly addressed in discussing various BCNs in the literature. The initial study performed by Van Zee *et al.* did not even address the issue of predicted probability cut off values³⁶. The Mayo study however, documented predicted probabilities in both their patient population and

that of the University of Michigan using both the MSKCC and Mayo BCNs ¹⁰. Interesting and unexplained variations between the two populations emerged. In applying the MSKCC BCN 8% of the Mayo population had a predicted probability of <10% as opposed to 41% of the smaller Michigan population (n=69) ¹⁰. Applying the Mayo BCN to the Mayo and Michigan populations, only 2.6% of the Mayo population had a predicted probability of <10% versus 5.7% of the Michigan population ⁹. A single Mayo patient (n=462) had a predicted probability of <5% ¹⁰.

The lack of any patients within clinically useful predicted probability groups in our population may be potentially explained by two factors, one pathologic and one surgical. In manipulating the MSKCC BCN (version B) two factors are very heavily weighted in determining the final predicted value of +ALND, SLN detection method and the number of -SLNs. Specifying SLN detection method as “serial sections” or “IHC” markedly lowers the end predicted value ¹⁷. As serial sectioning and IHC were not routinely performed in our population, this may have created a significant bias toward higher predicted values. The surgical component relates to the number of -SLN submitted for evaluation. In our patient population, an average of 0.8 -SLNs were removed per case (data not shown). In the retrospective MSKCC BCN study population the -SLN average was at least 1.45 -SLN per case ³⁶. Once again, increased numbers of -SLNs significantly lower predicted probability in the MSKCC BCN.

A surprising finding in our study was that the Mayo BCN was far less restrictive in our population than either version of the MSKCC BCN. 15.6% and 19.8% of our population fell within the limits of $\leq 5\%$ and $\leq 10\%$ predicted probabilities respectively (Table 11). No obvious explanation for this discordance is apparent. Unfortunately,

although the Mayo BCN is much more clinically useful in our population, it has not been validated in our study.

In examining our results from univariate and multivariate analysis, they correlate with the findings of established literature and are reported in Table 6, 8 and 10. The most significant finding from the point of view of this study is the significance of ECE in predicting ALN positivity (Table 9). This is due to the fact that some surgeons in our study institutions (personal observation, Dr. H. R. Wightman) are already offering chemotherapy and observation only to selected patients with a single SLN micrometastasis. This practice should be particularly discouraged in patients with ECE positive micrometastases, due to the increased likelihood of +ALNs in this group.

The third focus of our study was to determine whether micrometastases as a group within +SLNs are associated with a low risk of a +ALND. Regrettably, due to small numbers (n=12) few statistical conclusions could be drawn within this group. Of interest is the low percentage of micrometastases in our population (12.5%) in comparison with larger American studies ^{1, 11, 12, 23}. Specifically, micrometastases comprise 26% of the total patient population in the previously noted Rivers *et al.* study ²³. The reason for this difference is somewhat speculative. However, the absence of more rigorous histological examination in our population (serial sectioning and IHC) makes it very likely that many small micrometastases were simply reported as -SLNs. Additionally it is possible that some patients with micrometastases did not meet the selection criteria for this study. Inclusion in this study required a completion ALND. As noted above, some patients in the study institutions are not undergoing completion ALND, and it is probable that micrometastases are overrepresented in this group.

Our final hypothesis was that micrometastatic disease is rarely associated with high stage (N2) disease. The principle behind our final hypothesis is that limited metastatic disease (N1) in the axilla is not an indication for completion ALND, even in the presence of a +SLN. This is due to the fact that chemotherapy can eradicate small volume axillary disease. This position is supported by a National Surgical Adjuvant Breast Project conducted in the 1970s which demonstrated no survival advantage for axillary dissection versus observation in clinically negative lymph nodes ²³. If one accepts this concept, then the most important issue is the exclusion of high stage (N2) disease. We hypothesized that SLN micrometastases as a group would not demonstrate N2 disease in our population. This hypothesis is supported by Rivers *et al.* where only 1 of 74 (1.4%) of micrometastases demonstrated N2 disease with completion ALND ²³. Unfortunately, even in our small population of patients with micrometastases (n=12), 2 patients demonstrated N2 disease (16.7%), including one patient without ECE.

An unexplained finding in our study was the significantly lesser likelihood of a +ALND in the SBGH population only. This group is represented in our analysis as the “SLN on H&E only” subgroup, as intra-operative touch imprints were not performed at SBGH during the study period. The odds ratio for a +ALND at SBGH was 0.226 ([95%CI] 0.065-0.781) (Table 9). Any explanation of this finding is speculative. However, it is possible that patient selection criteria for SLNB differ between HSC, GH and SBGH.

Our study suffers from significant limitations. The most important of these is the small size of our study population (n=96). Particularly, only 12 patients with micrometastatic SLN disease were identified (Table 5). A second possible limitation is

bias introduced by our inclusion criteria. The HSC cohort consisted entirely of SLNBs that had undergone intra-operative assessment by imprint cytology. A small proportion of SLNBs at HSC are processed through routine histology only. Furthermore, in 23 +SLNB cases, completion ALNDs could not be found within the WRHA database. A proportion of these cases likely represents patients who elected to undergo chemotherapy and axillary observation as opposed to completion ALND. These cases were not included in our study. Patients being offered axillary observation only as opposed to completion ALND would likely be considered by their clinicians to have lower risk disease. Particularly, it is likely that they would have a higher rate of micrometastatic SLN disease than the overall population.

Our study would obviously be improved by larger patient numbers. A larger number of cases with micrometastases would likely allow us to draw clinically useful conclusions within this group. In the present study, ECE negative micrometastatic SLN disease was associated with +ALND in only one of ten cases, although this case did demonstrate N2 disease. Larger numbers would likely support the hypothesis that ECE negative micrometastatic SLNs are seldom associated with a +ALND. A larger study would be less likely to change our conclusions regarding validation of the published BCNs.

An additional improvement would be the analysis of patients with a +SLNB who did not undergo a completion ALND. This would allow separate analysis of this group, which may well demonstrate lower risk clinicopathologic variables than the overall population.

In summary, although a well established BCN (MSKCC BCN version B) was validated in our patient population, it demonstrated no clinical utility due to the absence of patients within clinically acceptable predicted probability values. Furthermore, micrometastatic disease in a SLN did not reliably exclude either low or high stage nodal disease in the completion ALND. We therefore recommend completion ALND in all cases of +SLNB, with the possible exception of patients with ECE negative micrometastatic disease.

X. CONCLUSIONS

This study has validated a well established BCN (MSKCC BCN version B) in our patient population. Unfortunately, no patients fell within accepted predicted probabilities for a negative completion ALND, indicating that the BCN is of no clinical utility for our population. Furthermore, we failed to support the hypothesis that micrometastatic disease in a +SLN was not associated with high (N2) stage metastatic nodal disease in the completion ALND. We also failed to identify any subgroup in this population that could reasonably be assured of a negative completion ALND after a +SLNB, with the possible exception of ECE negative micrometastatic SLN cases.

Table 1: Summary of tumor(T) clinical and pathological classifications of breast tumors based on facts from The International Union Against Cancer TNM Classification of Malignant Tumors 6th ed. 2002 ²⁴.

T-Primary Tumor (clinical and pathological classifications are interchangeable)			
TX	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
Tis	Carcinoma <i>in situ</i>	Tis(DCIS ¹)	Ductal carcinoma <i>in situ</i>
		Tis(LCIS ²)	Lobular carcinoma <i>in situ</i>
		Tis(Paget)	Paget disease of the nipple with no tumor
T1	Tumor ≤ 2 cm	T1mic	≤ 0.1 cm (microinvasion) greatest dimension
		T1a	$>0.1 - 0.5$ cm greatest dimension
		T1b	$>0.5 - 1$ cm greatest dimension
		T1c	$<1 - 2$ cm greatest dimension
T2	>2 to 5 cm greatest dimension		
T3	>5 cm greatest dimension		
T4	Tumor of any size with direct extension to the chest wall/skin	T4a	Extension to chest wall
		T4b	Skin edema and/or ulceration with/without satellite skin nodules
		T4c	Both 4a and 4b
		T4d	Inflammatory carcinoma ³

1. Ductal carcinoma *in situ*
2. Lobular carcinoma *in situ*
3. Characterized by “diffuse brawny induration of the skin with an erysipeloid edge, usually with no underlying mass” ²⁵.

Table 2: Summary of lymph node (N) clinical and pathological classifications of breast tumors based on facts from International Union Against Cancer TNM Classification of Malignant Tumors 6th ed. 2002 ²⁴

N-Regional Lymph Nodes (clinical and pathological classifications are distinct)				
NX	Regional lymph nodes cannot be assessed (previously removal)			
N0	No regional lymph nodes metastasis			
N1	Metastasis present in movable ipsilateral axillary lymph node(s)		pN1mic	Micrometastasis ($>0.2 \text{ mm} \leq 2 \text{ mm}$)
			pN1a	Metastasis in 1-3 axillary lymph nodes, at least one with metastasis size $>2 \text{ mm}$
			pN1b	Metastasis in internal mammary lymph nodes. Sentinel lymph node dissection reveals microscopic metastasis however, lymph nodes are not clinically apparent.
			pN1c	Metastasis in 1-3 axillary lymph nodes and internal mammary lymph nodes. Sentinel lymph node dissection reveals microscopic metastasis however, lymph nodes are not clinically apparent.
N2	N2a	Metastasis present in fixed axillary lymph nodes	pN2a	Metastasis in 4-9 axillary lymph nodes, at least one with metastasis size $>2 \text{ mm}$
	N2b	Metastasis present in clinically apparent internal mammary lymph nodes	pN2b	Metastasis in clinically apparent internal mammary lymph nodes, in the <i>absence</i> of axillary lymph node metastasis
N3	N3a	Metastasis present in infraclavicular lymph nodes	pN3a	Metastasis in ≥ 10 axillary nodes, at least one with metastasis size $>2 \text{ mm}$, <i>or</i> metastasis in infraclavicular nodes
	N3b	Metastasis present in internal mammary and axillary lymph nodes	pN3b	Metastasis present in clinically apparent internal mammary lymph nodes with >3 positive axillary lymph nodes. In addition, positive internal mammary lymph nodes with microscopic metastasis detected through sentinel lymph node dissection however, not clinically apparent.
	N3c	Metastasis present in supraclavicular lymph nodes	pN3c	Metastasis present in supraclavicular lymph nodes

Table 3: Summary of distant metastasis (M) clinical and pathological classifications of breast tumors based on facts from International Union Against Cancer TNM Classification of Malignant Tumors 6th ed. 2002 ²⁴

M-Distant Metastasis (clinical and pathological classifications are interchangeable)	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis is present
M1	Distant metastasis is present

Table 4: Required clinicopathologic variables for the Memorial Sloan Kettering Cancer Center Breast Cancer Nomogram versions A and B as defined in our study and the Mayo Clinic Breast Cancer Nomogram^{10, 17}

Variable	Memorial Sloan Kettering Cancer Center Breast Cancer Nomogram Version A	Memorial Sloan Kettering Cancer Center Breast Cancer Nomogram Version B	Mayo Clinic Breast Cancer Nomogram
Frozen section performed	Included	Excluded	Excluded
Patient age	Excluded	Excluded	Included
Pathologic size of tumor (cm)	Included	Included	Included
Tumor type: Ductal (specify grade I, II or III) Lobular	Included	Included	Excluded
LVI	Included	Included	Excluded
Multifocal	Included	Included	Excluded
Estrogen receptor status (+/-)	Included	Included	Included
Method of sentinel lymph node metastasis detection: Frozen section Routine histopathology Serial H&E* Immunohistochemistry	Included	Included (excludes frozen section)	Excluded
Number positive sentinel lymph nodes	Included	Included	Included
Number negative sentinel lymph nodes	Included	Included	Included
Sentinel lymph node extracapsular extension	Excluded	Excluded	Included
Size of sentinel lymph node metastasis	Excluded	Excluded	Included
Age/Estrogen receptor status positive	Excluded	Excluded	Included
Number positive sentinel lymph nodes/Extracapsular extension	Excluded	Excluded	Included

* Hematoxylin and eosin staining on routine histology

Table 5: Overall patient and tumor variables within our study population

Variable	Variable Divisions	Number of Cases (n=96)	% of Total Population	% of Proportion of Total Population With Positive Axillary Lymph Node Dissection
Age	<50	30	31	51
	≥50	66	69	42
Breast	Right	51	53	41
	Left	45	47	49
Breast tumor size	T1 (≤2.0 cm)	30	31	32
	T2 (>2.0, ≤ 5.0 cm)	53	55	45
	T3 (>5.0 cm)	13	14	74
Tumor grade	I	14	15	48
	II	58	60	40
	III	24	25	56
Pathology of tumor	Ductal carcinoma	86	90	46
	Lobular carcinoma	10	10	38
Tumor lymphovascular invasion	Present	50	52	54
	Absent	46	48	35
Number of sentinel lymph nodes removed	Single	36	38	48
	Multiple	60	63	43
Number of positive sentinel lymph nodes on routine histology (Hematoxylin and eosin staining)	Single	61	64	39
	Multiple	35	36	55
Number of negative sentinel lymph nodes on routine histology (Hematoxylin and eosin staining)	None	55	57	56
	Single	19	20	35
	Multiple	22	23	26
Size of sentinel lymph node metastasis	≤ 2 mm	12	13	24
	> 2, ≤ 5 mm	18	19	32
	> 5, ≤ 10 mm	26	27	41
	> 10 mm	40	42	60
Sentinel lymph node extracapsular extension	Present	46	48	56
	Absent	50	52	35
Multifocality	Present	21	22	46
	Absent	75	78	45
Estrogen receptor status	Positive	77	80	45
	Negative	19	20	45
Progesterone receptor status	Positive	64	67	44
	Negative	32	33	48
Axillary lymph node status	Positive	45	47	N/A*
	Negative	51	53	N/A*
Number positive axillary lymph nodes (Excluding sentinel lymph nodes)	≤4	31	32	N/A*
	>4	14	15	N/A*
Total number of positive lymph nodes (Total of all positive axillary and sentinel lymph nodes)	≤4	73	76	N/A*
	>4	23	24	N/A*

* Not applicable

Table 6: Univariate analyses testing associations between clinicopathologic variables and positive axillary lymph node status

Variables	Confidence Interval (95%)			Odds Ratio	P value (X ²)
Age (≥50 vs. <50)	0.2883	-	1.6312	0.6858	0.3926
Breast Left vs. Right	0.5213	-	2.6010	1.1645	0.7103
Lymphovascular Invasion Present vs. Absent	0.9575	-	4.9230	2.1711	0.0618
Multifocality Present vs. Absent	0.3942	-	2.7383	1.0390	0.9384
Estrogen Receptor Status Positive vs. Negative	0.3542	-	1.9385	0.9756	0.9616
Sentinel Lymph Node Detection Method Only H&E ¹ vs. Imprints & H&E ¹	0.0991	-	0.9083	0.3000	0.0276
Total Number Sentinel Lymph Nodes Submitted Multiple vs. Single	0.3575	-	1.8725	0.8182	0.6346
Sentinel Lymph Node Extracapsular Extension Present vs. Absent	1.1089	-	5.7553	2.5263	0.026
Sentinel Lymph Node Metastasis Size >2mm vs. ≤2 mm >5 mm vs. ≤5mm >10 mm vs. ≤10 mm ≤2 mm vs. (>2, ≤ 5mm) ≤2 mm vs. (>5, ≤ 10 mm) ≤2 mm vs. >10 mm	0.7586 1.1170 1.2929 0.293 0.481 1.167	- - - - - -	11.8636 7.0188 6.9612 7.681 10.066 21.423	3.0000 2.8000 3.0000 1.500 2.200 5.000	0.1045 0.0255 0.0095 0.6266 0.3095 0.0302
Breast Tumor Size ≤2 cm vs. >2, ≤5 cm ≤2 cm vs. >5 cm	0.070 1.492	- -	4.531 29.787	1.786 6.670	0.2223 0.013
Tumor Type Ductal vs. Lobular	0.3600	-	5.1884	1.3667	0.6453
Tumor Grade Grade I vs. Grade II Grade I vs. Grade III Ductal Only Grade I vs. Grade II Grade I vs. Grade III	0.219 0.372 0.248 0.459	- - - -	2.276 5.268 2.880 7.177	0.706 1.400 0.845 1.815	0.5598 0.6187 0.7876 0.3955
Tumor Type & Grade Ductal vs. Lobular, Grade II Ductal vs. Lobular, Grade I	0.2594 N/A ²	- -	5.6161 N/A ²	1.2069 0	0.8104 0.2994

1. Hematoxylin and eosin staining on routine histology
2. Not applicable

Table 7: Axillary lymph node positivity rates within subgroups for sentinel lymph node metastasis size

Subgroups for Sentinel Lymph Node Metastasis Size	Number of Cases in Subgroup	Axillary Lymph Node Positivity Rate (%)
≤ 2 mm	12	25%
>2 mm, ≤ 5 mm	18	33.3%
>5 mm, ≤ 10 mm	26	42.3%
>10 mm	40	62.5%

Table 8: Multivariate analyses testing associations between clinicopathologic variables and axillary lymph node status

Variable	Coefficient	Standard Error	P value	Odds Ratio	Confidence Interval		
Intercept	1.2412	2.0986	0.5542			-	
Age (by 10 year intervals)	-0.4785	0.2375	0.0439	0.620	0.389	-	0.987
Ductal carcinoma	0.0513	0.9143	0.9553	1.053	0.175	-	6.316
Lymphovascular invasion	0.0898	0.5615	0.8729	1.094	0.364	-	3.288
Sentinel lymph node detection on H&E* only	-1.3171	0.7155	0.0657	0.268	0.066	-	1.089
Extracapsular extension	0.5920	0.5574	0.2881	1.808	0.606	-	5.39
Sentinel lymph node metastasis size >5, ≤10 mm	0.5702	0.7690	0.4584	1.769	0.392	-	7.984
Sentinel lymph node metastasis ≥10 mm	1.4185	0.7334	0.0531	4.131	0.981	-	17.391
Tumor size >2, ≤5 cm	0.3422	0.6143	0.5776	1.408	0.422	-	4.694
Tumor size >5 cm	3.1209	1.0751	0.0037	22.666	2.756	-	186.407
Grade II	-0.7499	0.8830	0.3957	0.472	0.084	-	2.667
Grade III	-0.2581	0.9984	0.7960	0.773	0.109	-	5.467
Estrogen receptor positive	0.7792	0.8566	0.3630	2.180	0.407	-	11.682
Progesterone receptor positive	-0.6937	0.6871	0.3127	0.500	0.13	-	1.921

* Hematoxylin and eosin staining on routine histology

Table 9: Backward stepwise logistic regression of statistically significant clinicopathologic variables and axillary lymph node positivity

Variable	Odds Ratio	Confidence Interval		
Sentinel lymph node detection on H&E* only	0.226	0.065	-	0.781
Extracapsular extension	2.604	1.01	-	6.721
Sentinel lymph node metastasis ≥ 10 mm	3.089	1.195	-	7.988
Tumor size > 5 cm	6.807	1.479	-	31.33

* Hematoxylin and eosin staining on routine histology

Table 10: Receiver operating curve analysis and area under the curve for axillary lymph node positivity, Memorial Sloan Kettering Cancer Center breast cancer nomogram version A, version B and the Mayo breast cancer nomogram

Nomogram	Estimate of Area Under the Curve	Area Under the Curve Standard Error	Confidence Interval (95%)	Prevalence of Axillary Lymph Node Positivity	Cases (n)
Memorial Sloan Kettering Cancer Center Breast Cancer Nomogram version A	0.66597	0.06296	0.52392 - 0.77198	0.52632	76
Memorial Sloan Kettering Cancer Center Breast Cancer Nomogram version B	0.71198	0.05305	0.59174 - 0.80123	0.46875	96
Mayo Clinic Breast Cancer Nomogram	0.64532	0.05761	0.51810 - 0.74460	0.46875	96

Table 11: Predicted probability cut-off values at 5%, 10%, 20%, 30%, 40% and 50% using the Memorial Sloan Kettering Cancer Center breast cancer nomogram version A, B and the Mayo Clinic breast cancer nomogram on our study population

Predicted Probability of Positive Axillary Lymph Node(s)	Breast Cancer Nomogram	Number of Cases Within Cut-off Group	Proportion (%) of Total Population
≤ 5%	Memorial Sloan Kettering Cancer Center version A	0/76	N/A*
	Memorial Sloan Kettering Cancer Center version B	0/96	N/A*
	Mayo Clinic	15/96	15.6
≤ 10%	Memorial Sloan Kettering Cancer Center version A	0/76	N/A*
	Memorial Sloan Kettering Cancer Center version B	0/96	N/A*
	Mayo Clinic	19/96	19.8
≤ 20%	Memorial Sloan Kettering Cancer Center version A	0/76	N/A*
	Memorial Sloan Kettering Cancer Center version B	3/96	3.1
	Mayo Clinic	20/96	20.8
≤ 30%	Memorial Sloan Kettering Cancer Center version A	5/76	6.6
	Memorial Sloan Kettering Cancer Center version B	12/96	12.5
	Mayo Clinic	26/96	27.1
≤ 40%	Memorial Sloan Kettering Cancer Center version A	10/76	13.2
	Memorial Sloan Kettering Cancer Center version B	23/96	24.0
	Mayo Clinic	37/96	38.5
≤ 50%	Memorial Sloan Kettering Cancer Center version A	25/76	32.9
	Memorial Sloan Kettering Cancer Center version B	44/96	45.8
	Mayo Clinic	49/96	51.0

* Not applicable

**Receiver Operating Curve of Axillary Lymph Node
Positivity, Memorial Sloan Kettering Cancer Center Breast
Cancer Nomogram Version A**

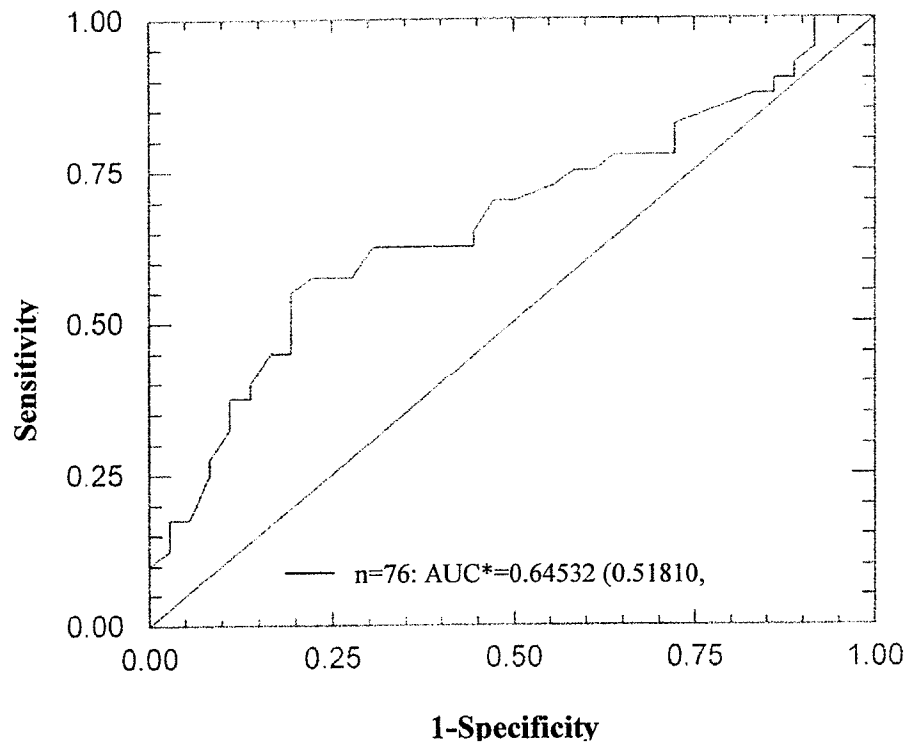


Figure 1: Receiver operating characteristic curve calculation for all (n=76) positive sentinel lymph node patients using the Memorial Sloan Kettering Cancer Center breast cancer nomogram version A

* Area under the curve

**Receiver Operating Curve of Axillary Lymph Node
Positivity, Memorial Sloan Kettering Cancer Center Breast
Cancer Nomogram Version B**

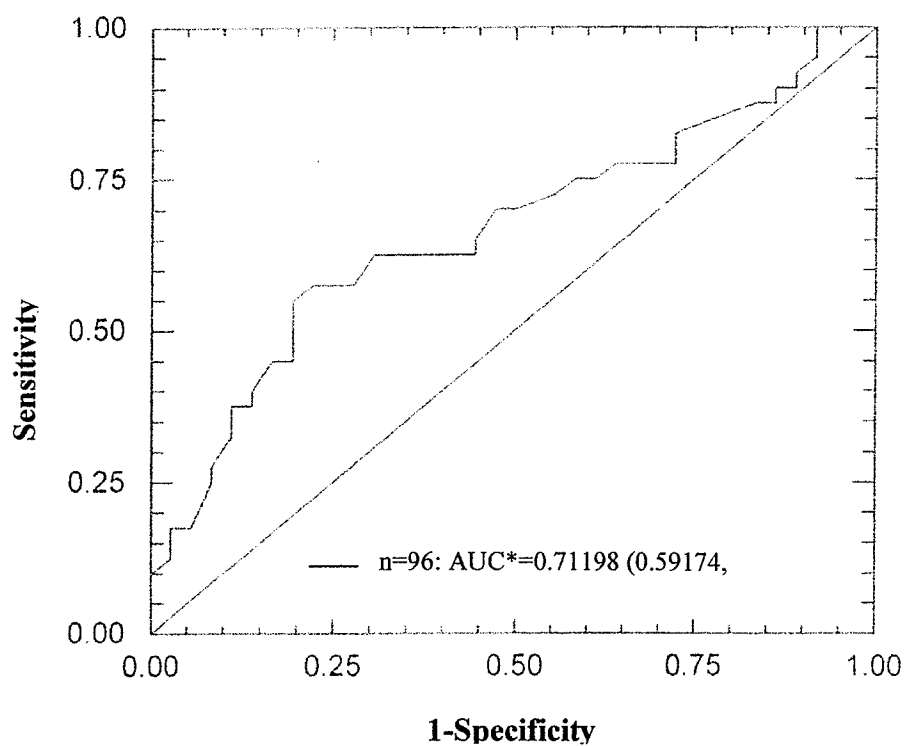


Figure 2: Receiver operating characteristic curve calculation for all (n=96) positive sentinel lymph node patients using the Memorial Sloan Kettering Cancer Center breast cancer nomogram version B

* Area under the curve

**Receiver Operating Curve of Axillary Lymph Node
Positivity, Mayo Clinic Breast Cancer Nomogram**

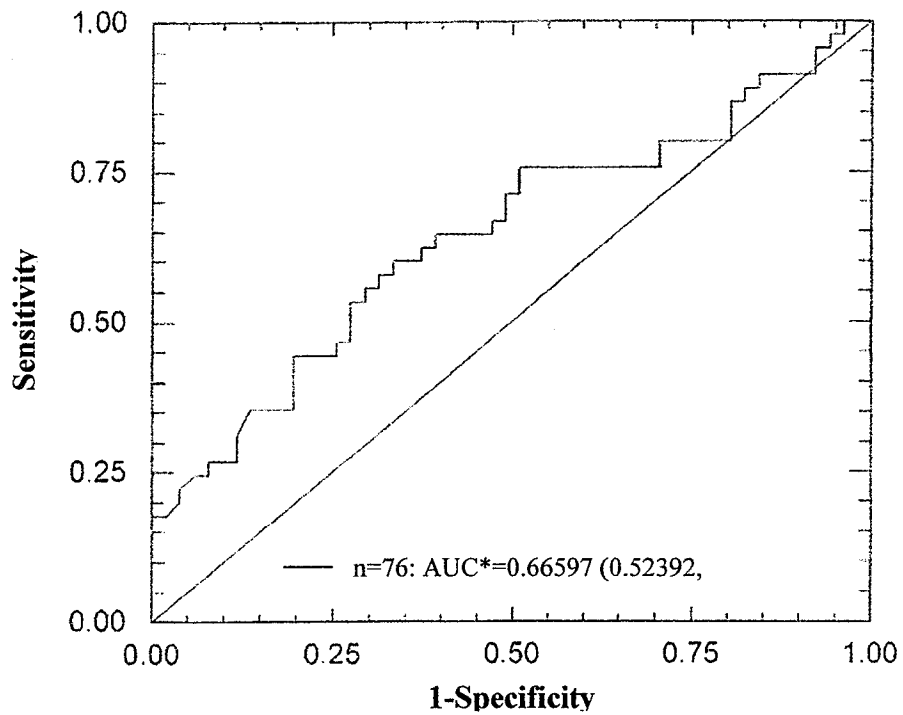


Figure 3: Receiver operating characteristic curve calculation for all (n=96) positive sentinel lymph node patients using the Mayo Clinic breast cancer nomogram

* Area under the curve

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