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Sentinel Node in Clinical Practice

*Implications for Breast Cancer Treatment and
Prognosis*

YVETTE ANDERSSON



ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2012

ISSN 1651-6206
ISBN 978-91-554-8316-6
urn:nbn:se:uu:diva-171078

Dissertation presented at Uppsala University to be publicly examined in Aulan, Ingång 21, Västmanlands Sjukhus, Västerås, Saturday, May 12, 2012 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English.

Abstract

Andersson, Y. 2012. Sentinel Node in Clinical Practice: Implications for Breast Cancer Treatment and Prognosis. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 754. 55 pp. Uppsala. ISBN 978-91-554-8316-6.

The introduction of sentinel lymph node biopsy (SLNB) has conveyed several new issues, such as the risk of false negativity, long-term consequences, the prognostic significance of micrometastases and whether ALND can be omitted in sentinel lymph node- (SLN) positive patients.

Archived SLN specimens from 50 false negative patients and 107 true negative controls were serially sectioned and stained with immunohistochemistry. The detection rate of previously unknown metastases did not differ between the false and the true negative patients. The risk of false negativity was higher in patients with multifocal or hormone receptor-negative tumours, or if only one SLN was found.

In a Swedish multicentre cohort, 2216 SLN-negative patients in whom ALND was omitted were followed up for a median of 65 months. The isolated axillary recurrence rate was only 1.0%, and the overall survival was high (93%).

The survival of 3369 breast cancer patients (2383 node-negative (pN0), 107 isolated tumour cells (pN0(i+)), 123 micrometastases (pN1mi) and 756 macrometastases (pN1)) was analysed. The 5-year cause-specific and event-free survival was worse for pN1mi and pN1 patients than for pN0 patients. There was no difference in survival between pN0(i+) and pN0 patients.

Tumour and SLN characteristics in 869 SLN-positive patients were compared between those with and without non-SLN metastases, and the Tenon score was calculated. The risk of non-SLN metastases was higher in case of SLN macrometastases (compared with micrometastases), a high positive/total SLN ratio and Elston grade 3 tumours, and increased with increasing tumour size. The area under the curve (AUC) for the Tenon score was 0.65, and the test thus performed inadequately in this population.

In conclusion, despite the risk of false negativity, SLNB with omission of ALND in SLN-negative patients appears to be safe even in the long term. The presence of micrometastases is of prognostic importance and should entail adjuvant treatment. The need for ALND in patients with SLN micro- and even macrometastases has been questioned, but the occurrence of non-SLN metastases is hard to predict, and strong evidence for the safe omission of ALND is lacking.

Keywords: breast cancer, sentinel node, micrometastases, survival, non-sentinel node metastases

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ISSN 1651-6206

ISBN 978-91-554-8316-6

urn:nbn:se:uu:diva-171078 (<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-171078>)

I get by...
I'm gonna try
with a little help from my friends

J. Lennon, P. McCartney

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I Andersson Y, Frisell J, Sylvan M, de Boniface J, Bergkvist L. Serial sectioning of breast cancer sentinel nodes does not significantly improve false negativity rate. Manuscript.
- II Andersson Y, de Boniface J, Jönsson P-E, Ingvar C, Liljegren G, Bergkvist L, Frisell J; on behalf of the Swedish Breast Cancer Group and the Swedish Society of Breast Surgeons. Axillary recurrence rate 5 years after negative sentinel node biopsy for breast cancer. *Br J Surg* **2012** Feb; 99(2): 226-231
- III Andersson Y, Frisell J, Sylvan M, de Boniface J, Bergkvist L. Breast cancer survival in relation to the metastatic tumor burden in axillary lymph nodes. *J Clin Oncol* **2010** Jun; 28(17): 2868-73
- IV Andersson Y, Frisell J, de Boniface J, Bergkvist L. Prediction of non-sentinel lymph node metastases: evaluation of the Tenon score. *Breast Cancer: Basic and Clinical Research* **2012**;6 31-38

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Abbreviations

ALND	Axillary lymph node dissection
cALND	Completion axillary lymph node dissection
AUC	Area under the curve
CSC	Cancer stem cell
EMT	Epithelial to mesenchymal transition
FNR	False negative rate
HE	Haematoxylin and eosin
IHC	Immunohistochemistry
ITC	Isolated tumour cells
MET	Mesenchymal to epithelial transition
OS	Overall survival
ROC	Receiver operating characteristics
SLN	Sentinel lymph node
SLNB	Sentinel lymph node biopsy

Introduction

Breast cancer is the most common cancer disease amongst European women, and every year just over 7000 Swedish women are diagnosed with the disease [1]. One in every 10 women in Sweden is at risk of developing breast cancer before 75 years of age. Older women account for the majority of the incidence, but almost 20% are less than 50 years old.

Despite an increasing incidence during the last 30-40 years, there is a slight decrease in the mortality rate since the 1980s, partly because of earlier diagnosis due to screening mammography and increased awareness of the disease, partly because of more effective treatment. It remains, however, that every year 1500 Swedish women die from breast cancer [1].

About 50% of patients are diagnosed by screening mammography, while the other 50% are symptomatic.

Treatment

The main treatment for primary breast cancer is surgery. Most women are treated by breast-conserving surgery, but 40-45% undergo mastectomy because of large or multifocal tumours, or at their own request. Almost all patients receive some adjuvant therapy, including systemic treatment and radiotherapy.

To decide on the appropriate systemic treatment, the primary tumour and the axillary lymph nodes are characterized by pathological examination. The size and mitotic activity of the tumour are measured, oestrogen, progesterone and HER-2 receptor status is determined, and the tumour is graded according to the Elston score. Furthermore, the lymph nodes are examined, and in the event of metastases, these are classified into macrometastases (>2 mm), micrometastases (>0.2 - 2 mm) and isolated tumour cells, ITCs (≤ 0.2 mm)[2] (Figure 1).

Patients with oestrogen receptor-positive tumours larger than 10 mm receive hormonal treatment, and those with axillary lymph node metastases, or with a combination of unfavourable tumour characteristics (large tumour, high Elston grade or mitotic activity, or progesterone receptor negativity) are considered for chemotherapy. If the tumour is HER-2 positive, trastuzumab is offered in combination with chemotherapy.

If breast-conserving surgery has been performed, radiotherapy to the breast is given. Furthermore, radiotherapy is given to the chest wall if the cancer is multifocal or extensive, and in the event of axillary lymph node metastases, regional lymph nodes are included in the radiation field.

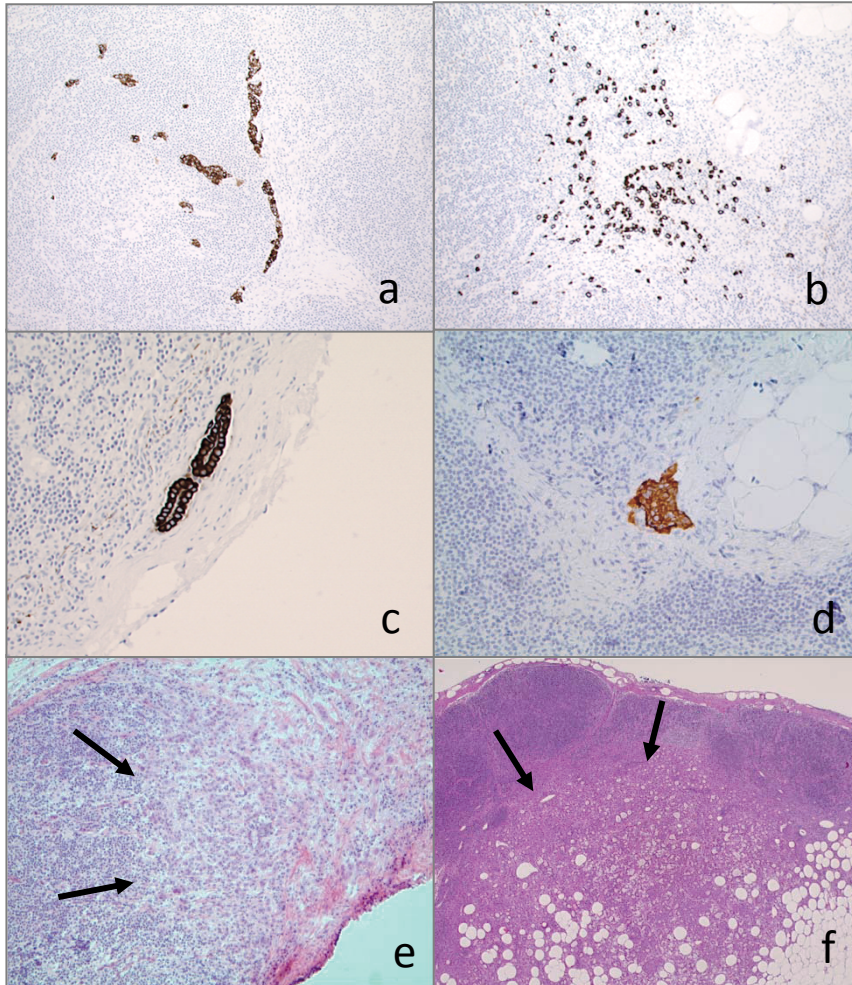


Figure 1. Sentinel lymph node metastases: immunohistochemistry- (IHC) stained isolated tumour cells (a, b and c), micrometastasis stained with IHC (d) and haematoxylin and eosin (HE) (e) and HE-stained macrometastasis (f).

Historical aspects of breast cancer surgery

Even though ancient Egyptian physicians more than 3500 years ago tried to treat breast cancer by cutting out the tumour, they found that amputating the breast did not usually prolong life and considered cancer a systemic disease [3].

In 460 BC, Hippocrates (Figure 2) introduced the “humoural theory”. The body consisted of four “humours” (blood, phlegm, yellow and black bile) which mirrored the building blocks of nature (air, water, fire and earth). Any imbalance between these humours caused illness [4]. Because of the black and hard appearance of an untreated tumour penetrating the skin, Hippocrates believed that breast cancer erupted from black bile and called the condition *karkinos* (Greek for crab), which later evolved into the term carcinoma [3].

In 200 AD, Galen succeeded Hippocrates as the dean of Greek medicine, and he and his apprentices addressed the black bile excess by recommending miscellaneous treatments like opium, rhubarbs, barley water, turpentine, sulphur, zinc oxide, dried vipers, lizard intestines, and also more classic treatments like blood-letting, laxatives and inducing vomiting [3]. During the following years, Galen continued in the Arab and Byzantine world while belief in witchcraft and sorcery prevailed in Medieval Europe, and medical problems were treated by shamans, monks, apothecaries and barbers. In the late middle ages, monastic scribes translated Arab texts into Latin and the humoural theories returned.



Figure 2. Hippocrates.

However, from the late 17th to the mid-18th century, there was a questioning of the humoural theory and, in the 1760s, no modern-thinking physician ordered remedies against black bile any more. Instead, surgery gained ground and the

mastectomy became the treatment of choice. During the 18th century, Hunter (b 1728) performed post-mortem dissections and described breast cancer's spread to nearby lymph nodes [3]. Surgeons became more certain that breast cancer was a localized disease, which could be cured as long as affected axillary lymph nodes were removed [4].

In 1846, William Thomas Green, an American dentist, used ether to anaesthetize a patient during surgery (resection of a facial tumour) for the first time. However, widespread use of anaesthesia was delayed by cultural assumptions about pain as a developer of a heroic character in women and "as a moral medication". Not until the 1890s did most surgeons use anaesthesia in all patients [3]. Anaesthesia, in combination with the advent of aseptic routines after the discoveries of Semmelweiss and Lister in the middle of the 19th century, allowed more radical surgery.

Sir William Stewart Halsted (1852-1922) made radical mastectomy the gold standard for the next 100 years [3-5]. The breast, with all of its skin, the greater part of the major (and sometimes the minor) pectoral muscle, and the contents of the axilla (exposing the subclavian vein and the brachial plexus) were removed, all in one piece [6].

In the beginning of the 20th century, several theories about the spread of cancer evolved, including Handley's "cancer permeation hypothesis", in which cancer spread centrifugally along the plane of the deep fascia and along the lymphatic vessels [3]. These theories, together with the discovery of antibiotics (penicillin 1928) and the possibility of blood transfusion (1937) paved the way for even more radical surgery. At its extreme, supraclavicular lymph nodes with a portion of the rib cage and the collar bone were removed, and occasionally the sternum was split to reach the mediastinal lymph nodes. At the end of the 19th century, oophorectomy was demonstrated to improve breast cancer prognosis, and after the discovery of oestrogen's tumour-promoting effect, adrenalectomies and even hypophysectomies were performed during the 1950s.

Following the advent of radiotherapy in the 1930s and 1940s, less radical surgery began to evolve, and surgeons like Keynes, McWirth and Patey proposed mastectomy with limited axillary dissection, or even simple mastectomy or lumpectomy, to be just as safe as more radical surgery. Breast cancer was again more and more considered to be a systemic disease, and following the introduction of chemotherapy after World War II the popularity of radical surgery decreased [3]. The safety of breast-conserving and less radical lymph node surgery was confirmed in several studies during the 1980s and 1990s [7-9]. Since then, Halsted's radical mastectomy has been replaced by simple mastectomy or breast-conserving lumpectomy and limited axillary lymph node dissection (ALND).

Sentinel lymph node biopsy

Axillary lymph node status is the most important prognostic factor in breast cancer [10-12]. However, due to increased awareness and the introduction of screening mammography, breast cancer is now diagnosed at an earlier stage and, subsequently, the incidence of axillary lymph node metastases has decreased dramatically. For patients without axillary metastases, ALND is of no value, and about 20 years ago surgeons started to look for alternative axillary staging methods. Ultrasound, colour Doppler examination [13] and computed tomography- (CT) based evaluation [14] of the lymph nodes were suggested. Different axillary sampling methods, including pectoral node biopsy, four-node sampling technique and triple-node biopsy, were practised [15-17].

The concept of sentinel lymph node biopsy (SLNB) was first described by Gould et al [18] who, in 1951, during a parotidectomy for a parotid tumour, by chance noticed a normal looking lymph node at the junction of the anterior and posterior facial vein. For some reason, the node was excised and sent for frozen section pathology and, surprisingly, was found to be tumour positive.

Cabanas elaborated the SLNB concept more systematically, and published his results in 1977 [19]. He used lymphangiograms to detect the lymph node most likely to be the primary site for metastases in penile carcinoma. A few years later SLNB was introduced in melanoma surgery [20, 21].

Injection of dye into the breast tissue was performed by Turner-Warwick to demonstrate the lymphatic drainage of the breast as early as in the late 1950s [22], but it was Krag (1993) and Giuliano (1994) who first described SLNB by injecting an isotope in breast cancer patients [23, 24]. Shortly thereafter, Albertini published a study using a combination of vital blue dye and isotope [25].

The sentinel lymph node (SLN) is defined as the first node receiving lymphatic drainage from the tumour. The site of the SLN differs slightly depending on the tumour location in the breast and the injection technique, but includes the axilla in 85-100% of cases [26, 27]. Using an intratumoural injection technique, Estourgie et al [26] found drainage to an internal mammary chain SLN in 10-52% of patients. Internal mammary chain drainage was more frequent in tumours situated medially in the breast, but occurred also from tumours in the outer quadrants. Drainage was also observed to supra-clavicular, infraclavicular, interpectoral, and intramammary nodes.

The major advantage of SLNB, leaving the rest of the axillary lymph nodes intact in the absence of SLNB metastasis, is the decrease in incidence and severity of postoperative arm morbidity (swelling, pain and decreased mobility) [28-30]. Another possible advantage is a more accurate staging, as the dye and radiocolloid guide the way to the lymph node most likely to contain metastasis, which might have been left behind in an ALND.

The introduction of SLNB has, however, also conveyed several new issues, and some of these are vividly debated.

Firstly, SLNB conveys a risk of false negativity (metastases in non-SLNs despite a negative SLN). This would mean that the patient is misclassified and may not receive the adjuvant treatment she should be offered. It would also mean that metastatic lymph nodes may be left behind. The rate has varied in different validation studies, from 0 to as high as 30-40% in some reports [31-33]. The location and grade of the primary tumour, the number of SLNs and non-SLNs, the experience of the surgeon, and whether or not combined blue dye and isotope injection technique is used, have been reported to affect the risk of false negativity [32, 34-43]. The effect of tumour multifocality on false negative rate has been debated [44-48].

Secondly, as the SLNB technique is relatively new, there are few long-term follow-up studies. Early reports of a high false negativity rate [31] raised concerns about a higher risk of axillary recurrence. Still, most follow-up studies on SLN-negative women in whom ALND was omitted have shown few axillary recurrences [49-57]. However, most of these studies have short follow-up periods, and the majority of data comes from single specialised centres.

Another issue is the clinical importance of micrometastases (>0.2-2 mm). During SLNB, only a few lymph nodes are dissected, which allows the pathologist to examine these more thoroughly. This has led to a substantial stage migration [58-60], mostly as a result from an increased identification of micrometastases. The prognostic significance of these is unclear. Several authors claim that they do not matter [61-64], while other studies have shown a worse prognosis for patients with micrometastases than for node-negative patients [65-70].

Finally, there is an issue of the need for completion ALND (cALND) in SLN-positive patients. According to the present guidelines, a cALND is recommended in the event of a SLN metastasis with a size of at least 0.2 mm [33]. However, 50-65% of SLN-positive patients have negative non-SLNs [71, 72], and do not benefit from cALND. Several authors have suggested nomograms and scoring systems to predict the risk of non-SLN metastases [73-79] and validation studies have demonstrated a varying predictive ability [80-86]. An advantage of one of these scores, the Tenon score [73], is that a fair estimation of all predictive variables can be made perioperatively.

Aims

- To study the risk factors for false SLN negativity and to evaluate if a more thorough examination of the SLNs decreases the false negativity rate
- To report the axillary recurrence rate in SLN-negative patients without cALND from the Swedish Sentinel Node Multicentre Cohort Study after 5 years of follow-up
- To study the prognostic significance of micrometastases in breast cancer patients
- To compare primary tumour and SLN characteristics between SLN-positive breast cancer patients with and without metastases in non-SLNs and to validate the Tenon score

Patients

Validation Study

Between March 1998 and December 2001, 675 consecutive women from 20 hospitals were included in a Swedish Multicentre Validation Study. Eligible patients had a palpable breast cancer but no axillary lymph nodes clinically suspicious of metastasis. Patients with locally advanced tumours and those with multifocal tumours on preoperative mammography were excluded. Patients with previous ipsilateral breast surgery or preoperative chemotherapy, pregnant women, and patients with known allergic reactions to blue dye or isotope were also excluded.

Each of the 37 participating surgeons had to perform at least 10 SLN procedures before entering patients in the study.

Cohort Study

Between September 2000 and January 2004, 3501 women (with 3535 breast tumours) were included in the Swedish Sentinel Node Multicentre Cohort Study. Patients with a unifocal, invasive breast cancer less than 3 cm in diameter were eligible for enrolment. Exclusion criteria were palpable regional lymph nodes, neoadjuvant chemo- or radiotherapy, pregnancy, known allergic reactions to blue dye or isotope, previous surgery in the ipsilateral breast, and preoperatively diagnosed tumour multifocality.

Twenty-six Swedish hospitals (9 university, 13 county, 1 private, and 3 community) and 131 surgeons contributed to accrual in this study.

Data management

After enrolment, data sheets were sent to a research unit, where they were computerized. Data sheets included information on primary tumour characteristics, number of sentinel and non-sentinel nodes, with and without metastases, and administered adjuvant therapy.

The studies were approved by the Ethics Committee of Karolinska Institutet, Stockholm, and each region's local ethics committee.

Paper I

To investigate whether an extended examination of the SLNs reveals metastases more often in patients with a false negative SLN than in those with a true negative SLN, the archived SLN specimens from 50 patients with a false negative SLN and from 107 patients in a true SLN-negative control group were collected. The patients with a false negative SLN were included from the validation (negative SLN and positive cALND, n=18) and cohort (negative SLN and positive cALND, n=13 or isolated axillary recurrence, n=19) study. A control group with true negative SLN was randomly chosen from the validation (n=39) and cohort (n=68) study.

To analyse risk factors for false negativity, tumour and SLN characteristics were compared with all SLN-positive patients from the validation (n=250) and cohort (n=954) study.

Paper II

Sentinel lymph node-negative patients who had no cALND were included from the cohort study. Patients who were followed up outside Sweden, had DCIS only, or who had distant metastases at the time of surgery were excluded (Figure 3), leaving a total of 2216 for evaluation of axillary recurrence. Patients who were diagnosed to have multiple foci of invasive tumours in the breast (n=94), or tumours larger than 3 cm (n=46) by the postoperative pathological exam were included if cALND was omitted. Median follow-up was 65 months (range 0-113).

Paper III

For survival analyses, 3369 patients were included from the cohort study (Figure 4). The patients were stratified in four groups, according to their lymph node status; 2383 (71%) were node-negative, 107 (3%) had ITCs, 123 (4%) had micrometastases, and 756 (22%) had macrometastases. Median follow-up time was 52 months (range 0-91 months).

Paper IV

To analyse risk factors for non-sentinel metastases, 869 SLN-positive patients who underwent cALND were included from the validation study.

Additionally, the incidence of axillary recurrence was compared with 86 patients from the same SLN cohort who were diagnosed with SLN metastases but did not undergo cALND.

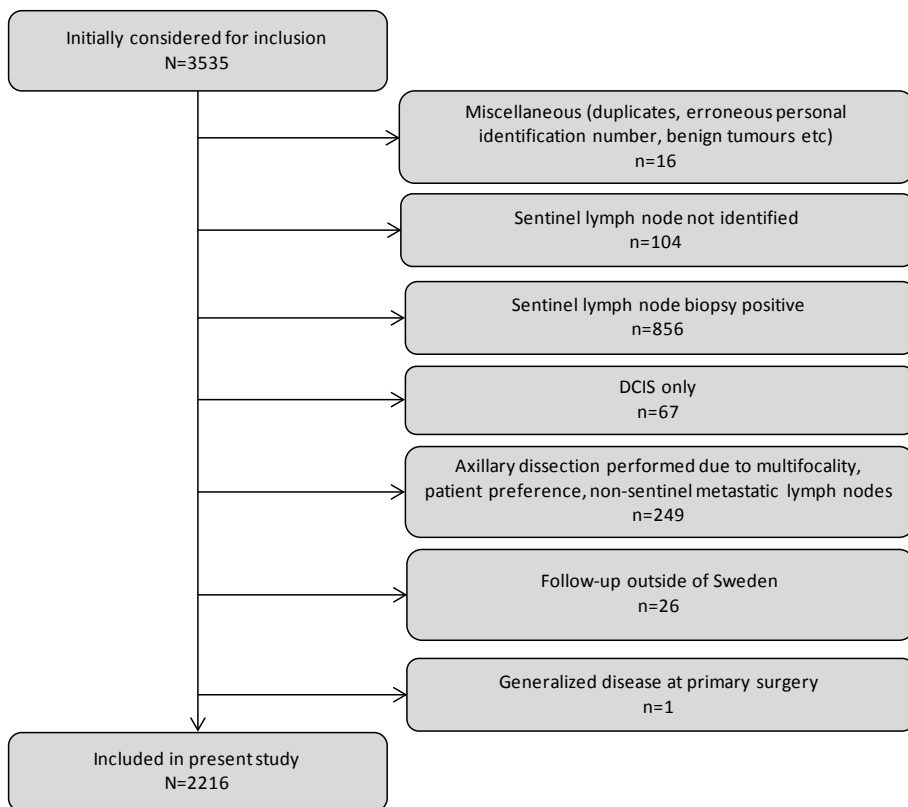


Figure 3. Flow chart for inclusion and exclusion in *Paper II* (5-year follow-up of sentinel lymph node-negative patients without completion axillary lymph node dissection). DCIS; ductal carcinoma in situ.

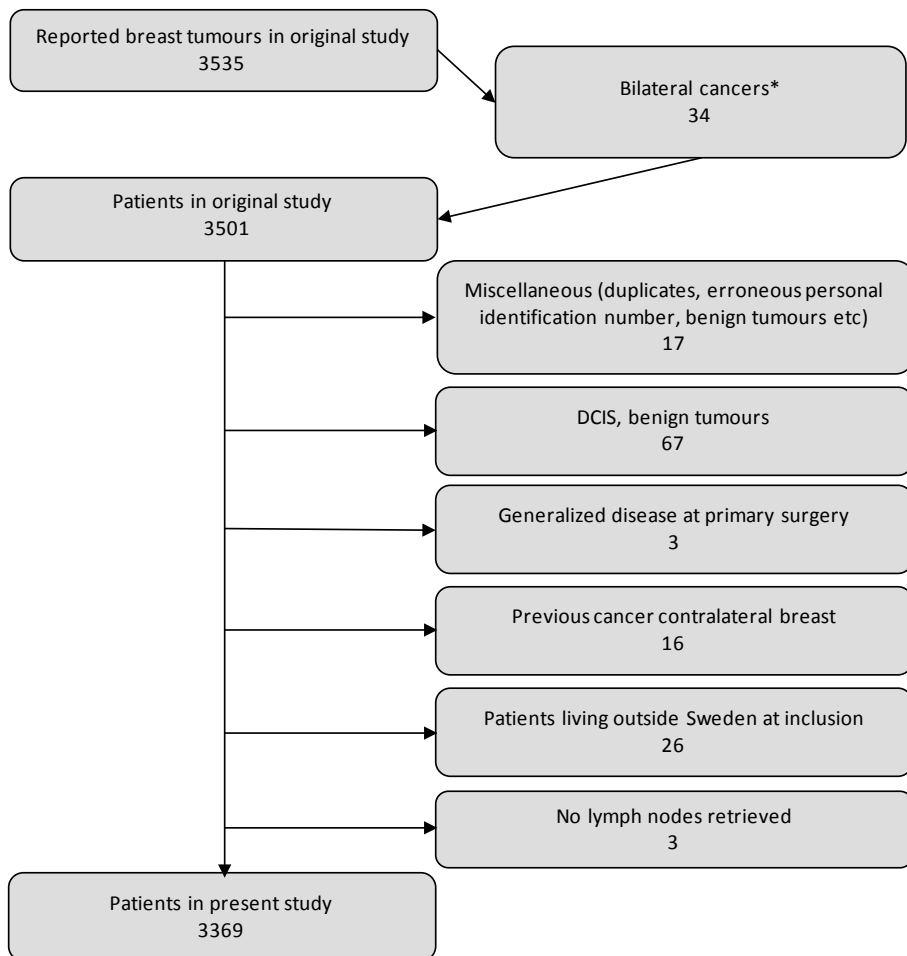


Figure 4. Flow chart for inclusion and exclusion in *Paper III* (prognostic significance of micrometastases). *; In case of bilateral cancers, only one cancer was included in the study. The cancer with the lowest lymph node stage, smallest tumour size or lowest tumour grade was excluded. DCIS; ductal carcinoma in situ.

Methods

Identification of sentinel node

Radioactive isotope (40-60 MBq Technetium-99 nanocolloid, Solco Nanocoll®; Nycomed, Amersham, UK) was injected peritumourally, sub- or intracutaneously 4-36 hours prior to surgery. Preoperative lymphoscintigraphic images (Figure 5) were obtained 5 and 45-60 minutes after injection, and if no SLN was identified the lymphoscintigraphy was repeated after 2-3 hours. Anterior and lateral views were taken, and the location of the SLN was marked on the skin.

Using the same injection technique, 1 ml blue vital dye (Patent Blue V®; Guerbet, Paris, France) was administered 5-15 minutes before incision (Figure 6).

During surgery, SLNs were identified by a handheld gamma probe. Hot and/or blue nodes were defined as SLNs.

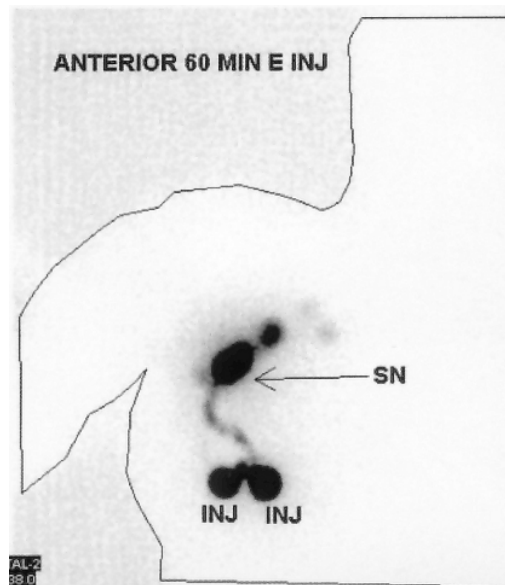


Figure 5. Lymphoscintigraphy.

Surgery

Surgery started with a separate axillary incision. The SLNs were identified and removed and sent for pathological examination. In the validation study, a cALND (level I and II) was performed in all patients, regardless of SLN status, while in the cohort study, cALND was performed only in the event of a positive SLN, if no SLN could be identified, or if the tumour was found to be multifocal on pathological examination.

Breast surgery was performed as a breast-conserving lumpectomy or simple mastectomy. Breast-conserving surgery was used in the majority of patients.

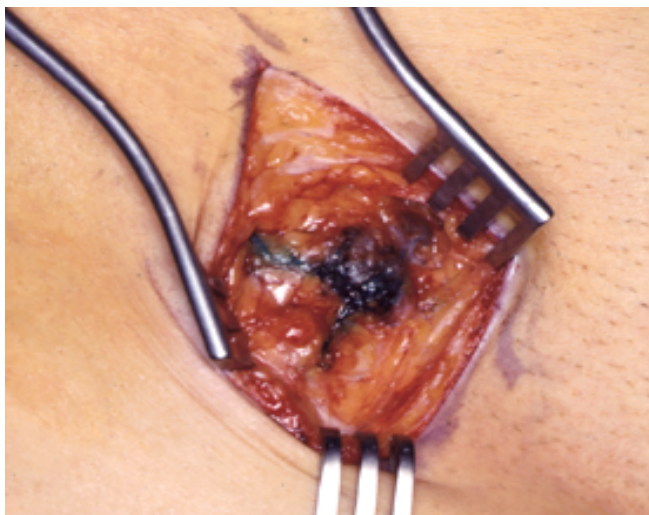


Figure 6. Patent Blue[®] sentinel lymph node with afferent and efferent lymph vessels.

Histopathological assessment

In the cohort study, frozen sections were obtained from all SLNs and examined during surgery. If a SLN was smaller than 4 mm, two sections were analysed separately. Nodes larger than 4 mm were bisected, and two sections from each half were analysed.

In both studies, at least three sections from the SLN or each part of a bisected node were prepared for definitive histopathology. Sections were stained with haematoxylin and eosin (HE). If no cancer cells were detected, immunohistochemistry (IHC) with cytokeratin antibodies was also performed in most cases. Non-SLNs were examined by routine staining (HE) according to the protocol of each pathology department.

For *study I*, the archived SLN specimens were sectioned at 0.2 mm levels. Two sections were prepared at each level and stained with HE and IHC, respectively. All sections were then assessed by the same pathologist.

Lymph node status was classified according to the revised American Joint Committee on Cancer Staging System for Breast Cancer (AJCC) [2]: node-negative (pN0), ITCs (≤ 0.2 mm, pN0(i+)), micrometastases (>0.2 - 2 mm) and macrometastases (>2 mm, pN1-2).

Adjuvant treatment

Adjuvant treatment combinations were given according to national and regional treatment guidelines, based on tumour characteristics, lymph node status, and surgical treatment. Patients with ITCs were regarded as lymph node-negative. If breast-conserving surgery had been performed, radiation therapy to the breast was given, which was extended to include the regional lymph nodes in case of axillary lymph node metastases.

Chemotherapy was offered to all patients with lymph node metastases or those with a combination of unfavourable primary tumour characteristics (large tumour, high Elston score, and progesterone negativity), after consideration of their general health. Endocrine therapy was offered to all patients with oestrogen- or progesterone receptor-positive tumours larger than 10 mm.

Follow-up

Patients in the cohort study were observed prospectively. The research protocol postulated follow-up with mammography and clinical examination, annually for 5 years and after 10 years. All follow-up data were reported to the study data base. Before data analyses, a list of all included patients was sent to all participating centres and returned to the research centre with updated information on events and latest follow-up dates. Furthermore, the authors were granted access at on-site visits to hospital files to update reported data.

Definitions

Lymph node recurrence was reported as either *axillary* or *extra-axillary* (supraclavicular or cervical). Axillary recurrence was considered *isolated* if the axilla was the sole initial site of recurrence, and *locoregional* if the patient developed an ipsilateral breast recurrence prior to, or concurrently with, the axillary recurrence. Local recurrence was defined as a relapse in the ipsilateral breast. Recurrences at separate sites were regarded as *synchronous* if they

were diagnosed within the same 2-month period. Recurrences outside the breast and the axilla were regarded as *generalized disease*.

In *study II*, centres contributing less than 150 SLNB procedures to the whole cohort study were defined as low experience centres, and those contributing more than 150 procedures were defined as high experience centres.

Tenon score

In *study IV*, the Tenon score was calculated for all patients by adding the point values for the presence of macrometastases in the SLN (yes = 2, no = 0), the histological tumour size in mm ($>20 = 3$, $11-20 = 1.5$, $<11 = 0$) and the ratio between positive and total SLNs ($1 = 2$, $0.5 = 1$, $<0.5 = 0$). The recommended threshold value for predicting negative non-SLNs is 3.5 or less [73].

Statistical methods

Counted from the date of the SLN biopsy, the breast cancer-, or cause-specific survival was calculated to the date of death due to breast cancer; event-free survival to the date of local, axillary, or distant recurrence, contralateral breast cancer, or death from any cause; and overall survival (OS) was calculated to the date of death. In the absence of any event, time was calculated from the date of the SLN biopsy to the date of last follow-up.

In *study I*, the detection rate of previously unknown SLN metastases was compared between patients with a false negative SLN and those previously found to have a true negative SLN, using a Chi-2 test. The size, Elston grade, hormone receptor status and localisation of the primary tumour, occurrence of multifocality, blue dye and isotope injection technique and the number of SLNs in patients with a false negative SLN and those with SLN metastases were compared in a univariate logistic regression model. All variables that demonstrated a statistically significant difference in univariate tests were then analysed in a multivariable regression model.

In *study II*, the primary endpoint was axillary recurrence rate. Secondary endpoints were overall recurrence rate, cause-specific, event-free and overall survival. All endpoints were calculated from Kaplan-Meier graphs. Cox proportional hazard regression analysis was used to assess the axillary recurrence hazard ratio for patients accrued in low compared with high experience centres.

In *study III*, the primary endpoints were cause-specific, event-free and overall survival and were calculated from Kaplan-Meier graphs. Cox proportional hazard regression analyses were used to assess the hazard ratio for adverse outcome for patients with ITCs, micrometastases and macrometastases compared with patients without lymph node metastases. Age and tumour

size, histologic grade of the tumour, and adjuvant treatment were adjusted for in the analyses.

In *study IV*, patients with positive non-SLNs were compared with those who had negative non-SLNs regarding age, size, histological type and grade of the primary tumour, oestrogen and progesterone receptor status, SLN status and ratio between number of positive and total number of SLNs in a univariate logistic regression model. All variables that demonstrated a statistically significant difference in univariate tests were then analysed in a multivariable regression model. A receiver operating characteristics (ROC) curve was drawn on the basis of the sensitivity and specificity of the Tenon score, and the area under the curve (AUC) was calculated.

The SPSS® (SPSS Inc., Chicago, Illinois) program was used for all analyses, and statistical significance was set at $P=.05$ for all tests.

Results

Paper I

Patient and tumour characteristics of patients with false and true negative SLNs are given in Table 1.

After serial sectioning, previously unknown SLN metastases were detected in 9 of 50 (18.0%) patients in the false negative group, and in 12 of 107 (11.2%) patients in the true negative group. The difference in the detection rate of previously unknown metastases was not statistically significant ($p=0.463$).

Hormone receptor status, number of SLNs and multifocality were significantly associated with false negativity. The risk of false negativity was higher if the tumour was hormone receptor-negative or multifocal, or if only one SLN was found. Three (14.3%) of 18 patients with isolated axillary recurrences had multifocal tumours.

Paper II

Patient and tumour characteristics are given in Table 2. Isolated tumour cells were diagnosed in 40 patients (1.8%).

Overall, there were 256 recurrences in 203 (9.2%) of the 2216 patients. Isolated axillary recurrences were diagnosed in 23 patients (1.0%) after a median of 25 months (range 4-87). The 5-year isolated axillary recurrence-free survival was 99.0% (95% CI 98.6-99.4). Locoregional axillary recurrences were found in an additional 14 patients. Thus, overall, 37 axillary recurrences (1.7%) were identified. There was no difference in axillary recurrence between patients treated in low compared with high experience centres.

Isolated axillary recurrences were reported in 3 of the 94 patients (2.6%) with multiple foci of invasive tumours but in none of the 46 patients with unifocal tumours larger than 3 cm.

The 5-year cause-specific survival was 97.2% (95% CI 96.5-98.0), event-free survival 88.8% (95% CI 87.7-90.2) and overall survival 93.1% (95% CI 92.0-94.2).

Table 1. Characteristics of patients with false and true negative SLNs in *Paper I*.

Characteristics	SLN status	
	False negative SLN	True negative SLN
N	50	107
Age (years)^a	58 (31-84)	62 (35-89)
Tumour size (mm)^b	23 (16)	15 (7)
Histotype^c		
ductal	37 (74.0)	79 (73.8)
lobular	8 (16.0)	8 (7.5)
mixed	1 (2.0)	1 (0.9)
other	0	9 (8.4)
missing	4 (8.0)	10 (9.3)
Tumour grade (Elston grade)^c		
1	4 (8.0)	31 (29.0)
2	23 (46.0)	43 (40.2)
3	22 (44.0)	28 (26.2)
missing	1 (2.0)	5 (4.7)
Oestrogen receptor^c		
positive	36 (72.0)	86 (80.4)
negative	14 (28.0)	20 (18.7)
missing	0	1 (0.9)
Progesterone receptor^c		
positive	26 (52.0)	69 (64.5)
negative	23 (46.0)	35 (32.7)
missing	1 (2.0)	3 (2.8)
Number of SLNs^a	1 (1-7)	2 (1-9)
Number of non-SLNs^{ad}	11 (1-24)	10 (1-20)

^a Median (range); ^b Mean (standard deviation); ^c Number (%); ^d In patients who had completion axillary lymph node dissection (N=32 in false negative, N=46 in true negative) SLN=sentinel lymph node

Table 2. Characteristics for 2216 SLN-negative patients in *Paper II*.

Characteristics		
<hr/>		
Age (years)^a		60 (23-94)
<hr/>		
Tumour size (mm)^b		15 (7)
<hr/>		
Histotype^c		
	ductal	1464 (66.1)
	lobular	255 (11.5)
	mixed	15 (0.7)
	other	164 (7.4)
	missing	318 (14.3)
<hr/>		
Tumour grade (Elston)^c		
	1	674 (30.4)
	2	1056 (47.7)
	3	410 (18.5)
	missing	76 (3.4)
<hr/>		
Oestrogen receptor^c		
	positive	1888 (85.2)
	negative	271 (12.2)
	missing	57 (2.6)
<hr/>		
Progesterone receptor^c		
	positive	1524 (68.8)
	negative	615 (27.8)
	missing	77 (3.4)
<hr/>		
Number of SLNs^a		2 (1-5)
<hr/>		
Adjuvant treatment^c		
	hormonal	1332 (60.1)
	radiotherapy	1668 (75.3)
	chemotherapy	224 (10.1)
<hr/>		

^a Median (range) ^b Mean (standard deviation) ^c Number (%)

SLN=sentinel lymph node

Paper III

Patient and tumour characteristics and data on adjuvant therapy are listed in Table 3. Contrary to the study protocol, SLNs were the only lymph nodes retrieved in 30 pN1mi patients (24.4%) and in 21 pN1 patients (2.8%).

Overall, there were 380 recurrences in 29 (8.8%) of the 3369 patients; 171 (7.5%) of the 2283 patients in the pN0 group, 7 (6.5%) of the 107 patients in the pN0(i+) group, 17 (13.8%) of the 123 patients in the pN1mi group, and 98 (13.0%) of the 756 patients in the pN1 group experienced recurrences.

During follow-up, 274 patients died. Of these, 153 were node-negative, 6 had ITCs, 10 had micrometastases, and 105 had macrometastases; 55, 2, 6, and 58 patients, respectively, from these four groups died of breast cancer. Compared with pN0 patients, 5-year cause-specific (Table 4) and event-free (Table 5, Figure 7) survival were significantly worse both for pN1mi and pN1 patients. The 5-year OS difference between patients with micrometastases and patients with node-negative disease was not statistically significant (Table 6). Cause-specific, event-free and overall survival did not differ between pN0(i+) and pN0 patients.

Paper IV

Patient and tumour characteristics are presented in Table 7. Most of the patients (n=691) had SLN macrometastases, but 20.0% (n=178) had metastases ≤ 2 mm (98 micrometastases and 80 ITCs). In 282 patients, the cALND was performed in a second session.

Non-SLN metastases were identified in 270 patients (31.3%). Eight (10.0%) of the 80 patients with SLNs containing ITCs, and 11 (11.2%) of the 98 patients with SLN micrometastases had non-SLN metastases. Of these, non-SLN macrometastases were revealed in 3 and 8 patients, respectively.

Tumour size and grade, SLN status and ratio between the number of positive SLNs and total number of SLNs were significantly associated with non-SLN status, both in uni- and multivariate analyses. Histotype was significant only in the univariate analysis. P-values for the association between different characteristics and non-SLN positivity are given in Table 7.

The risk of positive non-SLNs was 4.66 times higher for patients with SLN macrometastases than for those with SLN metastases ≤ 2 mm (95% CI 2.18-9.95, $P<0.001$) and 3.17 times higher for a high positive/total SLN ratio as defined in the Tenon score (95% CI 1.95-5.15, $P<0.001$). The hazard ratio for increasing tumour diameter (per millimetre) was 1.02 (95% CI 1.00-1.04, $P=0.035$) and for high tumour grade (Elston 3 vs. 1) 2.41 (95% CI 1.51-3.86).

We identified two small groups of patients in whom the risk of non-SLN metastases was less than 10%: pN1mi or pN0(i+) patients, either with a tu-

more smaller than 2 cm and Elston grade 1 or 2 (n=102), or with more than two SLNs removed (n=23).

The mean Tenon score was 5.3 in patients with non-SLN metastases and 4.5 in those without ($P<0.001$). Applying a threshold value of 3.5, the false negative rate was 13.8%, and 37 of 244 patients with a Tenon score 3.5 or less had non-SLN metastases. The area under the curve was 0.65 (95% CI 0.61-0.69) for all patients (Figure 8) and 0.63 (95% CI 0.59-0.67) for patients with SLN micro- and macrometastases.

In the study group, there were 10 (1.2%) isolated axillary recurrences after 56.3 months median follow-up. In a separate comparison group of 86 patients with SLN metastases in whom ALND was omitted (mean Tenon score 3.1), 1 (1.2%) patient had an isolated axillary recurrence after 51.8 months median follow-up.

Table 3. Characteristics according to lymph node status for patients in *Paper III*.

Lymph node status				
Characteristics	pN0	pN0(i+)	pN1mi	pN1
N	2383	107	123	756
Age (years)^a	60 (23 – 94)	56 (38 – 82)	59 (28 – 89)	57 (28 – 91)
Tumour size (mm)^b	15 (7)	17 (6)	17 (5)	20 (9)
Histotype^c				
ductal	1590 (66.7)	72 (67.2)	79 (64.2)	502 (66.4)
lobular	272 (11.4)	14 (13.1)	18 (14.6)	113 (14.9)
mixed	16 (0.7)	1 (0.9)	3 (2.5)	7 (0.9)
other	174 (7.3)	7 (6.5)	8 (6.5)	32 (4.3)
missing	331 (13.9)	13 (12.3)	15 (12.2)	102 (13.5)
Tumour grade (Elston)^c				
1	699 (29.3)	28 (26.2)	38 (30.9)	147 (19.4)
2	143 (48.0)	61 (56.9)	58 (47.2)	388 (51.3)
3	456 (19.1)	16 (15.0)	23 (18.7)	203 (26.9)
missing	85 (3.6)	2 (1.9)	4 (3.2)	18 (2.4)
Oestrogen receptor^c				
positive	2018 (84.7)	96 (89.7)	107 (87.0)	652 (86.2)
negative	306 (12.8)	10 (9.4)	12 (9.8)	97 (12.9)
missing	59 (2.5)	1 (0.9)	4 (3.2)	7 (0.9)
Progesterone receptor^c				
positive	659 (69.1)	79 (73.8)	87 (70.7)	536 (70.9)
negative	165 (27.6)	27 (25.3)	30 (24.4)	205 (27.1)
missing	79 (3.3)	1 (0.9)	6 (4.9)	15 (2.0)
Antihormonal therapy^c	1443 (60.6)	87 (81.3)	100 (81.3)	637 (84.3)
Radiation therapy^{cd}	1798 (75.4)	71 (66.4)	86 (69.9)	639 (84.5)
Chemotherapy^c	253 (10.6)	20 (18.7)	27 (22.0)	410 (54.2)
ALND^{ce}	361 (15.1)	73 (68.2)	93 (75.6)	735 (97.2)
Median number of pos./total LNs	0/2	1/8	1/10	2/12

pN0: lymph node-negative; pN0(i+): isolated tumour cells; pN1mi: micrometastases; pN1: macrometastases; ALND=axillary lymph node dissection; LNs= lymph nodes

^a Median (range) ^b Mean (standard deviation) ^c Number (%) ^d Breast and/or axilla ^e The goal of ALND was to retrieve at least 10 lymph nodes. The actual number of total retrieved lymph nodes varied between 1 and 44.

Table 4. Cause-specific survival for patients in *Paper III* according to lymph node status.

Lymph node status	5-year cause-specific survival^a (%) (95% CI)	Hazard ratio^b (95% CI)	P^b
No metastases	96.9 (96.0-97.8)	1	
Isolated tumour cells	97.4 (93.8-100)	0.94 (0.22-4.05)	0.938
Micrometastases	94.1 (89.4-98.8)	3.04 (1.19-7.77)	0.020
Macrometastases	91.8 (89.4-94.2)	3.33 (1.74-6.38)	<0.001

^a Calculated from Kaplan-Meier graph ^b Calculated from Cox regression model

Table 5. Event-free survival for patients in *Paper III* according to lymph node status.

Lymph node status	5-year event-free survival^a (%) (95% CI)	Hazard ratio^b (95% CI)	P^b
No metastases	87.1 (85.4-88.8)	1	
Isolated tumour cells	88.9 (82.3-95.4)	0.96 (0.53-1.84)	0.985
Micrometastases	79.6 (71.0-88.2)	1.71 (1.05-2.80)	0.032
Macrometastases	80.1 (76.8-83.5)	1.24 (1.24-2.43)	0.001

^a Calculated from Kaplan-Meier graph ^b Calculated from Cox regression model

Table 6. Overall survival for patients in *Paper III* according to lymph node status.

Lymph node status	5-year overall survival^a (%) (95% CI)	Hazard ratio^b (95% CI)	P^b
No metastases	92.4 (91.0-93.7)	1	
Isolated tumour cells	93.1 (87.8-98.5)	0.91 (0.39-2.11)	0.817
Micrometastases	90.7 (85.1-96.2)	1.48 (0.75-2.93)	0.258
Macrometastases	85.6 (82.7-88.5)	2.17 (1.42-3.31)	<0.001

^a Calculated from Kaplan-Meier graph ^b Calculated from Cox regression model

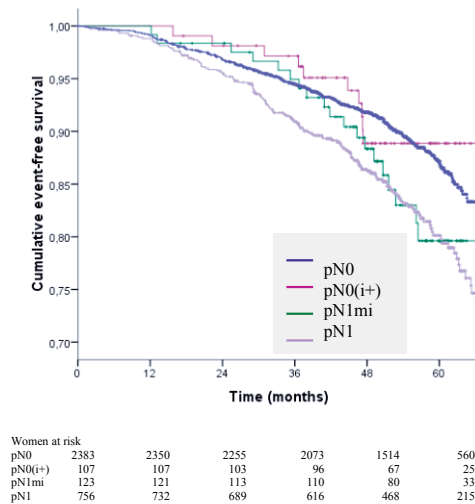


Figure 7. Kaplan-Meier graph demonstrating event-free survival for patients in *Paper III* according to lymph node status; pN0: lymph node-negative, pN0(i+): isolated tumour cells, pN1mi: micrometastases, pN1: macrometastases.

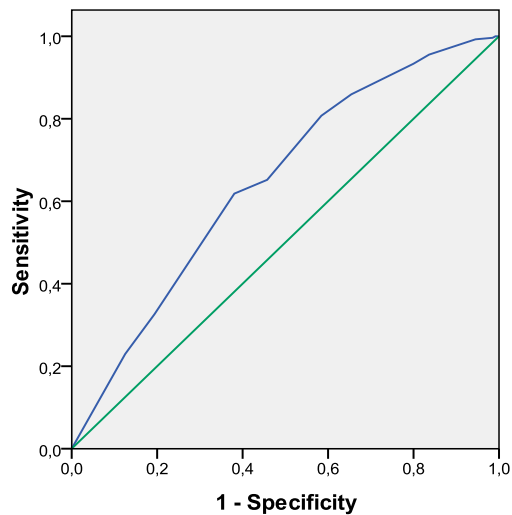


Figure 8. The receiver operating curve (ROC) calculated for the Tenon score for sentinel lymph node-positive patients in *Paper IV*; blue line, area under the curve (AUC) 0.65. The green, diagonal line represents AUC 0.5 (flipping a coin).

Table 7. Characteristics of non-SN positive and negative patients in *Paper IV*.

Non-sentinel lymph node status			
Characteristics	Positive non-SN	Negative non-SN	<i>P</i>
N	270	599	
Age (years)^a	57 (28-82)	57 (28-90)	0.481
Tumour size (mm)^b	19 (10)	17 (8)	<0.001
Histotype^c			0.035
ductal	173 (64.1)	408 (68.1)	
lobular	49 (18.1)	75 (12.5)	
mixed	2 (0.7)	10 (1.7)	
other	11 (4.1)	30 (5.0)	
missing	35 (13.0)	76 (12.6)	
Tumour grade (Elston)^c			<0.001
1	38 (14.1)	152 (25.3)	
2	138 (51.1)	304 (50.8)	
3	88 (32.6)	128 (21.4)	
missing	6 (2.2)	15 (2.5)	
Oestrogen receptor^c			0.502
positive	231 (85.6)	520 (86.8)	
negative	37 (13.7)	72 (12.0)	
missing	2 (0.7)	7 (1.2)	
Progesterone receptor^c			0.696
positive	185 (68.5)	425 (71.0)	
negative	80 (29.6)	160 (26.7)	
missing	5 (1.9)	14 (2.3)	
Number of SLNs^a	2 (1-9)	2 (1-8)	0.632
SLN status^c			<0.001
pN0(i+) ^d	8 (3.0)	72 (12.0)	
pN1mi ^e	11 (4.0)	87 (14.5)	
pN1 ^f	251 (93.0)	440 (73.5)	
Mean number of pos. SLNs/total SLNs	0.82	0.71	<0.001

^a Median (range) ^b Mean (standard deviation) ^c Number (%) ^d <0.2mm ^e 0.2-2mm ^f >2 mm
SLN=sentinel lymph node

Discussion

Paper I

In our study, we found similar rates of previously unknown SLN metastases after serial sectioning in false and true negative patients, indicating that omission of SLN serial sectioning was not a significant cause of false negativity. The rates are comparable to upstaging rates in previous studies [87-89].

There are inconsistencies and controversies regarding the pathological work-up of SLNs [90], and no generally applied guidelines exist. However, considering the similar upstaging rates in the false and true negative groups in our study, serial sectioning of SLNs is probably not cost-effective, and may subject a considerable number of patients to overtreatment.

Our study demonstrated a higher risk of false negativity in patients with multifocal or hormone receptor-positive tumours, and if only one SLN was found.

The higher false negative rate (FNR) in multifocal tumours was partly previously reported from the Swedish Multicentre Validation Study [91]. Additionally, in the Swedish Multicentre Sentinel Node Cohort Study [92], from which the rest of the patients were collected, multifocal tumour was a criterion for cALND. Hence, this group was probably somewhat biased.

However, 3 of 18 (14.3%) false negative cases with isolated axillary recurrence from the cohort study had multifocal tumours, and thus multifocal tumours seemed to be over represented in this group as well.

Several previous studies have also found a higher FNR in multifocal tumours [44, 46], while other authors conclude that SLNB is accurate in multifocal breast cancer [47, 48, 93-95]. However, in the review from Spillane and Brennan [47], several studies had a FNR exceeding 10%. Overall, there seems to be a tendency towards higher FNR in multi- than in unifocal breast cancer.

The association between the number of excised SLNs and FNR was strong in our study. This has been demonstrated by several previous studies [34, 35, 39, 42], and care should be taken not to leave any SLNs behind. However, excising too many SLNs would mean that the benefits of less arm morbidity with the SLNB technique would be lost and this has to be weighed in the balance. Up to four SLNs have been reported to increase accuracy [38, 43].

We observed a higher FNR in hormone receptor-negative tumours. The reason for this is unclear and, to our knowledge, this association has not been

previously reported. One theory could be that hormone receptor-negative tumours possess features that cause lymphatic vessel invasion, changing the route of lymphatic draining, but the association could also be purely by chance.

Paper II

In our large, prospective multicentre study of SLN biopsy as a single staging procedure in breast cancer patients, the axilla was the sole initial site of recurrence in 1.0% of the patients. This is in accordance with most previous studies on SLN-negative patients from highly specialised centres.

Despite early reports of high false negative rates up to 40%, an increasing number of follow-up studies demonstrate low axillary recurrence rates after SLN biopsy [49, 50, 96-100].

Kuijt and Roumen identified axillary recurrences in 5 of 100 SLN-negative breast cancer patients in which ALND had been omitted (median follow-up 6.5 years) [101]. Three of these recurrences were detected more than 2 years after surgery; the interval previously considered to reveal the majority of axillary recurrences. Based on these results, the authors calculate a life time axillary recurrence risk of 10% and thus suggest caution. Their study is very small, however, and does not present strong evidence against the substantial number of reports supporting the safety of SLNB.

In our study, 12 (52.2%) of the 23 isolated axillary recurrences were diagnosed more than 2 years after the SLNB, and three of these were found after more than 5 years. Optimally, a follow-up of 10 to 15 years of SLN-negative patients treated without ALND should be regarded as necessary to fully evaluate the safety of the method.

Most follow-up studies derive from highly specialised centres. According to previous experience, several new techniques have performed excellently in specialised centres but have been less successful when applied elsewhere. Thus, the results from these studies may not be applicable in smaller, non-specialised hospitals where, however, the majority of the breast cancer patients are treated.

Our study evaluates patients that were treated at 26 hospitals by 131 surgeons, of whom 106 contributed less than 50 procedures and 63 less than 10, and thus demonstrates the feasibility of SLNB as a standard staging procedure outside highly specialised hospitals.

Paper III

The clinical significance of lymph node micrometastases in breast cancer patients continues to be a subject of debate. Some earlier studies suggest that micrometastases have no prognostic significance [63, 64].

However, our study shows that patients with micrometastatic disease have a worse prognosis than node-negative patients, which is in accordance with several previous studies [70], including a large retrospective register study by Truong et al [102], and even suggest that the prognosis is similar to that in macrometastatic disease.

Although the majority of both pN1mi and pN1 patients was treated with adjuvant hormonal therapy, only just over 20% of pN1mi patients received chemotherapy, compared with 50% of pN1 patients. This could partly explain the lack of prognostic difference between the groups. Recently, de Boer et al [65] presented a large study confirming a shortened 5-year disease-free survival in women with micrometastatic disease. They also found an improved prognosis for patients with micrometastases who had received adjuvant treatment.

Taking these and our results into consideration, it is reasonable to believe that patients with micrometastases should benefit from adjuvant cytostatic and hormonal treatment.

A weakness of our study is that patients were treated at 26 different hospitals and, therefore, pathological examination of lymph nodes and adjuvant treatment may have differed. On the other hand, we believe that the multi-centre design best reflects the reality that most patients experience.

Another possible weakness of our study is that we did not perform serial sections of the lymph nodes, and some of the patients may have been misclassified. Also, because ALND was omitted in several patients with micrometastases, some of them may actually have had macrometastases. However, we estimate that the number of misclassified cases is low and does not affect the results.

Finally, another weakness is the small number of events that might have contributed to an inability to show a significantly worse OS in micrometastatic disease.

To decrease the risk of confounding factors and misclassification of lymph node stage, studies on SLN material are of great importance. A strength of our study is a large population in a prospective SLN cohort with a median follow-up of more than 4 years. Our results indicate that patients with micrometastases should be offered the same adjuvant treatment as those with macrometastases.

Paper IV

Several authors have, by creating nomograms and scoring systems, attempted to define a subset of SLN-positive patients in whom the risk of non-SLN metastases is negligible [73-78]. The Tenon score outperformed other scoring systems in a study by Coutant et al [81] and includes characteristics that can be estimated at the time of the SLN biopsy.

We validated the Tenon score in a Swedish multicentre cohort. The AUC limit for considering an acceptable ability is 0.70 and, as the AUC value for the Tenon score in our material was only 0.65, the performance of the score was inadequate.

Another validation study by Coutant et al also demonstrated a good accuracy of the Tenon score [103]. Both studies from this group evaluated French populations. A French data set was also used to develop the Tenon score. In contrast, validation studies in other populations demonstrate lower prediction accuracy (AUC 0.58-0.70) [83, 104, 105].

Unfortunately, we were not able to validate the Memorial Sloan- Kettering Cancer Center (MSKCC) nomogram [78] in our population as we had incomplete information about the occurrence of lymphovascular invasion. The MSKCC nomogram has, however, been validated in several other studies, and the AUC varied between 0.58 and 0.86 [81]. In three studies, the AUC was less than 0.70, possibly reflecting population differences in a similar way as for the Tenon score. This could represent differences in populations, surgical technique or pathological examination.

Predicting a negligible risk of non-SLN metastases in SLN-positive breast cancer patients thus seems complicated. However, the axillary recurrence rate, both in SLN-positive patients without cALND and in patients without any axillary procedure, has been lower than expected in the present and in previous studies [106-109]. Additionally, many studies have also reported a very low axillary recurrence rate in SLN-negative patients without cALND despite the fact that the SLNB false negative rate is known to be about 5-10% [49, 50, 98, 99]. This indicates that not all positive lymph nodes left behind will develop into clinically important metastases.

Conclusions

- Omission of SLN serial sectioning was not a significant cause of false negativity in our material. Care should be taken not to leave any SLNs behind, while the effect of multifocal tumours on false negativity is unclear.
- The axillary recurrence rate in SLN-negative breast cancer patients in whom ALND is omitted is low and SLNB is also a feasible staging procedure outside highly specialised centres.
- Breast cancer patients with lymph node micrometastases have a worse prognosis than lymph node-negative patients. The prognostic role of isolated tumour cells is unclear.
- The Tenon score performed inadequately in our material and we could, based on tumour and SLN characteristics, only define a very small group of patients in which negative non-SLNs could be predicted.

Future perspectives

In the view on breast cancer, the pendulum has swayed back and forth; from Hippocrates' humoral theory and systemic "treatment" with turpentine and dried vipers, to the 19th and the greater part of the 20th centuries' belief in breast cancer as a localized, centrifugally spreading disease and Halstead's radical mastectomy (or even more radical surgery), back to today's increasingly popular theory that breast cancer is, after all, a systemic disease from the start.

The nature of breast cancer disease has changed since the Halstead era, from very advanced tumours with extensive lymph node metastases to small, often screening-detected tumours, of which about 70-80% are lymph node-negative. Thus, the need for extensive surgery has decreased.

In more than half of the lymph node positive patients, metastases are found only in the SLNs [71]. Furthermore, several studies indicate that the axillary recurrence rate is much lower than expected even if metastatic lymph nodes are left behind [106-110]. This has prompted a debate about the necessity of cALND in SLN-positive patients, and an increasing number of authors propose that cALND should be omitted, at least in selected patients. Improved adjuvant systemic treatment is expected to treat possible metastases left behind.

In 2011, Giuliano et al [111] published the results of the ACO-SOG Z0011 study, randomizing SLN-positive patients to either cALND or no further axillary surgery. After a median follow-up of 6.3 years, survival was comparable for the patients who did and did not have cALND.

However, the Z0011 study was closed early, partly due to a low accrual rate, and the included patients had a low risk of recurrence. Therefore, one cannot rule out the possibility of a significant selection bias. There are also results from several other studies that call for caution when considering omission of cALND.

In a meta-analysis from the pre-SLN era, ALND improved survival compared with no axillary treatment [112]. Similar results were demonstrated in a retrospective study reporting the outcome for patients who underwent either ALND or axillary sampling, including axillary radiotherapy if any of the sampled nodes were positive [113]. The survival rate after 132 months was significantly worse for patients who did not have ALND (42% vs. 58%).

Furthermore, even though Park et al [114], in their retrospective study, conclude that it is reasonable to omit cALND in a low-risk subset of

SLN-positive patients, the axillary recurrence rate in SLN-positive patients who did not have cALND was 2% after only 30 months (compared with 0.4% in patients who had cALND), despite the fact that all patients had favourable tumour characteristics. Also strengthening the cause for caution is a recent analysis from the large Dutch MIRROR study [115], in which patients with SLN ITCs or micrometastases who had cALND were compared with those who did not. Not performing cALND in patients with SLN micrometastases was associated with an increased 5-year regional recurrence rate.

The question is: what is the appropriate position of the breast cancer pendulum? Is it now heading too far back again? The challenge is to optimize the surgical and adjuvant treatment by decreasing the morbidity from axillary surgery, without risking the prognosis of the patients. Breast cancer is a disease with a tendency for late relapses, and recurrences after up to 15-20 years are not uncommon. Thus, studies with corresponding follow-up times are required to fully evaluate the safety of omitting cALND.

Recently, there is increasing interest in the molecular mechanisms of tumour development and their progression into an invasive and metastatic state. According to the cancer stem cell (CSC) hypothesis, cancers arise in cell populations that either maintain or acquire the stem cell property of self-renewal. These CSCs drive the malignant process and also generate a population of non-renewing cells that form the bulk of the tumour [116, 117]. Through several pathways, the cells are gradually dedifferentiated from the centre of the tumour out towards the tumour-host interface, where some CSCs may evolve into migrating CSCs by epithelial to mesenchymal transition (EMT) [118]. These migrating CSCs have the ability of lymphatic or haematogenous dissemination. Arriving at a distant site they may undergo a reverse transition (mesenchymal to epithelial transition, MET) back to stationary CSCs and form metastatic colonies.

Cancer stem cells also have the ability to survive in a quiescent state [118, 119], and this may be an explanation for late breast cancer recurrences. Moreover, *in vitro* studies have suggested that CSCs are relatively resistant to chemotherapy, hormonal therapy and radiation [116, 118-121]. Thus, if lymph node metastases left behind contain treatment resistant CSCs with disseminating abilities, relying on adjuvant treatment could be hazardous in a long-term perspective.

For a future perspective, studies determining if metastases are able to metastasize themselves are desirable. If so, exploring characteristics in lymph node metastases should be done in an attempt to predict which metastases have metastatic abilities, and thus should be treated surgically. Further exploration of the related, interesting research area of evolving therapy directly targeted against CSCs is also warranted [122, 123].

Swedish summary

Bakgrund och syfte

Sedan mitten på 1900-talet har kirurgi vid bröstcancer utvecklats mot mindre radikala ingrepp. Istället för att operera bort hela bröstet genomgår nu majoriteten av bröstcancerpatienterna bröstbevarande kirurgi. Eventuell metastasering (spridning) till regionala lymfkörtlar är den faktor som har störst betydelse för patientens prognos, och därför utgör också så kallad lymfkörtelstaging av axillen (armhålan) en del av det kirurgiska ingreppet vid bröstcancer.

Sentinel node-biopsin, eller den så kallade portvaktskörtelmetoden, innebär att man opererar bort den första körteln som dränerar lymfvätska från det område i bröstet där tumören sitter. Detta medför betydligt mindre armbesvär än axillutrymning, där mellan 10 och 20 lymfkörtlar opereras bort, vilket tidigare var standardingreppet. Sentinel node-biopsin introducerades gradvis i Sverige i början av 2000-talet efter att först ha testats och validerats i olika studier.

Om sentinel node är frisk behöver man inte operera bort några ytterligare lymfkörtlar, och då de flesta patienter inte har axillmetastaser har denna nya metod förhindrat besvärande armsymtom för flera tusen kvinnor världen över. Metoden har dock medfört flera nya frågeställningar.

Det finns en risk att man inte hittar någon metastas i sentinel node, men att det ändå finns metastas i någon av de övriga körtlarna. Sentinel node är då falskt negativ. Om detta inträffar innebär det dels att patienten felklassificeras och kanske inte får den tilläggsbehandling hon borde få, dels att man riskerar att lymfkörtlar med metastas lämnas i axillen. Syftet med *delarbete I* var att ta reda på om en ännu mer detaljerad patologisk undersökning av sentinel node kan minska risken, samt att utvärdera om det finns några särskilda riskfaktorer för falsk negativitet.

Det finns flera studier som har följt patienter som har opererats med bara sentinel node-biopsi för att se hur det går för dessa. Eftersom metoden är relativt ny är det dock få studier som har en lång uppföljningstid. Nästan alla studier kommer också från högspecialiserade centra, där alla patienter har opererats på ett stort sjukhus. För att kontrollera att det här är en metod som kan användas allmänt inom ramen för rutinsjukvården behövs multicenterstudier. I *delarbete II* var syftet att göra en 5-årsuppföljning av de sentinel

node-negativa patienter som deltog i den svenska multicenterkohortstudien och som inte genomgick kompletterande axillutrymning.

Eftersom man nu opererar bort endast en eller ett par lymfkörtlar kan patologen undersöka dessa mycket noggrannare än tidigare, med tätare snitt och andra färgningar. Det innebär att man hittar även små metastaser, vilka delas in i isolerade tumörceller ($\leq 0,2$ mm) och mikrometastaser ($> 0,2-2$ mm). Den kliniska betydelsen av dessa har debatterats flitigt, och studier visar divergerande resultat. I *delarbete III* var syftet att utvärdera om dessa små metastaser påverkar prognosen hos bröstcancerpatienter.

Enligt nuvarande rutiner gör man en axillutrymning på patienter som har en positiv sentinel node-biopsi. Cirka 50-65 % av dessa patienter har dock ingen ytterligare metastas, och opereras därför ”i onödan”. Svårigheten är att veta vilka patienter som har metastaser i övriga körtlar, och det har tagits fram flera nomogram och poängsystem för att förutsäga denna risk. *Delarbete IV* syftade till att undersöka om patienter som har metastaser i övriga körtlar skiljer sig från de som inte har det vad beträffar olika egenskaper hos primärtumör och sentinel node, och som en del i detta utvärdera ett av de poängsystem som har tagits fram (Tenon score).

Delarbete I

Kvarvarande sentinel node-material tillhörande 50 patienter med falskt negativ sentinel node och 107 kontrollpatienter med sant negativ sentinel node undersöktes med seriesnittning (täta snitt med 0,2 mm mellanrum) och färgning med immunhistokemi. Tidigare okända sentinel node-metastaser upptäcktes hos 18 % av de falskt negativa patienterna, och hos 11 % av de sant negativa. Denna skillnad var inte statistiskt signifikant.

Egenskaper hos tumör och sentinel node jämfördes också mellan de falskt negativa och en grupp med 1204 patienter med positiv sentinel node. Risken för falsk negativitet var högre om tumören var multifokal (växte på flera ställen i bröstet) eller icke känslig för östrogen och progesteron, eller om man bara hittade en sentinel node under operationen.

Delarbete II

I en 5-årsuppföljning av de 2216 sentinel node-negativa patienter i den svenska multicenterkohortstudien som inte genomgått axillutrymning hade 1 % (23 patienter) fått återfall i axillen utan att först ha fått ett återfall i bröstet. Den sjukdomsfria överlevnaden var 89 % och den totala överlevnaden 93 %.

Delarbete III

Överlevnaden för 3369 patienter från den svenska multicenterkohortstudien analyserades och jämfördes mellan fyra grupper: körtelnegativa (71 %), pa-

tienter med isolerade tumörceller (3 %), patienter med mikrometastaser (4 %) och patienter med makrometastaser (>2 mm, 22 %).

Den sjukdomsfria 5-årsöverlevnaden var signifikant sämre för patienter med mikrometastaser än för körtelnegativa (80 % jämfört med 87 %) och lika låg som för patienter med makrometastaser. Även den cancerspecifika 5-årsöverlevnaden var signifikant sämre för patienter med mikrometastaser än för körtelnegativa.

Den totala 5-årsöverlevnaden var 91 % för patienter med mikrometastaser och 94 % för körtelnegativa, men denna skillnad var inte statistisk signifikant. Det var ingen skillnad i överlevnad mellan körtelnegativa och patienter med isolerade tumörceller.

Delarbete IV

De 869 sentinel node-positiva patienter som hade genomgått axillutrymning valdes ut från den svenska multicenterkohortstudien. Egenskaper hos primärtumör och sentinel node jämfördes mellan de som hade och de som inte hade metastaser i övriga körtlar.

Risken för att ha metastaser i övriga körtlar ökade om sentinel node innehöll makrometastas (jämfört med mikrometastas), om kvoten mellan antal positiva sentinel nodes och totalt antal sentinel nodes var hög och om tumören hade den högsta graden (Elstongrad 3). Risken ökade också ju större primärtumören var.

Area under the curve (samlat mått på ett tests känslighet och urskilningsförmåga) för Tenon score (beräknat på förekomst av makrometastas i sentinel node, tumörstorlek och kvoten mellan antalet positiva och totala antalet sentinel nodes) var 0,65. Värdet 0,50 motsvarar att singla slant och för att ett test ska anses adekvat bör värdet vara minst 0,70.

Slutsatser

Otillräcklig snittning av sentinel node verkar inte vara någon betydande orsak till falsk negativitet. Det är dock viktigt att vara noga med att inte lämna någon sentinel node kvar i axillen. Det verkar också finnas en tendens till högre risk för falsk negativitet hos patienter som har en multifokal bröstcancer.

Få sentinel node-negativa patienter som inte genomgått axillutrymning hade fått axillrecidiv efter 5 års uppföljning, och överlevnaden var hög. Sentinel node-biopsi är således en säker metod att använda i rutinsjukvård, oavsett storlek på sjukhus.

Patienter med mikrometastaser har nästan lika dålig prognos som patienter med makrometastaser och bör sannolikt ha motsvarande adjuvant behandling (tilläggsbehandling). Överlevnaden även för patienter med makrometastaser var dock hög i denna studie.

Tenon score gav en otillräcklig prediktion i vår population. Då även tidigare studier som utvärderat olika poängsystem har visat olika resultat beroende på vilken population som använts verkar det vara svårt att försöka förutsäga risken hos varje enskild patient.

Acknowledgements

I would like to express my sincere gratitude to all those who have helped and supported me in my work on this thesis. In particular, I wish to thank:

My mentor, **Leif Bergkvist**, knowledgeable, sensible, always there to help despite a busy schedule, for your almost indiscernible way of making me grow and rise to the occasion. This thesis would not have happened without you.

My co-tutor, **Jan Frisell**, for your kind and inspirational way of sharing your grand experience in surgical science.

My other co-tutor, **Jana de Boniface**, for your sharp-eyed scrutinizing of every draft, always questioning and improving and, together with Leif and Jan, making our scientific meetings productive and enjoyable.

Maria Sylvan and her team at the Department of Pathology in Danderyd for all your help, especially with the sentinel node material for *Paper III*. I would also like to thank **Gunilla Högborg** for your quick work in sectioning the rest of the sentinel nodes.

Co-authors **Göran Liljegren**, **Christian Ingvar** and **Per-Ebbe Jönsson**, for your support and for being the major pillars to the sentinel node project throughout its introduction and spread in Sweden.

Doctors, nurses and secretaries in surgical, oncological and pathology departments all over Sweden, for invaluable help with collection of follow-up data and sentinel node material.

Jerzy Leppert, former head of the Centre for Clinical Research in Västerås, for creating an environment where science may flourish, and for your inspiring way of thinking outside the box.

Mats Enlund, the present head of the Centre for Clinical Research, for believing in me and for providing time and resources for my research.

All the **personnel and researchers** at the Centre for Clinical Research, for your friendly support and coffee break company. In particular, I would like to

thank **Marie-Louise W-E**, for generously introducing me to the large sentinel node data base; **Maria** and **Michaela**, for your prompt and competent solutions to every practical and administrative problem; **Katarina**, the queen of tables and figures; and **Tony**, for guiding me in the strange world of computers. Additionally, special thanks to **Kent**, for your pedagogical help with statistical issues; **Eva S**, for pep talk and small talk; and to my Camargue roommates **Helena** and **Cecilia**.

Staffan, for giving me support and a lot of elbow space, and the rest of the excellent team at the Surgical Breast Unit in Västerås for making me enjoy my clinical work even more: **Eva**, **Elisabeth**, **Marica**, **Kathy**, **Karina**, **Ulrica**, **Christine**, **Olafur** and, of course, **Anders** – still going strong!

My **friends and colleagues** at the Surgical Department in Västerås, for making this an exciting and often fun place to work in. I will not list all the names, but many of you deserve extra appreciation. Special thanks to **Martin** and **Bengt**, for your encouragement and patient tutoring on my path of becoming a surgeon, and to **Johan** and **Abbas** for being there – shared troubles are less heavy to bear.

My parents, probably the world's most supportive, for everything – I know that you believe in me and would stand up for me whatever might happen.

My sister and friend, **Jessica**, for always being there for me and for forcing me to keep an open mind.

Last but not least: **Robert**, my haven in stormy weathers, for loving me the most when I deserve it the least, and simply for being you.

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Paper I



Serial sectioning of breast cancer sentinel nodes does not significantly improve false negativity rate

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Key words: breast cancer, sentinel node, false negativity

Draft: 12-03-07

Abstract

Aims

The aim of this study was to investigate whether an extended pathological examination of sentinel lymph nodes (SLNs) reveals metastases more often in patients with a false negative SLN than in those with a true negative SLN, and to explore tumour and SLN characteristics in these patients.

Methods and results

Archived SLN specimens from 50 false negative cases and 107 true negative controls were assessed after serial sectioning and immunohistochemical staining. Previously undiagnosed SLN metastases were discovered in 9 of 50 (18.0%) patients in the false negative group, and in 12 of 107 (11.2%) patients in the true negative group ($p=0.245$).

Tumour and SLN characteristics of 57 patients with a false negative SLN were compared with 1204 patients with a positive SLN by regression analysis. The risk of false negativity was higher if the tumour was hormone receptor-negative or multifocal, or if only one SLN was identified.

Conclusions

The omission of serial sectioning in SLN examination is not a clinically significant cause of false negativity whereas multifocality seems to increase the risk, as does excising only one SLN.

Introduction

Sentinel lymph node (SLN) biopsy has largely replaced axillary lymph node dissection (ALND) as a routine axillary staging procedure in breast cancer patients. Even though the follow-up in most studies has been relatively short, considering the often late occurrence of relapse in breast cancer disease, SLN biopsy (SLNB) is deemed to be safe and accurate [1-7]. However, there is an inherent risk of SLN false negativity, resulting in some patients being misclassified and potentially undertreated.

The false negative rate (FNR) is calculated by dividing the number of SLN-positive patients by the total number of axillary lymph node-positive patients [8]. Validation studies report rates varying between 0 and 30% [9-11]. The combined use of blue dye and isotope [12-15], removal of more than one SLN [13, 16-20], and increasing experience with the SLNB technique [9, 12, 15] are factors decreasing the FNR. An increased risk of false negativity has been suggested for histological grade 3 breast tumours and for patients diagnosed by a previous excisional biopsy [13, 17]. In several studies, the FNR was higher in patients with lateral than in those with medial tumours [14, 17, 18], probably because lateral isotope injection makes gamma probe detection in the adjacent axilla more difficult to perform. The effect of tumour multifocality on the FNR is debated [21-25], while most authors agree that tumour size has no impact [24, 26-28].

The SLNB technique, retrieving only one or a few lymph nodes per patient, allows the pathologist a more thorough examination than would be reasonable after a more extensive primary ALND. According to a review by Cserni et al [29], serial sectioning and immunohistochemistry (IHC) result in upstaging of 9-47% of those patients whose lymph nodes are negative on conventional haematoxylin and eosin (HE) staining. The SLNB has

thus conveyed a substantial stage migration [30, 31], mostly as a consequence of the increased identification of micrometastatic disease. The clinical importance of micrometastases has been extensively debated [32-39]. However, recent reviews have concluded that patients with micrometastatic disease have a worse prognosis than node-negative individuals [40-42]. Furthermore, at least 10% of patients with SLN micrometastases have additional metastases in non-SLNs [43-45].

In order to optimise the staging of breast cancer patients, it is thus important to minimise the FNR. The purpose of this study was to investigate whether an extended examination of the SLNs more often reveals previously undiagnosed metastases in patients with a false negative SLN than in those with a true negative SLN, and to explore tumour and SLN characteristics in these patients.

Patients and Methods

The Swedish Sentinel Node Multicentre Validation Study

Between March 1998 and December 2001, a total of 675 SLN procedures from 20 Swedish hospitals were included in the Swedish Sentinel Node Multicentre Validation Study. Patients and methods have been described in detail elsewhere [46]. Patients with a palpable, invasive breast cancer less than 3 cm in diameter were eligible for enrolment. Before entering patients in the trial, each surgeon had to perform at least 10 SLNB procedures. After the preoperative sub- or intradermal injection of 40 mBq Technetium-99 nanocolloid (Solco Nanocoll[®]; Nycomed, Amersham, UK) and 1-2 ml blue dye (Patent Blue V[®]; Guerbet, Paris, France), a SLNB was performed. The SLNB was followed by a standard ALND of level I and II in all patients. The false negative rate was 7.7%.

The Swedish Sentinel Node Multicentre Cohort Study

Between September 2000 and January 2004, a total of 3535 SLN procedures from 25 Swedish hospitals were included in the Swedish Sentinel Node Multicentre Cohort Study. Patients and methods have been described in detail elsewhere [47]. In short, patients with a unifocal, invasive breast cancer less than 3 cm in diameter were eligible for enrolment. After the preoperative sub- or intradermal injection of 40-60 mBq Technetium-99 nanocolloid (Solco Nanocoll[®]; Nycomed, Amersham, UK) and 1 ml blue dye (Patent Blue V[®]; Guerbet, Paris, France), a SLNB was performed. If no sentinel node could be identified, ALND of levels I and II was performed. A completion ALND (cALND) was performed in the event of a positive SLNB diagnosed per- or postoperatively. In 314 patients, a cALND was performed despite a negative SLN (if the primary tumour in the breast was found to be multifocal or larger than 3 cm on pathological examination, or at the patient's request). Patients were followed prospectively. The research protocol postulated annual follow-up by mammography and clinical examination.

For both studies, exclusion criteria were palpable regional lymph nodes, neoadjuvant chemo- or radiotherapy, pregnancy, known allergic reactions to blue dye or isotope, previous surgery in the ipsilateral breast and preoperatively diagnosed tumour multifocality. Both studies were approved by the ethics committee of Karolinska Institutet, Stockholm, and each region's local ethics committee. All patients gave written informed consent.

Pathological assessment

Frozen sections were obtained from each SLN and examined during surgery. If a sentinel lymph node was smaller than 4 mm, two sections were analysed separately. Nodes larger than 4 mm were bisected and two sections from each half analysed. For paraffin-embedded histopathology, at least three sections were prepared from the sentinel node or each part of a bisected node. Sections were stained

with haematoxylin and eosin (HE). If no cancer cells were detected, immunohistochemistry (IHC) with cytokeratin antibodies was also performed.

Non-sentinel lymph nodes were examined by routine staining (HE) according to the protocol of each pathology department.

The patients were classified into four lymph node stages according to the revised American Joint Committee on Cancer Staging System for Breast Cancer [48]: node-negative (pN0), isolated tumour cells (ITCs ≤ 0.2 mm, pN0(i+)), micrometastases (>0.2 -2 mm, pN1mi), and macrometastases (>2 mm, pN1).

The present study

Patients with a false negative SLN were included from the above mentioned studies. A false negative SLN procedure was defined either as a negative SLN followed by a positive ALND or as a negative SLN in a patient without cALND who developed an isolated axillary recurrence during follow-up. Twenty-one patients with a positive ALND were included from the validation study and 38 patients were included from the cohort study, 16 with a positive ALND and 22 with an isolated axillary recurrence. From both original studies, 119 patients with a true negative SLN were randomly chosen to form a control group (43 from the validation and 76 from the cohort study).

Archived SLN specimens from the 59 false negative cases and the 119 controls were collected from each pathology department. After serial sectioning, the occurrence of previously undiagnosed SLN metastases was compared between cases and controls.

Tumour and SLN characteristics in patients with a false negative SLN were also compared with all patients in the original studies who had a positive SLN (250 from the validation and 954 from the cohort study).

New pathological assessment

Archived SLN specimens were sectioned at 0.2 mm levels. Two sections were prepared at each level and then stained with HE and IHC, respectively. All sections were then assessed by the same pathologist (MS). Any metastatic deposit was measured and characterised.

Statistical analysis

The detection of previously undiagnosed SLN metastases was compared between patients with false and true negative SLNs using a Chi-2 test.

The size, Elston grade, hormone receptor status and localisation of the primary tumour, presence of multifocality, blue dye and isotope injection technique and the number of SLNs were compared in patients with a false negative SLN and those with SLN metastases using a univariate logistic regression model. All variables of statistical significance in univariate tests were then analysed in a multivariable regression model.

SPSS 20.0[®] was used for all analyses and statistical significance was set at the 0.05 level for all tests.

Results

From the false negative SLN group (N=59), 2 patients were excluded because SLN metastases had been found in the primary pathological examination according to patient file

review. From the control group (N=119), 2 patients were excluded because of erroneous personal identity numbers. Seven SLN specimens from the false negative group and 10 from the control group were unavailable for serial sectioning and these patients were excluded from the analyses of previously undiagnosed metastases. No patients were excluded from the SLN-positive group (N=1204). Thus, 157 patients (50 cases and 107 controls) were left for comparing the groups with false and true negative SLNs after serial sectioning and 1261 patients (57 cases and 1204 controls) for comparing characteristics between the groups with false negative and positive SLNs.

Serial sectioning

Patient and tumour characteristics for patients with false and true negative SLNs are given in Table 1. For the 19 false negative patients who did not have a cALND, the median time to axillary recurrence was 23 months (range 5-87). The median follow-up for the 68 patients in the control group who did not have a cALND was 65 months (range 27-96).

After serial sectioning, previously undiagnosed SLN metastases were detected in 9 of 50 (18.0%) patients in the false negative and in 12 of 107 (11.2%) in the true negative group. The difference in detection rate between the groups was not statistically significant ($p=0.245$).

After excluding patients without a cALND the detection rate in the true negative group was 10.9% (5/46, $p=0.463$).

Most of the previously unknown metastases were ITCs (N=14), 6 were micrometastases and only 1 was a macrometastasis (size 3 mm). For further details, see Table 3.

Correlation between clinicopathological characteristics and false negativity

The median number of SLNs was 1 (range 1-7) in the false negative group, and 2 (range 1-10) in the SLN-positive group. The median number of positive SLNs in the latter group was 1 (range 1-9). Completion ALND was performed in 37 (64.9%) patients with false negative SLNs and in 1118 (92.9%) with positive SLNs. Of the 24 false negative cases who had a positive cALND, 23 (95.8%) had macrometastases and 1 (4.2%) ITC, and of all 1204 SLN-positive patients, 251 (80.8%) had macrometastases, 123 (10.2%) micrometastases and 108 (9.0%) ITC.

The mean number of non-SLNs harvested by cALND was 10.2 (median 11, range 1-24) after a false negative SLNB and 11.2 (median 10, range 1-40) after a positive SLNB. The axillary tumour burden, defined as the total number of positive lymph nodes, was higher in the SLN-positive (mean 2.4) than in the false negative group (mean 2.0), but the difference was not statistically significant ($p=0.209$).

Hormone receptor status, number of SLNs and multifocality were significantly associated with false negativity in uni- and multivariable analyses (Table 2). Tumour Elston grade was significant only in univariate analysis.

The risk of false negativity was higher in hormone receptor- negative or multifocal tumours or if only one SLN was found. Three of 18 patients (14.3%) with an isolated axillary recurrence had multifocal tumours.

Discussion

The false negative rate (FNR) of the SLNB procedure averages 8-9% [11], resulting in some patients being misclassified and potentially undertreated. In this study, aiming to explore the causes of false negativity, we found similar rates of previously undiagnosed SLN metastases after serial sectioning in false and true negative patients, indicating that the omission of serial sectioning was not a significant cause of false negativity. However, the risk was higher in patients with multifocal or hormone receptor-negative tumours, and if only one SLN was found.

In the present study, the upstaging rates after serial sectioning and IHC in the false (18.0%) and true (11.2%) negative groups were comparable to those in previous studies [49-51]. In a pre-SLN review, Dowlatshahi et al. [49] found serial sectioning and IHC to detect metastases in 9-33% of patients whose lymph nodes were negative after routine sectioning. Certainly, since the pathological work-up of SLNs is more thorough, the upstaging rate is expected to be lower after serial sectioning of SLNs than of unselected axillary lymph nodes and, accordingly, Weaver et al [51] identified new metastases in 15.9% of previously negative SLNs after serial sectioning with IHC.

There are inconsistencies and controversies regarding the pathological work-up of SLNs [29], and no generally applied guidelines exist. However, considering the similar upstaging rates in the false and true negative groups in the present study, serial sectioning of SLNs is probably not cost-effective and may subject a considerable number of patients to over-treatment.

The accuracy of SLNB in multifocal tumours is not fully established. In the present study, almost 25% (N=14) of the patients with a false negative SLN had multifocal tumours, and their hazard ratio was significantly higher (3.15). This could partly be explained by the fact

that in the Swedish Multicentre Validation Study [46], from which 8 of the false negative patients with multifocal tumours were included, the FNR in patients with multifocal tumours was 21.0% (compared with 5.6% in patients with unifocal tumours). Furthermore, in the Swedish Multicentre Cohort Study [47], from which the rest of the patients were collected, multifocality was a criterion for cALND, which is why this group was probably biased. However, 3 of 18 (14.3%) false negative patients with isolated axillary recurrences from the cohort study had multifocal tumours. Thus, multifocal tumours seem to be overrepresented in this group too, even though the small numbers make a comparison difficult.

Several previous studies have also found a higher FNR in multifocal tumours[21] [23], while other authors conclude that SLNB is accurate in multifocal breast cancer, reporting false negative rates from 0 to 8% [25, 52-54]. However, the latter studies do not report any corresponding false negative rates for patients with unifocal tumours. In a recent review, Spillane and Brennan [24] conclude that SLNB is safe in multiple breast cancer. However, several studies in the review had a FNR exceeding 10%. Overall, there seems to be a tendency towards higher FNR in multi- than in unifocal breast cancer. While waiting for the results of ongoing studies, caution might be called for when considering SLNB in patients with multifocal tumours.

We also observed a higher FNR in hormone receptor-negative patients. The reason for this is unclear and, to our knowledge, this association has not been reported elsewhere. One theory could be that hormone receptor-negative tumours have features that cause lymphatic vessel invasion, changing the route of lymphatic draining. The association could also be purely by chance.

The association between the number of excised SLNs and the FNR was strong in the present study with a three times higher risk in patients with only one SLN *versus* more than one

removed. This has been demonstrated by several previous studies [13, 17-19]. Up to four SLNs have been reported to increase accuracy [16, 20].

Previous studies have indicated a higher risk of false negativity for tumours located in the lateral part of the breast [14, 17, 18]. We detected no such association in the present study. An explanation could be that the combined use of isotope and blue dye was practised in almost all patients, possibly facilitating the identification of SLNs when isotope injected laterally in the breast was interfering with the radioactive signal from the SLN.

A weakness of the present study is that not all patients had cALND. One could argue that the true negative controls who did not undergo cALND and after serial sectioning were found to have SLN metastases would have had an axillary recurrence if the follow-up had been long enough. In this case, the proportion of newly detected SLN metastases would probably be higher in the false negative group. However, none of these patients had a shorter follow-up than the median time to axillary recurrence in the patients with a false negative SLN. In fact, almost all patients had a much longer follow-up, and it is unlikely that more than a few of them would receive axillary recurrences with prolonged follow-up. Furthermore, excluding the true negative patients without cALND did not change the results.

Another possible weakness is that non-SLNs were only examined by routine staining according to the protocol of each pathology department. Considering that routine staining misses up to 33% of metastases [49], this would imply that some of the patients that were diagnosed to be true negative were actually false negative. We do not believe, however, that this has significantly influenced our results.

In conclusion, the omission of serial sectioning of SLN does not appear to be a significant cause of false negativity. By contrast, multifocality, hormone receptor negativity, and the extraction of only one SLN seem to increase the risk. To decrease the risk of false negativity,

care should be taken not to leave any SLNs behind, including careful palpation of the axillary wound.

Acknowledgements

The authors thank the medical staff in surgical, oncological and pathology departments at all participating hospitals for invaluable help with collection of follow-up data and sentinel node material.

The study was funded by grants from the Swedish Breast Cancer Association (BRO), the Swedish Cancer Society and Centre for Clinical Research Uppsala University.

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SLN status		
Characteristics	False negative SLN	True negative SLN
N	50	107
Age (years)^a	58 (31-84)	62 (35-89)
Tumour size (mm)^b	23 (16)	15 (7)
Histotype^c		
ductal	37 (74.0)	79 (73.8)
lobular	8 (16.0)	8 (7.5)
mixed	1 (2.0)	1 (0.9)
other	0	9 (8.4)
missing	4 (8.0)	10 (9.3)
Tumour grade (Elston grade)^c		
1	4 (8.0)	31 (29.0)
2	23 (46.0)	43 (40.2)
3	22 (44.0)	28 (26.2)
missing	1 (2.0)	5 (4.7)
Oestrogen receptor^c		
positive	36 (72.0)	86 (80.4)
negative	14 (28.0)	20 (18.7)
missing	0	1 (0.9)
Progesterone receptor^c		
positive	26 (52.0)	69 (64.5)
negative	23 (46.0)	35 (32.7)
missing	1 (2.0)	3 (2.8)
Number of SLNs^a	1 (1-7)	2 (1-9)
Number of non-SLNs^{a,d}	11 (1-24)	10 (1-20)

^a Median (range); ^b Mean (standard deviation); ^c Number (%); ^d In patients who had completion axillary lymph node dissection (N=32 in false negative, N=46 in true negative) SLN=sentinel lymph node

Table 1. Characteristics of patients with false or true negative sentinel lymph node biopsy.

SLN status						
Characteristics	False negative SLN	Positive SLN	P	Hazard ratio	95 % CI	P
N	57	1204	univariate	multivariate		multivariate
Age (years)^a	58 (31-84)	57 (23-90)	0.954			
Tumour size (mm)^b	23 (12)	17 (8)	0.115			
Histotype^c			0.794			
lobular	10 (17.6)	188 (15.6)				
other	43 (75.4)	788 (73.8)				
missing	4 (7.0)	128 (10.6)				
Tumour grade (Elston)^c			0.025			0.457
1 or 2	33 (57.9)	859 (71.3)		1		
3	23 (40.3)	320 (26.6)		1.26	0.68-2.34	
missing	1 (1.8)	25 (2.1)				
Hormone receptor^c			0.001			0.002
positive	41 (71.9)	1055 (87.7)		1		
negative	16 (28.1)	134 (11.1)		2.93	1.48-5.80	
missing	0	15 (1.2)				
Number of SLNs^c			<0.001			<0.001
1	35 (61.4)	395 (32.8)		3.27	1.86-5.73	
>1	22 (38.6)	809 (67.2)		1		
Tumour site^c			0.140			
UOQ	34 (59.6)	562 (46.7)				
other	20 (35.1)	518 (43.0)				
missing	3 (5.3)	124 (10.3)				
Multifocality^c			0.003			0.001
yes	14 (24.6)	133 (11.0)		3.15	1.63-6.09	
no	43 (75.4)	1071 (89.0)		1		
Injection site^d			0,235			
peritumoural	2 (3.5)	21 (1.7)				
dermal	46 (80.7)	824 (68.5)				
both	7 (12.3)	232 (19.3)				
missing	2 (3.5)	127 (10.5)				

^a Median (range); ^b Mean (standard deviation); ^c Number (%); ^d Isotope and/or Patent Blue; SLN=sentinel lymph node; UOQ=upper outer quadrant

Table 2. Univariate and multivariable analyses in patients with false negative or positive sentinel lymph node biopsy.

Subject	Study group	Tumour characteristics ^a	Type of metastasis	Comment	Previous IHC
1	case	28 mm, ductal grade 2, ++	micro	>200 tumour cells	yes
2	case	16 mm, ductal grade 2, ++	micro	1 mm	no
3	case	22 mm, ductal grade 3, ++	ITC		no
4	case	45 mm, lobular grade 2, +-	ITC		yes
5	case	18 mm, ductal grade 2, ++	ITC	tumour cells only in the lymph node sinuses	yes
6	case	29 mm, mixed grade 3, ++	micro	1 mm	yes
7	case	15 mm, lobular grade 1, ++	ITC		yes
8	case	14 mm, ductal grade 2, +-	ITC		yes
9	case	31 mm, ductal grade 2, +-	ITC	extensive metastases in fatty tissue surrounding metastatic non-SLNs	unknown
10	control	20 mm, lobular unknown grade, ++	ITC		no
11	control	14 mm, ductal grade 2, +-	ITC		no
12	control	9 mm grade 2, ++	micro	1 mm	no
13	control	15 mm grade 3, ++	ITC		no
14	control	4 mm grade 1, ++	macro	5 mm	no
15	control	16 mm, ductal grade 1, ++	micro	extensive periglandular metastasis	no
16	control	12 mm, ductal grade 3, ++	ITC	tumour cells only in the lymph node sinuses	no
17	control	15 mm, ductal unknown grade, ++	ITC		no
18	control	27 mm, ductal grade 1, ++	micro		yes
19	control	15 mm, ductal grade 1, ++	ITC		yes
20	control	21 mm, ductal grade 2, ++	ITC		no
21	control	19 mm, ductal grade 1, ++	ITC		unknown

^asize, histotype, Elston grade, receptor status; IHC=immunohistochemistry; ITC=isolated tumour cells; SLN=sentinel lymph node; ++: oestrogen- and progesterone-positive; +-: oestrogen-positive and progesterone-negative

Table 3. Description of previously unknown metastases in cases (false negative SLN) and controls (true negative SLN).

Paper II



Axillary recurrence rate 5 years after negative sentinel node biopsy for breast cancer

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Background: Sentinel lymph node (SLN) biopsy has replaced axillary lymph node dissection (ALND) as the standard axillary staging procedure in breast cancer. Follow-up studies in SLN-negative women treated without ALND report low rates of axillary recurrence, but most studies have short follow-up, and few are multicentre studies.

Methods: Between September 2000 and January 2004, patients who were SLN-negative and did not have ALND were included in a prospective cohort. Kaplan-Meier estimates were used to analyse the rates of axillary recurrence and survival. The risk of axillary recurrence was also compared in centres with high and low experience with the SLN biopsy (SLNB) technique.

Results: A total of 2195 patients with 2216 breast tumours were followed for a median of 65 months. Isolated axillary recurrence was diagnosed in 1.0 per cent of patients. The event-free 5-year survival rate was 88.8 per cent and the overall 5-year survival rate 93.1 per cent. There was no difference in recurrence rates between centres contributing fewer than 150 SLNB procedures to the cohort and centres contributing 150 or more procedures.

Conclusion: This study confirmed the low risk of axillary recurrence 5 years after SLNB for breast cancer without ALND.

Paper accepted 31 October 2011

Published online 19 December 2011 in Wiley Online Library (www.bjs.co.uk). DOI: 10.1002/bjs.7820

Introduction

Sentinel lymph node biopsy (SLNB) was introduced by Krag and Giuliano in the early 1990s^{1,2}. Following validation of its accuracy³, SLNB evolved to become the standard axillary staging procedure in women with breast cancer. In the Swedish validation study, the false-negative rate was 7.7 per cent⁴, which is comparable to that in other validation studies. In an early review, the median false-negative rate of SLNB was reported to be 7 per cent³, but was as high as 30–40 per cent in some of the included studies. This raised concerns regarding a higher risk of axillary recurrence following a negative SLNB result, if axillary lymph node dissection (ALND) is omitted.

In apparent support of this concern, Kujit and Roumen⁵ described axillary recurrence in five (5.0 per cent) of 100

sentinel lymph node (SLN)-negative patients in whom ALND was not done (median follow-up 6.3 years). In a randomized multicentre study by Zavagno and colleagues⁶, there was a higher incidence of locoregional recurrence and reduced disease-free survival after SLNB alone than if ALND was added, although the result was not statistically significant.

The majority of follow-up studies after negative SLNB have reported few axillary recurrences^{7–10}, although most of these studies had a relatively short follow-up^{7,10–13}, and only one included more than 800 patients. Most of the data were from specialized centres.

The present study presents data from a large multicentre cohort study of sentinel node-negative patients with breast cancer.

Methods

The Swedish Sentinel Node Multicentre Cohort Study

Between September 2000 and January 2004, women (with 3535 breast tumours) were included in the Swedish Sentinel Node Multicentre Cohort Study. Patients and surgical methods have been described in detail elsewhere¹⁴. In brief, women with unifocal, clinically node-negative (preoperative axillary ultrasonography was not mandatory), invasive breast cancer less than 3 cm in diameter were eligible for enrolment. Exclusion criteria were: neoadjuvant chemotherapy or radiotherapy, pregnancy, known allergic reaction to blue dye or isotope, previous surgery to the ipsilateral breast, and multifocal tumour diagnosed before surgery. Written informed consent was obtained from all women. The study was approved by the ethics committee of Karolinska Institute, Stockholm, and each participating region's local ethics committee (registration number: NCT01351974; <http://www.clinicaltrials.gov>). Twenty-six Swedish hospitals (9 university, 13 county, 1 private and 3 community) and 131 surgeons contributed to accrual in this study.

Identification of sentinel node and surgical procedure

The sentinel node was identified by means of isotope injection (Solco Nanocoll®; Nycomed, Amersham, UK) with preoperative lymphoscintigraphy, and blue dye (Patent Blue V®; Guerbet, Paris, France).

Pathological assessment

Frozen sections were taken from each SLN and examined during surgery. If a sentinel node was smaller than 4 mm, two sections were analysed separately. Nodes with a diameter of 4 mm or more were bisected, and two sections from each half analysed. According to the study protocol, at least three sections were prepared from the sentinel node or each part of a bisected node for definitive histopathological assessment. Sections were stained with haematoxylin and eosin. If no cancer cells were detected, immunohistochemistry with cytokeratin antibodies was also performed.

Non-sentinel nodes were examined by routine haematoxylin and eosin staining according to the protocol of each pathology department.

Treatment and follow-up

Patients with a negative SLN (including those with isolated tumour cells) and unifocal tumour did not proceed to axillary dissection.

Adjuvant treatment was given according to national and regional guidelines, based on tumour characteristics, lymph node status and surgical treatment. Patients with isolated tumour cells were regarded as lymph node-negative. Breast-conserving surgery was followed by radiation therapy. Chemotherapy was offered to all women with lymph node metastases or to those with a combination of unfavourable primary tumour characteristics (large tumour size, high Elston score, hormone receptor-negative), after consideration of their general health. Endocrine therapy was offered to all women with oestrogen or progesterone receptor-positive tumours larger than 10 mm.

Patients were followed prospectively, with annual mammography and clinical examination.

Shortly before data analysis, a list of all included patients was sent to all participating centres requesting updated information on events and latest follow-up dates. In addition, the authors were granted access to hospital files at onsite visits in order to update and validate reported data.

The present analysis

Sentinel node-negative patients without completion ALND were included from the Swedish sentinel node cohort. Patients who were followed up outside Sweden, who had ductal carcinoma *in situ* only, or who had distant disease at the time of surgery were excluded. Patients with multifocal tumours or tumours larger than 3 cm on postoperative histopathology were included in the present analysis if completion ALND had been omitted.

Definitions of recurrence

Lymph node recurrence was reported as either axillary or extra-axillary (supraclavicular or cervical). Axillary recurrence was considered to be isolated when the axilla was the sole initial site of recurrence, and as locoregional if the patient developed an ipsilateral breast recurrence before, or concurrently with, the axillary recurrence. Local recurrence was defined as a relapse in the ipsilateral breast. Recurrences at separate sites were regarded as synchronous if they were diagnosed within the same 2-month interval. Recurrences outside the breast and axilla were regarded as generalized disease.

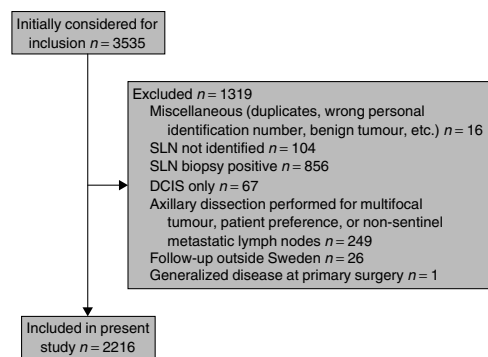


Fig. 1 Selection of procedures from the Swedish Sentinel Node Multicentre Cohort Study for inclusion in the present analysis. SLN, sentinel lymph node; DCIS, ductal carcinoma *in situ*

Statistical analysis

The primary endpoint was the rate of axillary recurrence. Secondary endpoints were overall recurrence, and cancer-specific, event-free and overall survival.

Kaplan–Meier curves were constructed for analysis of axillary recurrence and survival. From the date of SLNB, breast cancer-specific survival was determined to the date of death from breast cancer; event-free survival to the date of any recurrence, contralateral breast cancer or death from any cause; and overall survival to the date of death. In the absence of any event, time was calculated from the date of SLNB to the date of last follow-up.

Cox proportional hazard regression analysis was used to assess the axillary recurrence hazard ratio for patients accrued in centres with low experience (those contributing fewer than 150 SLNB procedures to the whole cohort) compared with high-experience centres (those contributing 150 or more SLNB procedures). SPSS® version 14.0 software (SPSS, Chicago, Illinois, USA) was used for all analyses, and statistical significance was set at the 0.05 level for all tests.

Results

Initially 3501 women with 3535 breast tumours were recruited in the Swedish Sentinel Node Multicentre Cohort Study. A total of 2195 SLN-negative women with 2216 tumours fulfilled the inclusion criteria (*Fig. 1*); median follow-up was 65 (range 0–113) months. Patient and tumour characteristics are shown in *Table 1*. Multiple foci of invasive tumour were detected on histopathology in 94 women (4.3 per cent), and 46 (2.1 per cent) had a tumour

Table 1 Patient and tumour characteristics

	No. of procedures* (n = 2216)
Patient age (years)†	60 (23–94)
Tumour size (mm)‡	15(7)
Histological subtype	
Ductal	1464 (66.1)
Lobular	255 (11.5)
Mixed	15 (0.7)
Other	164 (7.4)
Not known	318 (14.4)
Tumour grade (Elston score)	
1	674 (30.4)
2	1056 (47.7)
3	410 (18.5)
Not known	76 (3.4)
Oestrogen receptor status	
Positive	1888 (85.2)
Negative	271 (12.2)
Not known	57 (2.6)
Progesterone receptor status	
Positive	1524 (68.8)
Negative	615 (27.8)
Not known	77 (3.5)
No. of SLNs†	2 (1–5)
Adjuvant treatment	
Hormonal	1332 (60.1)
Radiotherapy	1668 (75.3)
Chemotherapy	224 (10.1)

*With percentages in parentheses unless indicated otherwise; values are †median (range) or ‡mean(s.d.). SLN, sentinel lymph node.

larger than 3 cm. SLNs with isolated tumour cells (smaller than 0.2 mm) were detected in 40 women (1.8 per cent).

In the whole sentinel node cohort, the median number of procedures performed at each of the 26 participating hospitals was 132 (range 1–302); 131 surgeons contributed a median of 9 (range 1–137) procedures. Sixty-three surgeons did fewer than ten procedures, 43 did 10–50, 22 did 51–100, and three surgeons carried out more than 100 procedures.

For the 2216 procedures in patients with negative sentinel nodes and no further axillary surgery, 25 hospitals contributed a median of 97 (range 1–193) procedures, with 121 surgeons each contributing a median of 7 (range 1–91) procedures.

Recurrence

Overall, 203 women (9.2 per cent) developed 256 recurrences. Isolated axillary recurrence was detected in 23 women (1.0 per cent) after a median follow-up of 25 (range 4–87) months. Twelve of the isolated axillary recurrences were diagnosed more than 2 years after surgery, three of these after more than 5 years (61, 77 and 87 months). The

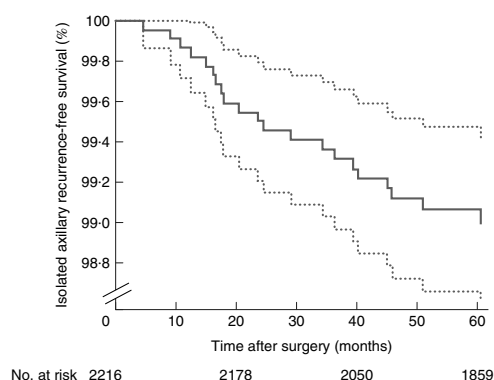


Fig. 2 Kaplan–Meier curve for isolated axillary recurrence-free survival, with 95 per cent confidence intervals, for patients initially sentinel lymph node-negative who did not have axillary lymph node dissection

5-year isolated axillary recurrence-free survival was 99.0 (95 per cent confidence interval (c.i.) 98.6 to 99.4) (Fig. 2). Locoregional axillary recurrence was found in an additional 14 women. Thus, overall, 37 axillary recurrences (1.7 per cent of patients) were identified. There was no difference in the rate of axillary recurrence between patients treated in centres with low or high experience ($P = 0.574$ for isolated and $P = 0.719$ for total axillary recurrence).

Isolated axillary recurrence was found in three (3 per cent) of the 94 women with multiple foci of invasive tumour, but in none of the 46 women with a unifocal tumour larger than 3 cm in diameter.

Local recurrence was reported in 57 women (2.6 per cent) and distant metastases were diagnosed in 119 (5.4 per cent), of which 14 (0.6 per cent) were extra-axillary lymph node recurrences. Contralateral breast cancer was diagnosed in 43 women (1.9 per cent).

Survival

During follow-up, 179 women (8.2 per cent) died. Of these, 69 deaths (3.1 per cent) were reported to be caused by breast cancer. At 5-year follow-up, the breast cancer-specific survival rate was 97.2 (95 per cent c.i. 96.5 to 98.0) per cent, event-free survival rate 88.8 (87.5 to 90.2) per cent, and overall survival rate 93.1 (92.0 to 94.2) per cent.

Discussion

In this large prospective multicentre study of SLNB as a single axillary staging procedure for breast cancer, the

axilla was the sole initial site of recurrence in 23 women (1.0 per cent). This is similar to the findings of most previous studies of SLN-negative patients from highly specialized centres^{11,15–20}, and in another Swedish study²¹ where the axillary recurrence rate was 1.0 per cent after 5 years, but in this case following ALND. The proportion of node-negative patients receiving additional adjuvant therapy has increased since the latter study, complicating such a comparison. However, the lack of a difference between the axillary recurrence rates after both ALND and SLNB seems to support the proposal that SLNB is a safe staging procedure. Despite early reports of high false-negative rates of up to 40 per cent, an increasing number of follow-up studies with longer follow-up have demonstrated low axillary recurrence rates after SLNB. SLNB is associated with substantially less arm morbidity than ALND^{22,23}, and is now considered to be the standard staging method.

Kuijt and Roumen⁵ identified axillary recurrence in five (5.0 per cent) of 100 patients with SLN-negative breast cancer in whom ALND had not been performed (median follow-up 6.5 years). Three of the recurrences were discovered more than 2 years after surgery, the interval previously considered to reveal the majority of axillary recurrences. Based on these results, the authors⁵ calculated a lifetime axillary recurrence risk of 10 per cent and thus suggested caution. Their study was, however, very small. In the present study, 12 of the isolated axillary recurrences were diagnosed more than 2 years after SLNB, three of which were found after more than 5 years. Follow-up of 10–15 years in these SLN-negative patients treated without ALND is necessary to evaluate the safety of the method fully.

Most previous follow-up studies derived from specialized centres. According to previous experience, some new techniques perform excellently in specialized settings but are less successful outside these centres. In the present study, patients were treated at 25 hospitals by 121 surgeons, with similar results. There are only four other multi-institutional follow-up studies of SLN-negative patients^{6,7,24,25}; none of these reported worse axillary recurrence rates than single-centre studies.

The present protocol included immunohistochemical examination of all SLNs, which may have led to detection of metastases not seen on routine examination. This could have contributed to the low axillary recurrence rate; however, comparable results from many other studies that did not use immunohistochemistry suggest that the effect of this technique on the present results was negligible.

So far, the evidence continues to support the value and safety of SLNB as the standard axillary staging procedure in breast cancer.

Acknowledgements

The authors are grateful for the effort of the responsible surgeons and staff at participating centres who have collected and followed these patients. Special thanks also go to Marie-Louise Walker-Engström for data management.

This study was funded by grants from the Swedish Breast Cancer Association (BRO), the Swedish Cancer Society and the Centre for Clinical Research, Uppsala University.

Disclosure: The authors declare no conflict of interest.

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Snapshot Quiz 12/04

Question. A 32-year-old man was referred with a short history of the illustrated finger changes and a swelling in the same arm at the site of a previously ligated arteriovenous fistula. What is the diagnosis?



The answer to the above question is found on p. 245 of this issue of *BJS*.

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Paper III



Breast Cancer Survival in Relation to the Metastatic Tumor Burden in Axillary Lymph Nodes

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Submitted June 10, 2009; accepted March 10, 2010; published online ahead of print at www.jco.org on May 10, 2010.

Supported by grants from the Swedish Breast Cancer Association, the Swedish Cancer Society, and the Centre for Clinical Research, Uppsala University.

Presented in part at the 14th Annual Meeting of the Swedish Surgeon's Society, August 17-21, 2009 Halmstad, Sweden.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/10/2817-2868/\$20.00

DOI: 10.1200/JCO.2009.24.5001

ABSTRACT

Purpose

The aim of this study was to determine the prognostic significance of lymph node micrometastases in patients with breast cancer.

Patients and Methods

Between September 2000 and January 2004, 3,369 patients with breast cancer were included in a prospective cohort. According to their lymph node status, they were classified in the following four groups: 2,383 were node negative, 107 had isolated tumor cells, 123 had micrometastases, and 756 had macrometastases. Median follow-up time was 52 months. Kaplan-Meier estimates and the multivariate Cox proportional hazard regression model were used to analyze survival.

Results

Five-year cause-specific and event-free survival rates were lower for patients with micrometastases (pN1mi) than for node-negative (pN0) patients (94.1% v 96.9% and 79.6% v 87.1%, respectively; $P = .020$ and $P = .032$, respectively). There was no significant survival difference between node-negative patients and those with isolated tumor cells. The overall survival of pN1mi and pN0 patients did not differ.

Conclusion

This study demonstrates a worse prognosis for patients with micrometastases than for node-negative patients.

J Clin Oncol 28:2868-2873. © 2010 by American Society of Clinical Oncology

INTRODUCTION

The introduction of the sentinel lymph node (SLN) biopsy has conveyed a substantial stage migration as shown by several authors.¹⁻³ This is mostly a result of an increased identification of micrometastatic disease and has thus intensified the debate on its prognostic significance.⁴⁻¹⁰ One of the few studies with a long follow-up (median, 18.9 years)⁶ found no difference in survival between patients with and without micrometastases. Recent studies suggest a worse prognosis for patients with micrometastases than for those without.^{8,11,12}

Most previous studies are based on the detection of micrometastases by re-examination of lymph nodes retrieved by standard axillary lymph node dissection (ALND). The significance of micrometastases found in SLN biopsies has been studied in a few recent studies.^{5,11,13-17} The results indicate a worse prognosis for patients with micrometastases than for those without, whereas the role of isolated tumor cells (ITCs) is still unclear.

The present prospective cohort study included 3,369 patients of whom 230 had metastases smaller

than 2 mm found at routine pathologic work-up of their SLNs and non-SLNs. The purpose of this study was to investigate the prognostic role of ITCs and micrometastases in axillary lymph nodes.

PATIENTS AND METHODS

The Swedish Sentinel Node Multicenter Cohort Study

Between September 2000 and January 2004, 3,501 women (with 3,535 breast tumors) from 25 Swedish hospitals were included in the Swedish Sentinel Node Multicenter Cohort Study. Patients and surgical methods have been described in detail elsewhere.¹⁸ In short, patients with a unifocal, invasive breast cancer less than 3 cm in diameter were eligible for enrollment. Exclusion criteria were palpable regional lymph nodes, neoadjuvant chemo- or radiotherapy, pregnancy, known allergic reactions to blue dye or isotope, previous surgery in the ipsilateral breast, and preoperatively diagnosed tumor multifocality. The trial was designed to analyze the risk of regional recurrence in the ipsilateral axilla, and therefore, patients with a previous contralateral breast cancer could be included. For the purpose of studying survival, however, they were excluded. After enrollment, data sheets, including information on

primary tumor characteristics, number of SLNs and non-SLNs with and without metastasis, and administered adjuvant treatment, were computerized. The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm, Sweden, and each region's local ethics committee. All patients gave written informed consent.

Surgical Procedure

After the preoperative injection of 40 to 60 mBq of technetium-99 nanocolloid (Solco Nanocol; Nycomed, Amersham, United Kingdom) and 1 mL of blue dye (Patent Blue V), SLN biopsy was performed. If no SLN could be identified, ALND of levels I and II was performed. A completion ALND was also performed in the event of a positive SLN biopsy, if lymph nodes clearly suspicious of metastasis were detected during surgery, or if the primary tumor in the breast was found to be multifocal on pathologic examination.

Pathologic Assessment

Frozen sections were obtained from each SLN and examined perioperatively. If an SLN was smaller than 4 mm, two sections were analyzed separately. Nodes larger than 4 mm were bisected, and two sections from each half were analyzed. According to the study protocol, at least three sections were prepared from the SLN or each part of a bisected node for definitive histopathology. Sections were stained with hematoxylin and eosin (HE). If no cancer cells were detected, immunohistochemistry with cytokeratin antibodies was also performed. Non-SLNs were examined by routine staining (HE) according to the protocol of each pathology department.

Treatment and Follow-Up

Adjuvant treatment combinations were given according to national and regional treatment guidelines, based on tumor characteristics, lymph node status, and surgical treatment. Patients with ITCs were regarded as lymph node negative. If breast-conserving surgery had been performed, radiation therapy to the breast was given, which was extended to include the regional lymph nodes in case of axillary lymph node metastases.

Chemotherapy was offered to all patients with lymph node metastases or those with a combination of unfavorable primary tumor characteristics (large tumor, high Elston score, and progesterone receptor negativity), after consideration of their general health. Endocrine therapy was offered to all patients with estrogen receptor- or progesterone receptor-positive tumors larger than 10 mm. The actual reported adjuvant treatment is controlled for in the analyses.

Patients were observed prospectively. The research protocol postulated an annual follow-up with mammography and clinical examination.

Shortly before data analysis, a list of all included patients was sent to all participating centers and returned to the research center with updated information on events and latest follow-up dates. Furthermore, the authors were granted access to hospital files at on-site visits to update reported data.

Present Analysis

For the present analysis, 163 tumors from the database were excluded for reasons given in Figure 1, leaving a total of 3,369 patients for evaluation. Those pathology reports describing SLN micrometastases after routine pathologic work-up were scrutinized by a pathologist and three surgeons to differentiate between micrometastases and ITCs. When any doubts remained, original slides were re-examined and reclassified by a pathologist (MS). On the basis of the largest metastasis in either SLNs or non-SLNs, the following four groups of patients (classified according to the revised American Joint Committee on Cancer Staging System for Breast Cancer¹⁹) were identified: node negative (pN0), ITCs [< 0.2 mm, pN0(i+)], micrometastases (0.2 to 2 mm, pN1mi), and macrometastases (> 2 mm, pN1). The groups were compared regarding cause-specific survival, event-free survival, overall survival (OS), and the incidence of distant and regional recurrence.

Statistical Analysis

Cox proportional hazard regression analyses were used to assess the hazard ratio for adverse outcome for patients with ITCs, micrometastases, and macrometastases compared with patients without lymph node metastases. Age and tumor size (continuous variables), histologic grade of the tumor, ALND, and adjuvant treatment (categorical variables) were adjusted for in the

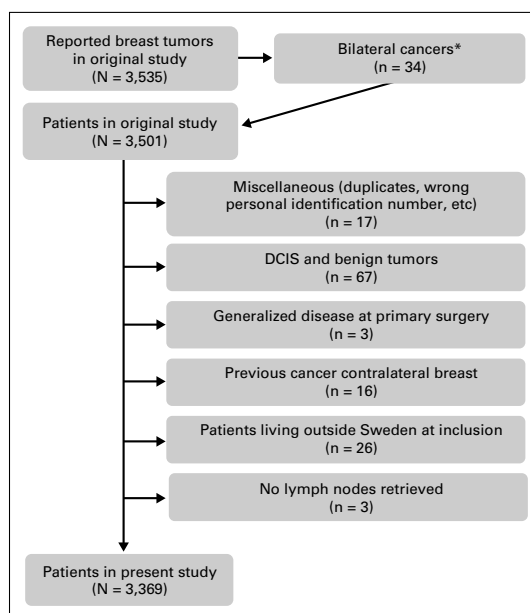


Fig 1. CONSORT diagram for patient inclusion and exclusion. (*) In case of bilateral cancers, only one cancer was included in the study. The cancer with lowest lymph node stage, smallest tumor size, or lowest tumor grade was excluded. DCIS, ductal carcinoma in situ.

analyses. Analyses were also performed after stratification in two age groups (younger or older than age 50 years).

The study end points were cause-specific survival, event-free survival, and OS and were calculated from Kaplan-Meier estimates. Measured from the date of the SLN biopsy, cause-specific survival was calculated to the date of death as a result of breast cancer; event-free survival was calculated to the date of local, axillary, or distant recurrence, contralateral breast cancer, or death from any cause; and OS was calculated to the date of death. In the absence of any event, time was calculated from the date of SLN biopsy to the date of last follow-up. SPSS version 14.0 (SPSS, Chicago, IL) was used for all analyses, and statistical significance was set at $P = .05$ for all tests.

RESULTS

Overall, 3,369 patients with breast cancer met the inclusion criteria; 2,383 (71%) were node negative, 107 (3%) had ITCs, 123 (4%) had micrometastases, and 756 (22%) had macrometastases (Appendix Fig A1, online only). Median follow-up time was 52 months (range, 0 to 91 months).

Patient and tumor characteristics are listed in Table 1. Patients with macrometastases were given chemotherapy twice as frequently as those with micrometastases. Although pN0(i+) patients were regarded as lymph node negative according to the study protocol, they received chemotherapy and hormonal therapy more often than pN0 patients (Table 1). Nearly all estrogen receptor- or progesterone receptor-positive patients received hormonal therapy. However, hormonal therapy was omitted in 320 patients with estrogen

Table 1. Demographic, Clinical, and Tumor Characteristics According to Nodal Involvement

Characteristic	Lymph Node Status							
	No Metastases (n = 2,383)		Isolated Tumor Cells (n = 107)		Micrometastases (n = 123)		Macrometastases (n = 756)	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Age, years								
Median	60		56		59		57	
Range	23-94		38-82		28-89		28-91	
Tumor size, mm								
Mean	15		17		17		20	
Standard deviation	7		6		5		9	
Histotype								
Ductal	1,590	66.7	72	67.2	79	64.2	502	66.4
Lobular	272	11.4	14	13.1	18	14.6	113	14.9
Mixed	16	0.7	1	0.9	3	2.5	7	0.9
Other	174	7.3	7	6.5	8	6.5	32	4.3
Missing	331	13.9	13	12.3	15	12.2	102	13.5
Tumor grade (Elston)								
1	699	29.3	28	26.2	38	30.9	147	19.4
2	143	48.0	61	56.9	58	47.2	388	51.3
3	456	19.1	16	15.0	23	18.7	203	26.9
Missing	85	3.6	2	1.9	4	3.2	18	2.4
Estrogen receptor status								
Positive	2,018	84.7	96	89.7	107	87.0	652	86.2
Negative	306	12.8	10	9.4	12	9.8	97	12.9
Missing	59	2.5	1	0.9	4	3.2	7	0.9
Progesterone receptor status								
Positive	165	69.1	79	73.8	87	70.7	536	70.9
Negative	659	27.6	27	25.3	30	24.4	205	27.1
Missing	79	3.3	1	0.9	6	4.9	15	2.0
Antihormonal therapy	1,443	60.6	87	81.3	100	81.3	637	84.3
Radiation therapy*	1,798	75.4	71	66.4	86	69.9	639	84.5
Chemotherapy	253	10.6	20	18.7	27	22.0	410	54.2
ALND†	363	15.2	73	68.2	93	75.6	735	97.2
LNs								
Median total No.	2		8		10		12	
Median No. positive	0		1		1		2	

Abbreviations: ALND, axillary lymph node dissection; LNs, lymph nodes.

*Breast and/or axilla.

†The goal of ALND was to retrieve at least 10 lymph nodes. The actual number of total retrieved lymph nodes varied between one and 44.

receptor–positive tumors larger than 10 mm, of whom eight had micrometastases and 26 had macrometastases.

The median number of retrieved SLNs was two for all groups, and the median number of positive SLNs was one for all metastatic groups. Completion ALND in a second operation was performed in 328 patients. This was a result of SLN metastases found on either postoperative HE staining (n = 150) or immunohistochemistry (n = 106). The remaining completion ALNDs were performed for other reasons, such as postoperatively diagnosed multifocality or a patient's own request. ALND performed in 305 patients with negative SLN revealed macrometastases in 14 patients. Contrary to the study protocol, SLNs were the only lymph nodes retrieved in 30 pN1mi patients (24.4%) and in 21 pN1 patients (2.8%).

In 11 patients with SLN micrometastasis, completion ALND revealed an additional non-SLN metastasis (seven macrometastases and four micrometastases). Likewise, a non-SLN metastasis was found in six patients with SLN ITCs (three macrometastases and three micrometastases).

Overall, there were 380 recurrences in 295 patients. One hundred seventy patients (7.1%) in the pN0 group, seven patients (6.5%) in the pN0(i+) group, 17 patients (13.8%) in the pN1mi group, and 98 patients (13.0%) in the pN1 group experienced recurrence (for details, see Appendix Fig A1).

Survival

During follow-up, 274 patients died. Of these, 153 were node negative, six had ITCs, 10 had micrometastases, and 105 had macrometastases; 55, two, six, and 58 patients, respectively, from these four groups died of breast cancer.

Compared with pN0 patients, cause-specific survival and event-free survival were significantly worse both for pN1mi and pN1 patients (Figs 2A and 2B, Tables 2 and 3). Five-year OS was not significantly shorter for patients with micrometastases compared with patients with node-negative disease (Table 4, Fig 2C). However, when analyzing only women younger than age 50 years (573 patients, of

Prognosis of Micrometastases in Breast Cancer

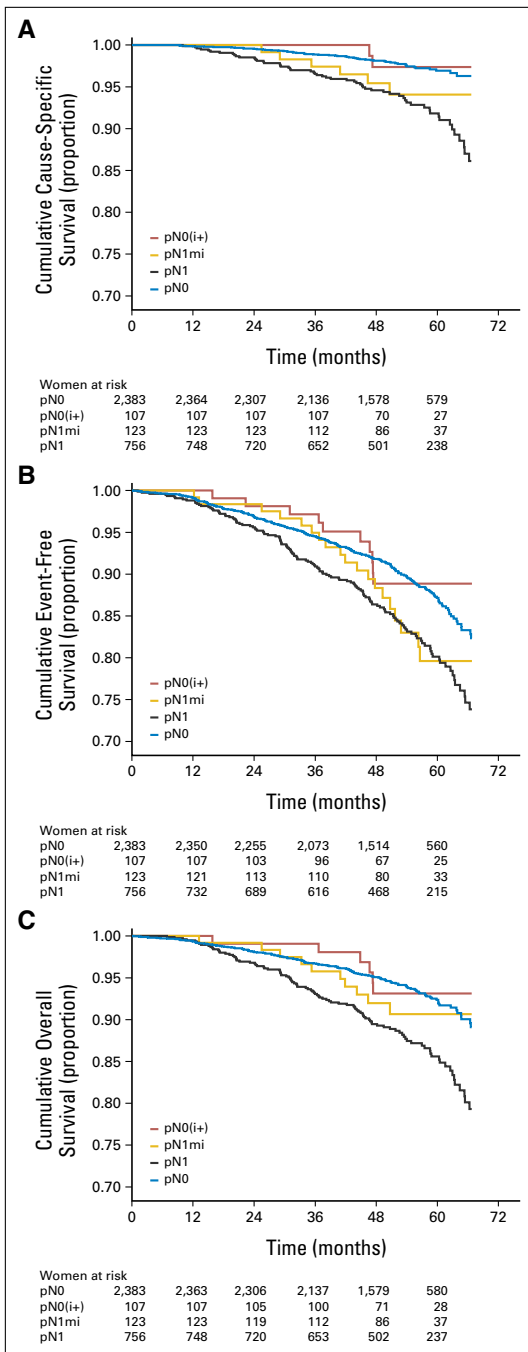


Fig 2. (A) Cause-specific survival, (B) event-free survival, and (C) overall survival for patients with invasive breast cancer with node-negative disease (pN0), isolated tumor cells [pN0(i+)], micrometastases (pN1mi), and macrometastases (pN1).

Table 2. Five-Year Cause-Specific Survival According to Nodal Involvement

Lymph Node Status	5-Year Cause-Specific Survival*		Hazard Ratio†	95% CI	P†
	Rate (%)	95% CI (%)			
No metastases	96.9	96.0 to 97.8	1		
Isolated tumor cells	97.4	93.8 to 100	0.94	0.22 to 4.05	.938
Micrometastases	94.1	89.4 to 98.8	3.04	1.19 to 7.77	.020
Macrometastases	91.8	89.4 to 94.2	3.33	1.74 to 6.38	< .001

*Calculated from Kaplan-Meier estimates.

†Calculated from Cox regression model.

whom 22 had micrometastases), OS was significantly worse for patients with micrometastases than for patients with no metastases (hazard ratio = 7.62; 95% CI, 1.63 to 35.59; $P = .010$). Survival did not differ between pN0(i+) and pN0 patients (Figs 2A to 2C, Tables 2 to 4).

DISCUSSION

The clinical significance of lymph node micrometastases in patients with breast cancer is a subject of debate. This prospective cohort study shows that patients with micrometastatic disease have a prognosis similar to patients with macrometastatic disease, which is in accordance with a retrospective register study including 62,551 women by Truong et al.⁹

In contrast, some earlier studies suggest that micrometastases have no prognostic significance. More than 10 years after breast cancer surgery, Millis et al⁶ and Nasser et al⁷ re-examined additional sections from lymph nodes of 477 and 159 patients, respectively, and concluded that micrometastases are of no prognostic significance.

Gobardhan et al¹⁴ argue that micrometastatic lymph node involvement in itself should not be an indication for adjuvant chemotherapy because survival was not shorter in 81 patients with micrometastases than in 423 node-negative patients. The risk of distant recurrence, however, was higher.

Our study includes a large population with a long follow-up and indicates that the prognosis for patients with micrometastases is almost equivalent to that of patients with macrometastases. Although the majority of both pN1mi and pN1 patients were treated

Table 3. Five-Year Event-Free Survival According to Nodal Involvement

Lymph Node Status	5-Year Event-Free Survival*		Hazard Ratio†	95% CI	P†
	Rate (%)	95% CI (%)			
No metastases	87.1	85.4 to 88.8	1		
Isolated tumor cells	88.9	82.3 to 95.4	0.96	0.53 to 1.84	.985
Micrometastases	79.6	71.0 to 88.2	1.71	1.05 to 2.80	.032
Macrometastases	80.1	76.8 to 83.5	1.24	1.24 to 2.43	.001

*Calculated from Kaplan-Meier estimates.

†Calculated from Cox regression model.

Table 4. Five-Year Overall Survival According to Nodal Involvement

Lymph Node Status	5-Year Overall Survival*		Hazard Ratio†	95% CI	P†
	Rate (%)	95% CI (%)			
No metastases	94.4	91.0 to 93.7	1		
Isolated tumor cells	93.1	87.8 to 98.5	0.91	0.39 to 2.11	.817
Micrometastases	90.7	85.1 to 96.2	1.48	0.75 to 2.93	.258
Macrometastases	85.6	82.7 to 88.5	2.17	1.42 to 3.31	< .001

*Calculated from Kaplan-Meier estimates.

†Calculated from Cox regression model.

with adjuvant hormonal therapy, only just over 20% of pN1mi patients received chemotherapy, compared with 50% of pN1 patients. This could partly explain the lack of prognostic difference between the groups.

Recently, de Boer et al¹¹ presented a large study confirming a shortened 5-year disease-free survival in women with micrometastatic disease. They also found an improved prognosis for patients with micrometastases who had received adjuvant treatment.

Taking these and our results into consideration, it is reasonable to believe that patients with micrometastases may benefit from systemic cytostatic and hormonal treatment. Because evidence for the impact of adjuvant chemotherapy on the prognosis of these patients is lacking, this hypothesis will have to be tested in a randomized clinical trial.

Contrary to earlier reports, most of the more recent studies demonstrate a worse prognosis for patients with micrometastases, which was found to be intermediate between macrometastatic and nonmetastatic disease in a review by Wada and Imoto.¹⁰ However, most of the studies included in the review were based on small patient populations (< 600 patients in four of seven studies). Only two of the studies^{12,20} comprised more than 1,500 patients. Both demonstrated worse OS in micrometastatic than in node-negative disease. Recently, this has been supported by further studies with larger patient populations.^{5,11}

Cummings et al²¹ suggest that micrometastases have a prognostic significance in premenopausal but not in postmenopausal women, whereas Cote et al²² argue the opposite. Our study shows a significantly worse OS only in pN1mi patients younger than age 50 years compared with pN0 patients. This may implicate a greater prognostic significance in premenopausal women, but the number of younger patients with micrometastases was small ($n = 22$). Another possible explanation is the presence of competing risks. Patients with micrometastatic disease may be assumed to experience recurrence and eventually die later than patients with macrometastatic disease. In older patients, other competing causes of death might interfere with the natural course of breast cancer.

This study did not demonstrate a worse prognosis in patients with ITCs compared with pN0. One possible explanation may be the relatively short follow-up. If ITCs are regarded as the early detection of true metastases, an effect could be expected after 10 to 15 years. Patients with ITCs would thus seem to have a better prognosis simply because their metastases are detected earlier (lead time bias). However, it is also possible that patients with ITCs have a truly better prognosis, being cured by the surgical intervention alone. Cox et al⁵ found a decreased survival in patients with ITCs when axillary dissection was

omitted. Another possible explanation is that patients with ITCs received adjuvant chemotherapy and hormonal therapy more often than node-negative patients.

The diagnostic reproducibility of ITCs and micrometastases has been moderate, as shown by Cserni et al.²³ Definitions have been debated, and there are still inconsistencies,²⁴ for example, in how to classify a lymph node with more than one small group of cancer cells.

To decrease the risk of confounding factors and misclassification of lymph node stage, studies on SLN material are of great importance. To our knowledge, only a few previous studies are based on SLN material. Four of these included less than 705 patients.^{13-15,17} Cox et al⁵ and Hwang et al¹⁶ reported on 2,108 and 3,360 patients, respectively, but because the SLN procedure was relatively novel, follow-up was less than 30 months. The study by de Boer et al¹¹ included 2,707 patients with a follow-up of 5.1 years. All three reports indicate a worse prognosis in patients with micrometastases but are retrospective register studies.

A strength of our study is a large population in a prospective SLN cohort with a median follow-up time of more than 4 years. A weakness of our study is that patients were treated at 25 different hospitals, and therefore, pathologic examination of lymph nodes and adjuvant treatment may have differed. This is a problem we share with most multicenter studies, and we have no reason to believe that possible differences between the centers in our study were exceptional. In addition, we believe that the multicenter design best reflects the reality most patients experience.

Another possible weakness of our study is that we did not perform serial sections of the lymph nodes, and some of the patients may have been misclassified. In a recent review, Rutgers²⁵ argues that 10% to 20% of lymph nodes initially reported as negative are actually positive when examined more thoroughly. However, our study is based on SLN biopsy material, and because this method conveys a more accurate examination of axillary lymph nodes than routine pathology, the risk of misclassification should be smaller.

Furthermore, it can be argued that, because ALND was omitted in more patients with micrometastases than macrometastases, some of them may actually have had macrometastases, thus worsening the prognosis for this group. However, we estimate that the number of misclassified cases is low and does not affect the results.

Finally, another weakness is the small number of events. The overall prognosis found in the present cohort was excellent, even for patients with macrometastases (5-year OS, 85.2%). The small number of events might have contributed to an inability to show a significantly worse OS in micrometastatic disease, except for patients younger than age 50 years. Still, despite few events and more intense adjuvant treatment for pN1mi, the lines in the survival graphs diverge between node-negative patients and patients with micrometastases.

In conclusion, this study demonstrates a similar prognosis in patients with micrometastatic and macrometastatic disease, indicating that patients with micrometastases should be offered the same adjuvant treatment. This hypothesis should be tested in a randomized clinical trial.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Jan Frisell, Leif Bergkvist
Financial support: Jan Frisell, Leif Bergkvist
Administrative support: Yvette Andersson, Leif Bergkvist
Provision of study materials or patients: Yvette Andersson, Jan Frisell, Leif Bergkvist

Collection and assembly of data: Yvette Andersson, Jana de Boniface, Leif Bergkvist
Data analysis and interpretation: Yvette Andersson, Jan Frisell, Maria Sylvan, Jana de Boniface, Leif Bergkvist
Manuscript writing: Yvette Andersson
Final approval of manuscript: Yvette Andersson, Jan Frisell, Maria Sylvan, Jana de Boniface, Leif Bergkvist

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Paper IV



Prediction of Non-Sentinel Lymph Node Status in Breast Cancer Patients with Sentinel Lymph Node Metastases: Evaluation of the Tenon Score

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Abstract

Introduction: Current guidelines recommend completion axillary lymph node dissection (cALND) in case of a sentinel lymph node (SLN) metastasis larger than 0.2 mm. However, in 50%–65% of these patients, the non-SLNs contain no further metastases and cALND provides no benefit. Several nomograms and scoring systems have been suggested to predict the risk of metastases in non-SLNs. We have evaluated the Tenon score.

Patients and Methods: In a retrospective review of the Swedish Sentinel Node Multicentre Cohort Study, risk factors for additional metastases were analysed in 869 SLN-positive patients who underwent cALND, using uni- and multivariate logistic regression models. A receiver operating characteristic (ROC) curve was drawn on the basis of the sensitivity and specificity of the Tenon score, and the area under the curve (AUC) was calculated.

Results: Non-SLN metastases were identified in 270/869 (31.1%) patients. Tumour size and grade, SLN status and ratio between number of positive SLNs and total number of SLNs were significantly associated with non-SLN status in multivariate analyses. The area under the curve for the Tenon score was 0.65 (95% CI 0.61–0.69). In 102 patients with a primary tumour <2 cm, Elston grade 1–2 and SLN metastases ≤2 mm, the risk of non SLN metastasis was less than 10%.

Conclusion: The Tenon score performed inadequately in our material and we could, based on tumour and SLN characteristics, only define a very small group of patients in which negative non-sentinel nodes could be predicted.

Keywords: breast cancer, sentinel node, metastases

Breast Cancer: Basic and Clinical Research 2012;6 31–38

doi: [10.4137/BCBCR.S8642](https://doi.org/10.4137/BCBCR.S8642)

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Introduction

Sentinel lymph node biopsy (SLNB) has widely replaced conventional axillary lymph node dissection (ALND) as routine axillary staging in breast cancer surgery. The SLNB method is accurate and safe¹⁻⁴ and conveys substantially less postoperative morbidity than ALND.^{5,6}

Current guidelines recommend completion axillary lymph node dissection (cALND) in case of a sentinel lymph node (SLN) metastasis larger than 0.2 mm.⁷ However, in 50%–65% of patients, the non-SLNs contain no further metastases⁸ and cALND provides no benefit. In addition, several studies have demonstrated the incidence of regional recurrence to be much lower than expected when axillary surgery was omitted,⁹⁻¹² and the need for cALND in all SLN-positive patients has been questioned. It remains, though, that even in the case of only SLN isolated tumour cells, the incidence of non-SLN metastases has been reported to be as high as 20%.¹³ The benefit of ALND on survival is debated. In a meta-analysis from the pre-SLN era, ALND improved survival,¹⁴ but in some latter, randomized, pre-SLN studies, the survival was similar in the ALND and the no ALND groups.^{15,16} Giuliano et al¹⁷ reported that SLNB alone does not result in inferior survival in SLN-positive patients, and in a recent review it was concluded that there is a potential role for avoiding ALND in selected SLN-positive patients.¹⁸

Several authors have suggested nomograms and scoring systems to predict the risk of non-SLN metastases, with the aim of aiding in the decision of further surgery.¹⁹⁻²⁵ Validation studies have demonstrated a reasonably accurate predictive ability,²⁶⁻³³ although far from perfect. In a comparison by Coutant et al, the Memorial Sloan-Kettering Cancer Center nomogram and Tenon score outperformed other predictive models.²⁷ An advantage of the Tenon score is that a fair estimation of all predictive variables can be made perioperatively, allowing the surgeon to decide whether cALND should be performed directly following frozen section of the SLN.

In the present study we evaluated the Tenon score in a large Swedish multicentre SLN cohort.

Patients and Methods

The Swedish sentinel node multicentre cohort study

Between September 2000 and January 2004, 3501 women (with 3535 breast tumours) from 25 Swedish

hospitals were included in the Swedish Sentinel Node Multicentre Cohort Study. Patients and surgical methods have been described in detail elsewhere.³⁴ In short, patients with a unifocal, invasive breast cancer less than 3 cm in diameter were eligible for enrolment. Exclusion criteria were palpable regional lymph nodes, neoadjuvant chemo- or radiotherapy, pregnancy, known allergic reactions to blue dye or isotope, previous surgery in the ipsilateral breast, and preoperatively diagnosed tumour multifocality. After enrolment, data sheets were sent to a research unit, where they were computerised. Data sheets included information on primary tumour characteristics, number of sentinel and non-sentinel lymph nodes with and without metastasis, and size of metastasis. The research protocol postulated annual follow-ups with mammography and clinical examination. Incidence of recurrences and survival were prospectively followed up by a research assistant via reports from the participating centres and on-site visits.

The study was approved by the ethics committee of Karolinska Institutet, Stockholm, and each region's local ethics committee. All patients gave written informed consent.

Surgical procedure

After the preoperative sub- or intradermal injection of 40–60 mBq Technetium-99 nanocolloid (Solco Nanocoll®) and 1 ml blue dye (Patent Blue V®), SLNB was performed. If no sentinel node could be identified, ALND of levels I and II was performed. A cALND was performed in the event of a positive SLNB diagnosed peri- or postoperatively, or if the primary tumour in the breast was found to be multifocal on pathological examination.

Pathological assessment

Frozen sections were obtained from each SLN and examined during surgery. If a sentinel lymph node was smaller than 4 mm, two sections were analysed separately. Nodes larger than 4 mm were bisected, and two sections from each half analysed. For paraffin-embedded histopathology, at least three sections were prepared from the sentinel node or each part of a bisected node. Sections were stained with haematoxylin and eosin (HE). If no cancer cells were detected, immunohistochemistry (IHC) with cytokeratin antibodies was also performed.



Non-sentinel lymph nodes were examined by routine staining (HE) according to the protocol of each pathology department.

At the time of inclusion into the cohort, pathologists rarely differentiated micrometastases from isolated tumour cells. To update the classification of metastases for the present study according to the revised American Joint Committee on Cancer Staging System for Breast Cancer,³⁵ all pathology reports describing SLN micrometastases after routine pathological work-up were scrutinised by a breast pathologist and three surgeons. When differentiation was not possible from the original report, original slides were re-examined and reclassified by the pathologist. Four groups of patients were identified based on the finding in the SLN: node-negative (pN0), isolated tumour cells (<0.2 mm, pN0(i+)), micrometastases (0.2 – 2 mm, pN1mi), and macrometastases (>2 mm, pN1).

The present analysis

For the present study, a positive SLN was defined as any SLN containing tumour cells, including isolated tumour cells. Patients who had at least one positive SLN and underwent cALND were included from the prospective database. Patients with positive non-SLNs were compared with those who had negative non-SLNs regarding age, size, histological type and grade of the primary tumour, oestrogen and progesterone receptor status, the number of positive and negative SLNs and SLN status.

The Tenon score was calculated for all patients by adding the point values for the presence of macrometastases in the SLN (yes = 2, no = 0), the histological tumour size in mm ($>20 = 3$, 11 – $20 = 1.5$, $<11 = 0$) and the ratio between positive and total SLNs ($1 = 2$, 0.5 – $1 = 1$, $<0.5 = 0$).¹⁹ Applying the recommended threshold value of 3.5 or less, the predicted non-SLN status was compared with the actual status.

The incidence of axillary recurrence was compared with a smaller group of patients from the same SLN cohort who were diagnosed with SLN metastases but did not undergo cALND ($n = 86$).

Statistical analysis

Continuous variables (age, tumour size and number of SLNs), dichotomous variables (oestrogen and progesterone receptor status) and categorical variables (histological type and grade of the tumour, SLN status

and ratio score between number of positive and total number of SLNs as defined in the Tenon score) were analysed in a univariate logistic regression model. All variables that demonstrated a statistically significant difference in univariate tests were then analysed in a multivariable regression model. A receiver operating characteristic (ROC) curve was drawn on the basis of the sensitivity and specificity of the Tenon score, and the area under the curve (AUC) was calculated.

SPSS 14.0® software was used for all analyses and statistical significance was set at the 0.05 level for all tests.

Results

Patient and tumour characteristics are given in Table 1. We identified 869 patients with SLN metastases where cALND was performed (282 cALNDs in a second session). Most of these patients ($n = 691$) had SLN macrometastases, but 20% (178/869) had metastases of ≤ 2 mm (11% micrometastases (98/869) and 9% isolated tumour cells (80/869), Table 2).

Additional axillary metastases

Non-SLN metastases were identified in 270/869 (31.3%) patients, and 251/691 (36.3%) of the pN1, 11/98 (11.2%) of the pN1mi, and 8/80 (10.0%) of the pN0(i+) patients. Of these, non-SLN macrometastases were revealed in 8/11 (72.7%) of the pN1 mi and 3/8 (37.5%) of the pN0(i+) patients. The proportions of positive non SLNs according to total number of SLNs and number of positive SLNs are given in Table 3.

We identified two groups of patients where the risk of non-SLN metastases was less than 10%. In pN1mi or pN0(i+) patients with a tumour smaller than 2 cm and Elston grade 1 or 2 ($n = 102$), it was 6.7%, and in pN1mi or pN0(i+) patients with more than two SLNs removed ($n = 23$), it was 6.0%.

Correlation between clinicopathologic characteristics and positive non-SLNs

Tumour size and grade, SLN status and ratio between the number of positive SLNs and total number of SLNs were significantly associated with non-SLN status, both in uni- and multivariate analyses. Histotype was significant only in the univariate analysis. *P*-values for the association between different characteristics and non-SLN positivity are given in Table 1.



Table 1. Patient and tumour characteristics in 869 SLN-positive patients who underwent completion axillary lymph node dissection.

Non-sentinel lymph node status			
Characteristics N	Positive non-SN 270	Negative non-SN 599	P ^d
Age (years) ^a	57 (28–82)	57 (28–90)	0.481
Tumour size (mm) ^b	19 (10)	17 (8)	<0.001
Histotype ^c			0.035
Ductal	173 (64.1)	408 (68.1)	
Lobular	49 (18.1)	75 (12.5)	
Mixed	2 (0.7)	10 (1.7)	
Other	11 (4.1)	30 (5.0)	
Missing	35 (13.0)	76 (12.6)	
Tumour grade (Elston grade) ^c			<0.001
1	38 (14.1)	152 (25.3)	
2	138 (51.1)	304 (50.8)	
3	88 (32.6)	128 (21.4)	
Missing	6 (2.2)	15 (2.5)	
Oestrogen receptor ^c			0.502
Positive	231 (85.6)	520 (86.8)	
Negative	37 (13.7)	72 (12.0)	
Missing	2 (0.7)	7 (1.2)	
Progesterone receptor ^c			0.696
Positive	185 (68.5)	425 (71.0)	
Negative	80 (29.6)	160 (26.7)	
Missing	5 (1.9)	14 (2.3)	
Number of SLNs ^a	2 (1–9)	2 (1–8)	0.872
Mean number of pos. SLNs/total SLNs	0.81	0.71	<0.001

Notes: ^aMedian (range); ^bMean (standard deviation); ^cNumber (%); ^dUnivariate analysis.

Abbreviation: SLN, sentinel lymph node.

The risk of positive non-SLNs was 4.66 times higher for patients with SLN macrometastases than for those with SLN micrometastases (95% CI 2.18–9.95, $P < 0.001$) and 2.79 times higher for a high positive/total SLN ratio as defined in the Tenon score (95% CI 1.69–4.60, $P < 0.001$). The hazard ratio for increasing tumour diameter (per millimetre) was 1.02 (95% CI 1.00–1.04, $P = 0.035$) and for high tumour grade (Elston grade 3 vs. 1) 2.41 (95% CI 1.51–3.86, $P < 0.001$).

Tenon score

The mean Tenon score was 5.29 in patients with non-SLN metastases and 4.49 in those without ($P < 0.001$). Applying a threshold value of 3.5, the specificity was

Table 2. Sentinel lymph node (SLN) status in 869 SLN-positive patients who underwent completion axillary lymph node dissection.

Non-sentinel lymph node status			
SLN status ^a	Positive non-SN N = 270	Negative non-SN N = 599	
pN0(i+) ^b	8 (3.0)	72 (12.0)	78
pN1 mi ^c	11 (4.0)	87 (14.5)	98
pN1 ^d	251 (93.0)	440 (73.5)	691

Notes: ^aNumber (%); ^b<0.2 mm; ^c0.2–2 mm; ^d>2 mm.

34.6% and the sensitivity was 85.9%. The false negative rate was thus 14.1% (38/245 patients with a Tenon score 3.5 or less had non-SLN metastases).

The area under the curve was 0.65 (95% CI 0.61–0.69) for all patients (Fig. 1), 0.63 (95% CI 0.59–0.67) for patients with SLN micro- and macrometastases, 0.57 (95% CI 0.44–0.70) for patients with SLN metastases of ≤ 2 mm, and 0.54 (95% CI 0.37–0.72) for pN1 mi patients only.

Axillary recurrences

In the study group, there were 10/869 (1.2%) isolated axillary recurrences (8/691 (1.2%) in pN1 and 2/98 (2.0%) in pN1mi patients) after 56.3 months median follow-up. Almost all patients (860/869, 99.0%) had

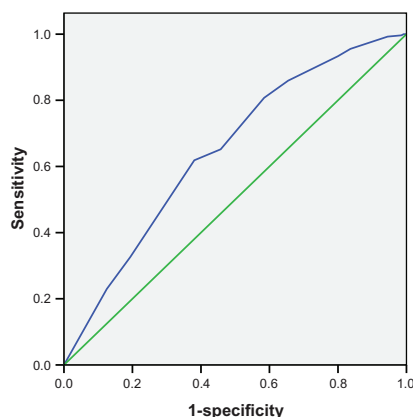


Figure 1. The receiver operation curve (ROC) for 869 sentinel lymph node-positive patients calculated for the Tenon score; blue line, area under the curve (AUC) 0.65. The green, diagonal line represents AUC 0.5 (flipping a coin).

**Table 3.** Cross tabulation of number of positive sentinel lymph nodes (SLNs) by total number of SLNs removed. Each cell represents the proportion of patients with positive non SLN.

Number of positive SLNs								
Number of SLNs	1	2	3	4	5	6	7	N
1	86/281 (31%)							281
2	53/196 (27%)	56/130 (43%)						326
3	14/92 (15%)	15/43 (35%)	17/30 (57%)					165
4	4/33 (12%)	4/15 (27%)	2/6 (33%)	6/8 (75%)				62
5	2/8 (25%)	1/5 (20%)	2/3 (67%)	3/3 (100%)	1/2 (50%)			21
6	0/2 (0)	1/3 (33%)	0/1 (0)	—	—	1/2 (50%)		8
7	—	0/1 (0)	0/2 (0)	—	—	—	—	3
8	0/1 (0)	1/1 (100%)	—	—	—	—	—	2
9	—	—	—	—	—	—	1/1 (100%)	1

Note: N, number of patients.

adjuvant treatment (81.5% radiation, 83.2% hormonal treatment and 49.4% chemotherapy).

In a separate group of 86 patients with SLN metastases in whom ALND was omitted (mean Tenon score 3.11), 1/86 (1.2%) patient had an isolated axillary recurrence after 51.8 months median follow-up. In this group, 82/86 (95.3%) had adjuvant treatment (68.6% radiation, 86.0% hormonal treatment and 5.8% chemotherapy).

Discussion

Several authors have, by creating nomograms and scoring systems, attempted to define a subset of SLN-positive patients in whom cALND could safely be omitted. The Tenon score outperformed other scoring systems in a study by Coutant et al²⁷ and includes characteristics that can be estimated at the time of the SLN biopsy. In the present study, we evaluated the Tenon score in a Swedish multicentre cohort. The AUC was only 0.65 and the performance of the score was thus inadequate in our patient cohort.

A validation study demonstrating good accuracy of the Tenon score was also presented by Coutant et al,³⁶ with both studies from this group evaluating French populations. A French data set was also used

to develop the Tenon score. In contrast, validation studies in other populations and also a recent French validation study demonstrate less prediction accuracy (AUC 0.58–0.70),^{29,37–39} which the results from our study are in accordance with. This could represent differences in populations, surgical technique or pathologic examination.

Unfortunately, we were not able to validate the Memorial Sloan-Kettering Cancer Center (MSKCC) nomogram²⁴ in our population as we had incomplete information about the occurrence of lymphovascular invasion. The MSKCC nomogram has, however, been validated in several other studies, and the AUC varied between 0.58 and 0.86.^{27,39} In three studies the AUC was less than 0.70 (the limit used for considering an acceptable predictive ability), possibly reflecting population differences in a similar way as for the Tenon score.

Several studies have tried, but have been unable, to define a subgroup in which cALND can safely be omitted.^{8,13,40–43} In a meta-analysis by Degnim et al,⁸ no subgroup had less than a 10% risk of non-SLN metastases.

We could identify two groups of patients in whom the risk of non-SLN metastases was less than 10%. However, these were very small subgroups (n = 102 and 23, respectively) that were not pre-planned in the study



and their clinical significance is therefore questionable. The results of our study, and most of the previously published, similar studies, indicate that the evaluation of primary tumour and SLN characteristics is not sufficient to decide whether to proceed with further axillary surgery.

Interestingly, only one of 86 SLN-positive patients in whom cALND was omitted had an isolated axillary recurrence. In accordance, a low incidence of axillary recurrence was previously demonstrated in other studies.^{10,12,44} In a review by Rutgers, the 2- to 3-year risk of axillary recurrence in SLN-positive patients was 0 to 1.4% if the axilla was left untreated.⁴⁵ In part, this could be explained by better prognostic factors in the patients that did not have cALND, but many studies have reported the axillary recurrence rate to also be lower than expected in SLN-negative patients. Since the false negative rate is known to be about 5%–10%,^{4,46–48} this indicates that not all positive lymph nodes left behind will develop into clinically significant metastases.

Recently, in a report on 97 314 patients who had breast cancer surgery between 1998 and 2005, Bilimoria et al⁴⁹ found no significant difference in axillary recurrence or survival for SLN-positive patients who underwent SLNB alone compared with those who had cALND. It is, however, a retrospective study and the completeness of follow-up was not reported. Furthermore, between 1998 and 2000 the number of excised lymph nodes was almost as high in the SLNB as in the ALND group.

Additionally, the American College of Surgeons Oncology Group (ACO-SOG) Z0011 trial found no higher incidence of axillary recurrence⁵⁰ and comparable survival¹⁷ in SLN-positive patients randomised to omission of cALND compared with those who completed an ALND after a median follow-up of 6.3 years. However, only 891 of the planned 1900 patients were accrued and the study was closed early. Considering the low accrual rate (despite many participating centres, several of these probably with large patient volumes), one cannot rule out the possibility of a significant selection bias, and included patients were at low risk for recurrence. Furthermore, all patients received whole-breast irradiation, including the lower part of the axilla.

We therefore believe that it is too early to abandon ALND for all SLN-positive patients.

Conclusion

The Tenon score performed inadequately in our material and we could only define a very small group of patients in which negative non-SLNs could be predicted.

Role of the Funding Source

The funding sources had no input in the study design, in the collection, analysis and interpretation of data, or in the writing of the manuscript.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Acknowledgements

The authors thank the medical staff at all participating hospitals for the inclusion and follow-up of patients. Special thanks are due to Marie-Louise Walker-Engström at the Centre for Clinical Research of Uppsala University, Central Hospital, Västerås, for excellent data management.

The study was funded by grants from the Swedish Breast Cancer Association (BRO), the Swedish Cancer Society and the Centre for Clinical Research of Uppsala University.

Ethical Approval

The study was approved by the ethics committee of Karolinska Institutet, Stockholm, and each region's local ethics committee. All patients gave written informed consent.

Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.



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