Sentinel Node in Clinical Practice

Implications for Breast Cancer Treatment and Prognosis

YVETTE ANDERSSON
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


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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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.


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## Contents

Introduction ................................................................................................... 11  
Treatment .................................................................................................... 11  
Historical aspects of breast cancer surgery .............................................. 13  
Sentinel lymph node biopsy ..................................................................... 15  

Aims .............................................................................................................. 17  

Patients .......................................................................................................... 18  
Validation Study ...................................................................................... 18  
Cohort Study ............................................................................................. 18  
Paper I ......................................................................................................... 19  
Paper II ....................................................................................................... 19  
Paper III ..................................................................................................... 19  
Paper IV .................................................................................................... 19  

Methods ........................................................................................................ 22  
Identification of sentinel node ................................................................. 22  
Surgery ....................................................................................................... 23  
Histopathological assessment .................................................................. 23  
Adjuvant treatment .................................................................................... 24  
Follow-up .................................................................................................. 24  
Definitions ................................................................................................ 24  
  Tenon score ............................................................................................ 25  
Statistical methods ..................................................................................... 25  

Results ........................................................................................................... 27  
Paper I ......................................................................................................... 27  
Paper II ....................................................................................................... 27  
Paper III ..................................................................................................... 30  
Paper IV .................................................................................................... 30  

Discussion ..................................................................................................... 36  
Paper I ......................................................................................................... 36  
Paper II ....................................................................................................... 37  
Paper III ..................................................................................................... 38  
Paper IV .................................................................................................... 39  

Conclusions ................................................................................................... 40
Future perspectives ..........................................................................................41

Swedish summary ..........................................................................................43
  Bakgrund och syfte ..................................................................................43
  Delarbete I ..............................................................................................44
  Delarbete II ............................................................................................44
  Delarbete III ..........................................................................................44
  Delarbete IV ...........................................................................................45
  Slutsatser ...............................................................................................45

Acknowledgements .......................................................................................47

References .....................................................................................................49
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALND</td>
<td>Axillary lymph node dissection</td>
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<tr>
<td>cALND</td>
<td>Completion axillary lymph node dissection</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<td>CSC</td>
<td>Cancer stem cell</td>
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<td>EMT</td>
<td>Epithelial to mesenchymal transition</td>
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<tr>
<td>FNR</td>
<td>False negative rate</td>
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<td>HE</td>
<td>Haematoxylin and eosin</td>
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<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
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<tr>
<td>ITC</td>
<td>Isolated tumour cells</td>
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<tr>
<td>MET</td>
<td>Mesenchymal to epithelial transition</td>
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<td>OS</td>
<td>Overall survival</td>
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<tr>
<td>ROC</td>
<td>Receiver operating characteristics</td>
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<td>SLN</td>
<td>Sentinel lymph node</td>
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<td>SLNB</td>
<td>Sentinel lymph node biopsy</td>
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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 karkinon (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.

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, McWirtner 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 supraclavicular, 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.

![Image of a surgical site](image.png)

*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*.

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

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*.

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

<sup>a</sup> Median (range)  <sup>b</sup> Mean (standard deviation)  <sup>c</sup> 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-SLN 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-
mourn 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.

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

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.

<table>
<thead>
<tr>
<th>Lymph node status</th>
<th>5-year cause-specific survival&lt;sup&gt;a&lt;/sup&gt; (%) (95% CI)</th>
<th>Hazard ratio&lt;sup&gt;b&lt;/sup&gt; (95% CI)</th>
<th>&lt;sup&gt;P&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No metastases</td>
<td>96.9 (96.0-97.8)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Isolated tumour cells</td>
<td>97.4 (93.8-100)</td>
<td>0.94 (0.22-4.05)</td>
<td>0.938</td>
</tr>
<tr>
<td>Micrometastases</td>
<td>94.1 (89.4-98.8)</td>
<td>3.04 (1.19-7.77)</td>
<td>0.020</td>
</tr>
<tr>
<td>Macrometastases</td>
<td>91.8 (89.4-94.2)</td>
<td>3.33 (1.74-6.38)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Calculated from Kaplan-Meier graph  <sup>b</sup>Calculated from Cox regression model

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

<table>
<thead>
<tr>
<th>Lymph node status</th>
<th>5-year event-free survival&lt;sup&gt;a&lt;/sup&gt; (%) (95% CI)</th>
<th>Hazard ratio&lt;sup&gt;b&lt;/sup&gt; (95% CI)</th>
<th>&lt;sup&gt;P&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No metastases</td>
<td>87.1 (85.4-88.8)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Isolated tumour cells</td>
<td>88.9 (82.3-95.4)</td>
<td>0.96 (0.53-1.84)</td>
<td>0.985</td>
</tr>
<tr>
<td>Micrometastases</td>
<td>79.6 (71.0-88.2)</td>
<td>1.71 (1.05-2.80)</td>
<td>0.032</td>
</tr>
<tr>
<td>Macrometastases</td>
<td>80.1 (76.8-83.5)</td>
<td>1.24 (1.24-2.43)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Calculated from Kaplan-Meier graph  <sup>b</sup>Calculated from Cox regression model

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

<table>
<thead>
<tr>
<th>Lymph node status</th>
<th>5-year overall survival&lt;sup&gt;a&lt;/sup&gt; (%) (95% CI)</th>
<th>Hazard ratio&lt;sup&gt;b&lt;/sup&gt; (95% CI)</th>
<th>&lt;sup&gt;P&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No metastases</td>
<td>92.4 (91.0-93.7)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Isolated tumour cells</td>
<td>93.1 (87.8-98.5)</td>
<td>0.91 (0.39-2.11)</td>
<td>0.817</td>
</tr>
<tr>
<td>Micrometastases</td>
<td>90.7 (85.1-96.2)</td>
<td>1.48 (0.75-2.93)</td>
<td>0.258</td>
</tr>
<tr>
<td>Macrometastases</td>
<td>85.6 (82.7-88.5)</td>
<td>2.17 (1.42-3.31)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Calculated from Kaplan-Meier graph  <sup>b</sup>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*.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Positive non-SN</th>
<th>Negative non-SN</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>270</td>
<td>599</td>
<td></td>
</tr>
<tr>
<td>Age (years)²</td>
<td>57 (28-82)</td>
<td>57 (28-90)</td>
<td>0.481</td>
</tr>
<tr>
<td>Tumour size (mm)²</td>
<td>19 (10)</td>
<td>17 (8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Histotype</td>
<td></td>
<td></td>
<td>0.035</td>
</tr>
<tr>
<td>ductal</td>
<td>173 (64.1)</td>
<td>408 (68.1)</td>
<td></td>
</tr>
<tr>
<td>lobular</td>
<td>49 (18.1)</td>
<td>75 (12.5)</td>
<td></td>
</tr>
<tr>
<td>mixed</td>
<td>2 (0.7)</td>
<td>10 (1.7)</td>
<td></td>
</tr>
<tr>
<td>other</td>
<td>11 (4.1)</td>
<td>30 (5.0)</td>
<td></td>
</tr>
<tr>
<td>missing</td>
<td>35 (13.0)</td>
<td>76 (12.6)</td>
<td></td>
</tr>
<tr>
<td>Tumour grade (Elston)²</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1</td>
<td>38 (14.1)</td>
<td>152 (25.3)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>138 (51.1)</td>
<td>304 (50.8)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>88 (32.6)</td>
<td>128 (21.4)</td>
<td></td>
</tr>
<tr>
<td>missing</td>
<td>6 (2.2)</td>
<td>15 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Oestrogen receptor²</td>
<td></td>
<td></td>
<td>0.502</td>
</tr>
<tr>
<td>positive</td>
<td>231 (85.6)</td>
<td>520 (86.8)</td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>37 (13.7)</td>
<td>72 (12.0)</td>
<td></td>
</tr>
<tr>
<td>missing</td>
<td>2 (0.7)</td>
<td>7 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Progesterone receptor²</td>
<td></td>
<td></td>
<td>0.696</td>
</tr>
<tr>
<td>positive</td>
<td>185 (68.5)</td>
<td>425 (71.0)</td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>80 (29.6)</td>
<td>160 (26.7)</td>
<td></td>
</tr>
<tr>
<td>missing</td>
<td>5 (1.9)</td>
<td>14 (2.3)</td>
<td></td>
</tr>
<tr>
<td>Number of SLNs²</td>
<td>2 (1-9)</td>
<td>2 (1-8)</td>
<td>0.632</td>
</tr>
<tr>
<td>SLN status²</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pN0(i+)</td>
<td>8 (3.0)</td>
<td>72 (12.0)</td>
<td></td>
</tr>
<tr>
<td>pN1mi</td>
<td>11 (4.0)</td>
<td>87 (14.5)</td>
<td></td>
</tr>
<tr>
<td>pN1</td>
<td>251 (93.0)</td>
<td>440 (73.5)</td>
<td></td>
</tr>
<tr>
<td>Mean number of pos. SLNs/total SLNs</td>
<td>0.82</td>
<td>0.71</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

² Median (range) ³ Mean (standard deviation) ⁴ Number (%) ⁵ <0.2mm ⁶ 0.2-2mm ⁷ >2 mm ⁸ 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 lifetime 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’ humoural 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 (≤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 undersöks med seriesnittning (tät 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 urskiljningsförmåga) 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

Otilräcklig 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.
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1. www.socialstyrelsen.se.


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine.