Breast cancer and axillary lymph node status

Pre-operative clinicopathological predictors of axillary metastasis

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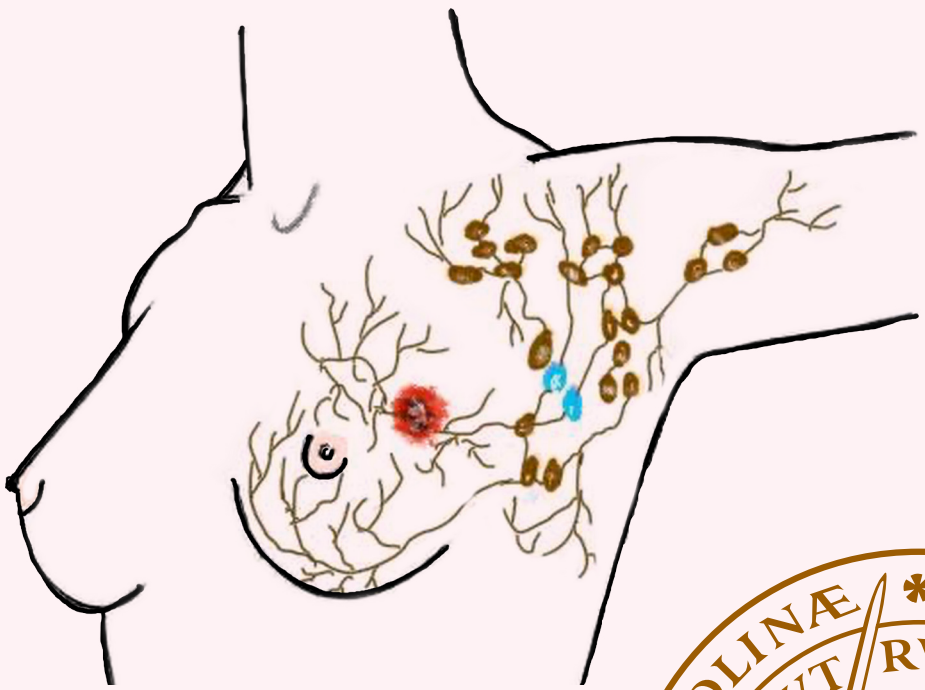

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Breast cancer and axillary lymph node status. Pre-operative clinicopathological predictors for axillary metastasis

Abstract

Axillary lymph node (ALN) status is one of the most important prognostic factors in primary breast cancer. Axillary staging is an essential step in the management of breast cancer. However, there is a growing interest in examining whether it is possible to omit axillary staging in patients with a low risk of nodal metastasis. The aim of this thesis was to determine the clinicopathological predictors associated with the presence or absence of the ALN metastases.

Study I The first aim was to determine the accuracy of ALN physical status in relation to the presence of metastases as revealed by histopathological examination. The second aim was to compare the tumour size as assessed by physical examination, with the size obtained by histopathological examination. This study included 2537 patients in Malmö, diagnosed with breast cancer during the years 1987-2002. Information was retrieved from the South Swedish Breast Cancer Group (SSBCG) registry.Out of 674 women with ALN metastases according to histological examination, only 206 patients had palpable lymph nodes. Sensitivity was 30% and specificity was 93%. There were 812 tumours larger than 20 mm according to histopathological examination, while only 665 tumours were larger than 20 mm by clinical examination. Sensitivity was 81% and specificity was 80%.

Study II The aim was to determine predictors for metastasis to sentinel node (SN). This study included 2552 patients with breast cancer recruited during 1 Jan 2008 to 31 Dec 2013 in Malmö and Lund. The information was retrieved from the Swedish National Quality Registry for Breast Cancer (NKBC). Tumours detected by mammography screening (0.63; 0.51-0.80) and negative hormonal status for oestrogen (0.64; 0.42-0.99) were associated with a lower risk for SN metastases.Tumours > 20 mm (1.84; 1.47-2.33). Multifocality (1.90; 1.45-2.47) and lymphovascular invasion (LVI) (3.74; 2.66-5.27) were predictors associated with a high risk for SNs metastases.

Study III The first aim was to identify clinicopathological determinants associated with non-sentinel node (non-SN) metastasis. The second aim was to determine the impact of the size of SN metastases and the number of SNs with macro-metastases on metastatic involvement in non-SNs. Data from NKBC was used, 602 patients in Lund and Malmö during 2008-2013 were included. All had metastases in SNs and had undergone completion axillary lymph node dissection (c-ALND). In all, 211 patients (35%) had metastases and 391 patients (65%) had no metastases in non-SNs. Lobular type (1.73; 1.01-2.97) and multifocal tumours (2.20; 1.41-3.44) had a high risk of non-SNs metastases. The presence of macro-metastases in the SN and the number of SNs with macro-metastases, regardless of the number of SNs removed by surgery, increased the risk of finding non-SNs with metastases.

Study IV The first aim was to validate the performance of the Skåne University Hospital nomogram (SUS nomogram) in an independent cohort.The second aim was to assess if routine data from a clinical registry was as useful as manually retrieved clinical records.This study included 2939 patients who had undergone ALN procedures in Malmö and Lund 2008-2013. Of these,1008 patients had metastases (34.3%) and 1931 patients (65.7%) had no metastases in the ALN. The area under the curve (AUC) in both centres was almost identical to the original value (0.74). The calibration diagram showed a good agreement between predicted probability and observed metastases for both centres. Routine data from NKBC was as useful as manually retrieved clinical records.

Conclusions Estimation of axillary metastasis by clinical examination gave a large proportion of false-positive and false-negative results. Similarly, tumour size had a high possibility of under- and over estimation. SN metastasis was less likely to occur in breast cancer diagnosed by screening mammography and in tumours with negative oestrogen status. Tumours larger than 20 mm, multifocality, and LVI were factors associated with high risk of SN metastasis. Lobular type and multifocal tumours had a high risk of non-SNs metastases.The total number of SNs removed by surgery had no impact on finding metastases in non-SNs. The presence of macro-metastases in SNs and the number of SNs with macro-metastases had a positive association with the presence of metastases in non-SNs. The SUS nomogram showed a good prediction of the SN metastasis and it performed adequately in an independent cohort. Routine data from NKBC was as useful as manually retrieved clinical records.

Key words: Breast cancer, axillary lymph node, sentinel node, non-sentinel node, axillary metastasis predictor

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To Bayan, Sandra, Liza, Allan and Laura

It always seems impossible until it´s done
Nelson Mandela

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List of Papers

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

I. **Majid S**, Tengrup I, Manjer J: **Clinical assessment of axillary lymph nodes and tumour size in breast cancer compared with histopathological examination: a population-based analysis of 2537 women.** *World J Surg.* 2013 Jan;37(1):67-71 *

II. **Majid S**, Rydén L, Manjer J: **Predictive factors for sentinel node metastases in primary invasive breast cancer: A population-based cohort study of 2552 consecutive patients.** *World J Surg Oncol.* 2018 Mar 12;16(1):54 **

III. **Majid S**, Rydén L, Manjer J: **Determinants for non-sentinel node metastases in primary invasive breast cancer. A population-based cohort study of 602 consecutive patients with sentinel node metastases.** *BMC Cancer.* 2019 Jun 25;19(1):626 **

IV. **Majid S**, Bendahl P-O, Huss L, Manjer J, Rydén L, Dihge L: **Validation of The SUS nomogram for preoperative prediction of a disease-free axilla in patients with breast cancer.** *Manuscript.*

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Thesis at a glance

Paper	Research Aims	Material and Methods	Results and Conclusions
I	The first aim of this study was to determine the accuracy of ALN physical status in relation to the presence of metastases as revealed by histopathological examination. The second aim was to compare the tumour size as assessed by physical examination, with the size obtained by histopathological examination.	Data was collected from the SSBCG. The cohort included 2537 patients with breast cancer in Malmö during 1987-2002. The physical ALN status was compared with the results of the histopathological examination for the presence of metastases. Tumour size by physical examination was compared with the tumour size after histopathological examination.	Out of 674 women with axillary lymph nodes metastases according to histological examination, only 206 had palpable lymph nodes at clinical examination. Sensitivity was 30% and specificity was 93%. There were 812 tumours larger than 20 mm according to histopathological examination, but only 665 of these tumours were larger than 20 mm by clinical examination. Sensitivity was 81% and specificity was 80%. The estimation of axillary metastases by clinical examination led to a large proportion of false-positive and false-negative results. Tumour size estimated by clinical examination was associated under- and over estimation in comparison to the tumour size measured by histopathological examination.
II	To determine predictive factors for metastasis to SN in primary invasive breast cancer.	3979 patients with primary breast cancer in Malmö and Lund for the period 1 Jan. 2008- 31 Dec. 2013 retrieved from NKBC. 2552 patients had undergone SNB. The risk of metastases to SNs was examined in relation to potential clinicopathological factors. Binary logistic regression was used, adjusted analyses yielded an odds ratio with a 95% confidence interval.	Tumours detected by screening mammography and tumours with negative hormonal status for oestrogen were associated with lower risk for SN metastasis. Tumours larger than 20 mm had a high risk of metastasising to SNs. Multifocality and LVI were also strong predictive factors for SN metastasis. This knowledge is useful in clinical practice and might help in identifying patients with node-negative or node-positive tumours.
III	The first aim of this study was to identify clinicopathological determinants associated with non-SNs metastases. The second aim was to determine the impact of the number of SNs with macro-metastases and the size of SN metastases on metastatic involvement in non-SNs.	602 patients with primary invasive breast cancer who had undergone SNB and c-ALND in Lund and Malmö during 2008-2013. All had micro- and/or macro-metastases in SNs. NKBC registry was used. The risk of metastases in non-SNs was analysed in relation to clinicopathological determinants. Additionally, we compared the association between the number of SNs and the size of metastases in SNs with the risk of metastases to non-SNs. Binary logistic regression was used, odds ratios with 95% confidence intervals were analysed.	211 patients (35%) had metastases in non-SNs and 391patients (65%) had no metastases in non-SNs. Lobular type and multifocal tumours had a high risk of non-SNs metastases. The total number of SNs had no impact on diagnosis of metastases in non-SNs.The presence of macro-metastases in SNs was associated with a high risk of metastases to non-SNs. The number of SNs with macro-metastases, regardless of the number of SNs removed by surgery, increases the risk of finding non-SNs with metastases. This information is valuable when considering whether or not to omit c-ALND.
IV	To validate the performance of the SUS nomogram in an independent cohort with invasive breast cancer. An additional aim was to assess if it was sufficient to use routinely collected data from NKBC registry vs. manually retrieved information from medical records.	2939 patients from Malmö and Lund diagnosed with invasive breast cancer 2008-2013. Clinicopathological determinants corresponding to predictors in the SUS nomogram were retrieved from the NKBC registry. Multiple imputation (MI) was applied. AUC and a comparison of observed and predicted values assessed discriminatory performance and calibration.	N+ was found in 1008 patients. AUC was 0.75 for Lund and 0.73 for Malmö. Original AUC was 0.74. The predicted N0 was similar to observed values, indicating an overall good calibration. The SUS nomogram provided a good prediction of disease-free axilla in our validation cohort. Routine data from NKBC was as useful as manually retrieved clinical records.

Abbreviations

ACOSOG	American College of Surgeons Oncology Group
ALN	Axillary Lymph Node
ALND	Axillary Lymph Node Dissection
AUC	Area Under the Curve
c-ALND	Completion Axillary Lymph Node Dissection
CB	Core Biopsy
CI	Confidence Interval
CT	Computerized Tomography
ER	Estrogen Receptor
EUSOMA	European Society of Breast Cancer Specialists
FISH	Fluorescent In Situ Hybridization
FNB	Fine Needle Biopsy
HR	Hormone Receptor
HER2	Human Epidermal Growth Factor Receptor 2
IHC	Immunohistochemistry
ITC	Isolated Tumour Cell
INCA	Information Network for Cancer Care
KVAST	Swedish Society of Pathology (Kvalitets- och standardiseringskommittén)
LP	Linear Predictive
+LR	Positive likelihood Ratio
-LR	Negative likelihood Ratio
LVI	Lymphovascular Invasion
MI	Multiple Imputation
MRI	Magnetic Resonance Imaging
NHG	Nottingham Histological Grading
NKBC	National Quality Registry for Breast Cancer
non-SN	Non-Sentinel Node
NPV	Negative Predicative Value
OR	Odds Ratio
PMM	Predictive Mean Matching
PPV	Positive Predicative Value
PGR	Progesterone Receptor
RCC-Syd	Regional Cancer Center in southern Sweden
RR	Relapse Rate
ROC	Receiver Operating Characteristic
RT	Radiotherapy
SN	Sentinel Node
SNB	Sentinel Node Biopsy
SPSS	Statistical Package for the Social Sciences
SSBCG	South Swedish Breast Cancer Group
TNM	Tumour Node Metastasis
TN	Triple Negative

Populärvetenskaplig sammanfattning (summary in Swedish)

Bröstcancer är den vanligaste cancersjukdomen bland kvinnor i världen. I Sverige insjuknar varje timme cirka en kvinna i bröstcancer. Fler än 90 000 kvinnor lever idag med en bröstcancerdiagnos. Cirka 1500 kvinnor dör årligen i bröstcancer (4 kvinnor varje dag) dock blir överlevnad allt bättre i Sverige, för närvarande är femårsöverlevnaden cirka 92%. Vid diagnos i tidigt stadium när sjukdomen enbart håller sig till bröstvävnad är femårsöverlevnaden så gott som 100 % varför det är mycket viktigt att diagnosticera sjukdomen i tidigt skede före lokal eller fjärrspridning. Genom klinisk undersökning kan större bröstcancerknutor diagnosticeras vid palpation av brösten. Klinisk bedömning av lymfkörtlarna i armhålan är däremot svår med undantag av vissa fall när körtlarna är förstorade och därmed kan dessa körtlar vara kännbara dock i samtliga fall krävs alltid en så kallad trippeldiagnostik således klinisk, radiologisk och patologisk diagnos.

Bröstcancerspridning (metastas) sker genom spridning av cancerceller till närliggande lymfkörtlar i armhålan eller direkt via blodbanan till olika kroppsorgan, vanligast lungor, skelett eller lever. Bröstcancer är en mångfacetterad sjukdom och spridningsprocessen kan styras av olika faktorer och kan variera från fall till fall. En mycket viktig och värdefull information, gällande sjukdomens förlopp och dess behandling, är förekomst av lymfkörtelspridning vid diagnos av bröstcancer. Tidigare opererades bort rutinmässigt alla lymfkörtlar i armhålan (ca 10–20 körtlar, kallas för axillutrymning) på cancersidan även vid små tumörer med friska körtlar men sedan början av tvåtusentalet har man kunnat utföra en ny teknik kallas för portvaktskörtel diagnostik (sentinel node, SN) då borttagning av endast 1–4 körtlar i armhålan. Med denna teknik kan man identifiera om det förekommer lymfkörtelspridning i armhålan och därmed gör mindre omfattande kirurgi med mindre risk för komplikationer så som armsvullnad, smärta, och rörelsebegränsning som kan förekomma efter axillutrymning. Dessutom har man på senare tid kunnat identifiera två olika samlingar av tumörceller, s.k. mikrometastaser (0.2-2.0 mm) och makrometastaser (>2mm) i SN. Det är oklart vilken klinisk signifikans dessa har och om det är nödvändigt att utföra en axillutrymning vid begränsade mikro eller makrometastaser i portvaktskörtlar. Dagens kunskap talar för att mer än 70 % av bröstcancerpatienter har friska portvaktskörtlar och att det är möjligt att identifiera de här patienterna och därmed möjlighet att undkomma/begränsa axillkirurgi genom tumörens kliniska och biologiska egenskaper. Således mindre omfattande axillkirurgi kan möjligen bidra till lägre patient sjuklighet och lägre kostnad för sjukvården.

Denna avhandling består av fyra olika studier. Studierna handlar om att kunna upptäcka lymfkörtelspridning hos bröstcancerpatienter genom att studera kliniska and biologiska egenskaper av cancertumören. Att identifiera bröstcancerpatienter

med hög/låg risk för cancerspridning i lymfkörtlar i armhålan kan ge möjligheten till att minska risken för både under- och överbehandling kirurgiskt.

I den första studien har vi försökt att utreda möjligheten att kunna bedöma armhålan gällande cancerspridning genom enbart klinisk undersökning. Dessutom har vi försökt att bedöma hur pass pålitligt en klinisk undersökning är gällande tumörstorleken jämfört med den slutliga patolog rapporten. Studien baseras på alla kvinnor diagnostiserade med bröstcancer i Malmö mellan åren 1987–2002. Totalt ingick 2579 patienter med invasiv eller *in situ* (förstadium) bröstcancer. Vi såg att tre av fyra kvinnor med kännbara lymfkörtlar hade spridning i armhålan medan en av tre kvinnor utan kännbara körtlar ändå hade cancerspridning i armhålan. Således klinisk undersökning av brösten och lymfkörtlar kan avvika betydligt från det slutliga patolog utlåtandet och detta kan innebära att val av behandling baserad på kliniskt fynd skulle kunna leda till såväl över- som underbehandling.

Vår andra studie undersökte möjligheten att kunna upptäcka cancerspridning i portvaktskörtlar före behandling genom att identifiera olika kliniska och biologiska egenskaper av bröstcancertumörer. Denna studie baserad på alla kvinnor med invasiv bröstcancer i Malmö och Lund diagnostiserades under åren 2008–2013. Sammanlagd inkluderades 2552 patienter som var opererade med portvaktskörtel biopsi. Materialet inhämtat från bröstcancerregistret i södra Sverige (RCC-SYD, NKBC). Resultaten visade att kvinnor som hade fått bröstcancerdiagnosen genom screeningmammografi och östrogenhormonnegativa tumörer hade mindre risk för cancerspridning i portvaktskörtlar. Däremot hade bröstcancertumörer större än 20 mm och tumörer som bestod av mer än en härd (multifokal) med kärlinväxt högre risk för cancerspridning i portvaktskörtlar. Detta antyder att dessa tumöregenskaper skulle kunna användas för att identifiera lågriskpatienter för cancerspridning.

I den tredje studien ville vi fortsätta bedöma risken och möjligheten att kunna identifiera cancerspridning i övriga körtlar i armhålan, således körtlar som kallas för icke-portvaktskörtlar (non-SN). Vi inkluderade alla bröstcancerpatienter från Malmö-Lund för åren 2008–2013 som hade cancerspridning i portvaktskörtlar och opererades med kompletterande körtelutrymning i armhålan, sammantaget identifierades 602 patienter. Materialet inhämtat från bröstcancerregistret i södra Sverige (NKBC). Vi undersökte riskfaktorer för cancerspridning i relation till olika kliniska och biologiska egenskaper hos bröstcancertumörer. Vi såg i denna studie att 65 % av bröstcancerpatienter som hade blivit opererad med kompletterande utrymning av lymfkörtlar i armhålan inte hade cancerspriding, dessutom kunde vi identifiera två särskilda grupper av patienter med högre risker för cancerspridning nämligen patienter med lobulärcancer (cancer i mjölkproducerande körtlar) samt patienter med multifokalcancer. Vidare har vi noterat att antalet portvaktskörtlar som opereras bort påverkar inte risken att hitta cancerspridning i icke-portvaktskörtlar däremot har vi funnit att antalet portvaktskörtlar med makrometastaser ökar risken att hitta cancerspridning i övriga icke-portvaktskörtlar. Resultaten av denna studie har visat att majoriteten av bröstcancerpatienter med

cancerspridning i portvaktskörtlar har friska kvarvarande körtlar i armhålan. Dessa patienter har ingen nytta av att bli opererade med mer omfattande kirurgi i armhålan då det finns mer risk för komplikationer så som lymfödem (armsvullnad) och armsmärta efter armhålskirurgi.

I arbete 4 använde vi samma databas och material som i arbete 2 och 3, dvs NKBC registret. I studien försökte vi att validera ett befintligt nomogram framtaget av vår forskargrupp (Dihge och kollegor), detta nomogram (benämnt SUS nomogram) är konstruerat för att kunna identifiera cancerspridning i lymfkörtlar lokaliserade i armhålan (axillmetastaser) före operation hos patienter med låg risk för cancerspridning. Detta i sin tur kan leda till att minskat antal onödiga operationer för patienter utan lymfkörtelmetastaser och på så sätt även minska komplikationer och lidande för patienterna. Vi inkluderade 2939 patienter från Malmö-Lund med diagnostiserad invasiv bröstcancer under perioden januari 2008 till december 2013. Vi fann att 1801 patienter (61,3%) som hade blivit opererade med portvakts körtelbiopsi (SN biopsi) och 1115 patienter (37,9%) hade genomgått lymfkörtelutrymning. Totalt hade 1008 patienter (34,3%) cancerspridning i armhålan och 1931 patienter (65,7%) hade ingen spridning i körtlarna. Således den sist nämnda patientgruppen hade ingen nytta av operationen med risk för onödiga komplikationer. Vidare har vi kunnat visa att nomogrammet fungerar bra och kan användas i praktiken med möjligheten att skräddarsy en behandlingsstrategi för varje patientgrupp med syfte att minimera över- eller underbehandling gällande kirurgiska ingrepp i armhålan. Dessutom har studie 4 visat att tillgängliga NKBC data är pålitliga då resultatet vi fick fram var likvärdigt med resultat av SUS nomogram där forskningsdata var framtagen manuellt från patientjournaler.

Sammanfattningsvis visar resultaten av dessa studier att enbart klinisk undersökning av bröstcancerpatienter inte är tillräcklig för att kunna bedöma förekomst av cancerspridning i armhålan. Tumörstorleken uppmätt kliniskt med palpation kan skilja sig från histopatologisk storlek med risk för över- eller underskattning beroende på patientens ålder. Genom att studera olika kliniska och biologiska egenskaper av bröstcancer kan man identifiera patienter med lägre eller högre risk för cancerspridning i portvaktskörtlar och därmed kunna ge skräddarsy behandling till varje patientgrupp. Kunskapen om att veta att majoriteten av bröstcancerpatienter med spridning i portvaktskörtlar inte har cancerspridning i övriga icke-portvaktskörtlar kan påverka vår syn och hantering av armhålskirurgi i framtiden. Genom att använda kunskapen om olika biologiska egenskaper av bröstcancern och genom implementering av pålitliga nomogram skulle man kunna identifiera bröstcancerpatienter med lägre risk för cancerspridning i armhålan. Att undvika onödiga operationer på friska körtlar kan minimera patientlidande och komplikationer så som lymfödem.

Introduction

Breast cancer is the most common form of malignancy in women in Sweden and worldwide [1]. ALN status, i.e. the presence vs. absence of metastatic involvement of ALN, is one of the most important and powerful prognostic factors for prediction of clinical outcome in patients with primary invasive breast cancer. Additionally, ALN status determines the extent of the adjuvant/neoadjuvant therapy[2-4].

The assessment and prediction of ALN status for the possible metastatic involvement is difficult. Non-invasive methods for prediction of ALN status by clinical examination or by different imaging facilities such as ultrasonography, mammography, computerised tomography (CT), and magnetic resonance imaging (MRI) have been confirmed to be associated with a low sensitivity [5, 6]. Historically, ALN status has been determined by ALND as a part of mastectomy [7]. However, this concept has undergone radical changes since the introduction of SNB in the late 1990s and early 2000s. The SN is the first lymph node that cancer cells drained to from the primary tumour. SNB has dramatically minimised the need for ALND and subsequently there has been a notable decrease in the incidence of complications secondary to the ALND e.g. pain, swelling, lymphedema and sensory/motoric dysfunction in the ipsilateral upper extremity [8, 9].

Lymph node metastases are classified as macro-metastasis (> 2 mm), micro-metastasis (> 0.2 - ≤2 mm) and isolated tumour cells (ITC ≤ 0.2 mm) [10, 11]. The majority of patients with breast cancer present with small primary tumours with no involvement of the ALN and it has been confirmed that >65% of breast cancer patients who have undergone SNB and/or ALND have a disease-free axilla [4, 12]. Moreover, clinical trials have repeatedly shown that there is no need of ALND in patients with negative SN [13-16]. Nowadays it is routine to leave lymph nodes behind in case the SN contains ITC (<0.2mm) and/or larger tumour deposits, called micro-metastasis (0.2-2.0 mm) [1, 17-19]. Moreover, some trials have reported that c-ALND made no contribution to a better survival and it has been suggested that it is safe to omit c-ALND even if the SN (maximum two nodes) contains tumour deposits larger than 2 mm (macro-metastasis) [17, 20]. Consequently, there is a growing interest in examining the possibility of avoiding axillary surgical staging in selected patients with low risk for axillary metastases [20]. However, the identification of low risk patients through an accurate prediction of the ALN status pre-operatively is difficult and is the subject of an ongoing debate. It has been suggested that identification of the clinicopathological predictors for ALN

metastases and implementation of a nomogram constructed for this purpose might facilitate the selection of breast cancer patients with low risk for ALN involvement [21].

Epidemiology

The incidence of breast cancer is increasing all over the world. It was estimated that there were 641,000 women with breast cancer in 1960, and over two million women in 2018 [1]. The incidence of breast cancer varies around the world; less-developed countries have the lowest incidence and the greatest incidence is seen in the more-developed countries [22].

In Sweden there were 8755 reported women with newly diagnosed breast cancer in 2018, which is equivalent to about 30% of all cases of malignant tumours [23]. The median age was 64 years, and the younger age group <40 years represented 5% of cases. The majority of cases were diagnosed by screening mammography (64.5%). SNB was performed in 5800 cases, ALND in 913 cases. Axillary metastases were found in about 24% of cases post-operatively. The intrinsic or molecular subtypes of breast cancer, which were based on the genes a cancer expresses, the luminal type (Luminal A and/or Luminal B, see section; definitions of intrinsic subtypes of breast cancer, page 52), represented the main category (75%), and less common subtypes were HER2 positive (13.5%) and triple negative (TN) 9% [23]. The 5-years survival was 92% and 10-years survival was 85% in 2016 [24].

The Breast

Anatomy

The breasts, also called the mammary glands, cover the pectoralis major muscles. They can extend from the sub-clavicle area to the sixth rib, i.e. the breasts cover much of the chest area and the front chest wall [25-27]. At the sides of the chest, the breast tissue can extend into the axilla, and can reach as far back as the latissimus dorsi muscle [25]. The mammary gland is composed of different layers of tissues: adipose tissues, glandular tissues, and other components such as connective tissues, vascular tissues and lymphatic tissues [25, 26].

The suspensory Cooper's ligaments are fibrous tissue prolongations that radiate from the superficial fascia to the skin. The superficial tissue layer is separated from the skin by 0.5–2.5 cm of subcutaneous fat [25, 26]. The breast contains 14–18 lobes which are connected and drained in to the nipple. The milk ducts measured about

2.0–4.5 mm and they are surrounded by dense connective tissue that supports the glands. Milk exits the breast through the nipple, which is surrounded by a pigmented area of skin called the areola. The size of the areola can vary widely. Sweat glands, known as Montgomery´s glands, are located in the areola. The function of these gland is to secrete oily fluid that lubricates and protects the nipple during breastfeeding [25, 26, 28, 29]

The size of the breast varies among women. A breast can have a volume of 100-1500 ml or more. There is also variation in the tissue composition ratios of the breast. There are breasts with more glandular tissues than adipose or connective tissues. The fat-to-connective-tissue ratio determines the density of the breast. During the life cycle, breasts change shape, size, and weight due to hormonal changes during puberty, the menstrual cycle, pregnancy, breastfeeding, and menopause [25, 26, 29-31].

The breast is an apocrine gland; it produces milk. The main units of the breast are the terminal duct lobular units, which produce the fatty breast milk. They are distributed throughout the body of the breast. About two-thirds of the lactating tissue is within 30 mm of the base of the nipple [25, 31]. The terminal ducts drain the milk from the lobular into 14–18 ducts and then to the nipple. Sensation in the breast is provided by peripheral nervous system innervation by means of the anterior and lateral cutaneous branches of the fourth-, fifth-, and sixth intercostal nerves. The T-4 (Thoracic spinal nerve 4), supplies sensation to the nipple-areola complex [31].

The main blood supply of the breast is divided between the medial and lateral sides; the arterial supply to the medial part of the breast is through the internal thoracic artery which is a branch of the subclavian artery [25, 32]. The lateral part of the breast is supplied by the following vessels; the lateral thoracic, the thoracoacromial branches which originate from the axillary artery, and the lateral mammary branches which originate from the posterior intercostal arteries (coming from the aorta). The veins of the breast correspond with the arteries, draining into the axillary and internal thoracic veins [32].

Physiology

The main regulators of breast development are the steroid hormones, oestrogen, progesterone, and growth hormone (GH). Oestrogen and progesterone are produced mainly by the ovaries and released into the body in fluctuating amounts with each menstrual cycle [33, 34].

Development of the breasts during the prenatal period of life is independent of sex hormones [33, 34]. Until puberty, the tubule networks remain rudimentary and the male and female breasts do not show any differences. Oestrogen during puberty in females, through activation of oestrogen-α specifically causes growth of and transformation of the tubules into the matured ductal system of the breasts [28, 33].

The ducts elongate under the influence of oestrogen and terminal end buds, penetrate into the fat and branch as the ducts elongate, forming a tree-like network of branched ducts that is embedded into the entire fat of the breast. Additionally, estrogen causes stromal tissue to grow and the nipple-areolar complex to increase in size and adipose tissue to accumulate [26, 33, 35, 36]. Progesterone, similarly to oestrogen, affects the development of the breasts throughout the women´s life but progesterone contributes to ductal development to a lesser extent than oestrogen [37]. Both GH and oestrogen are required for progesterone to affect the breasts, because oestrogen primes the breasts by inducing the expression of the progesterone receptor in the epithelial tissue. In contrast to the progesterone receptor (PR), the estrogen receptor (ER) expression in the breast is stable and differs relatively little in the context of reproductive status, menstrual cycle, or hormonal therapy [33, 38].

Lymphatic system

The lymphatic system has an essential role in systemic immunity and returning tissue fluid and macromolecules to the circulation. Lymphatic drainage has an important role in the pathology and treatment of breast cancer [39]. Cancer metastasis is mediated by malignant cells traveling within the lymphatic system to different parts of the body [39].

The lymph fluid

Lymph fluid is composed of interstitial fluid, proteins, clotting factors and leukocytes. Approximately 10% of the fluid in the interstitial space must be returned to the venous system to maintain fluid balance [39]. Lymphatic capillaries contain a single-layer endothelium with loose junctions in the basement membrane. This membrane helps the entry of fluid, cells, and macromolecules. Lymphatic capillaries drain into collecting vessels and lymph nodes which finally drain into larger regional lymphatic trunks [39].

Lymph nodes

Lymph nodes are encapsulated bean-shaped structures that filter microorganisms and tumour cells.[34, 39] The lymph nodes are essential for cellular immunity. Afferent lymphatic vessels drain into sinuses between germinal centres within the node. These germinal centres contain macrophages, which collect foreign material, including the radiolabelled colloids and dyes used to localise lymph nodes [34].

Lymphatic drainage of the breast

Lymphatics in the breast parenchyma originate in the interstitial interlobular tissue and within the walls of the lactiferous ducts. The lymphatic drainage of the breast is mainly to the ipsilateral axillary nodes, occurring with a probability of 98.2% [40, 41]. All lymphatics of the breast drain mainly along a subdermal plane into the

axilla, while the superficial lymphatics of the nipple and areola collect in the subareolar lymph plexus and the deep part of the breast [39]. The medial part of the breast drains into lymphatic vessels that perforate the deep fascia to drain into the internal mammary nodes [34]. Sporadic drainage to the subclavicular, supraclavicular, or interpectoral mammary nodes may happen, occurring with a probability of 1.7%, 3.1% and 0.7% respectively [40].

Axillary lymph nodes

The axillary lymph node chain can be divided into six groups (Fig.1), [25, 26]. The apical axillary group is also known as the subclavicular group. It contains 8-12 nodes between the superior border of the pectoralis minor and the clavicle. This group receives drainage from all other levels of axillary nodes and drains into the subclavian trunk, then in to the thoracic duct on the left and the right lymphatic trunk on the right side [25]. The brachial group consists of 4-6 nodes medial and posterior to the axillary vein, and receives the drainage from the upper extremity and drains into the apical axillary group [25]. The central group lies deep in the pectoralis minor within the adipose tissue of the axilla. It contains about 4-5 nodes and receives drainage from the breast, the brachial group, the pectoral group, and the subscapular group. The subscapular group, also known as the posterior group, consists of 5-7 nodes on the lateral edge of the scapula. It receives drainage from the neck, shoulder, and trunk. The interpectoral group, also known as Rotter's nodes [25], consists of 1-4 nodes between the pectoralis major and minor muscles and receives lymph drainage from the breast, draining into the apical axillary and pectoral group [25]. The pectoral group, also known as the anterior or external mammary group, contains 5-6 nodes along the lateral thoracic vessels. It receives drainage from the lateral side of the breast and abdominal wall, and drains into the central group. There are lymph nodes outside the axilla that are involved in the lymphatic drainage of the breast [25]. The infraclavicular group lies in the region bordered by the clavicle, deltoid and pectoralis major muscles. The internal mammary nodes are also known as the parasternal group. These nodes lie along the internal mammary artery and vein within the intercostal spaces and deep in the parietal pleura. Perforating lymphatics accompany perforating branches of the internal mammary artery through the pectoralis muscle. Variations in blood supply to the breast through these perforators explain why, in all quadrants of the breast, cancer has the potential to metastasise through internal mammary lymphatics [25, 26, 42].

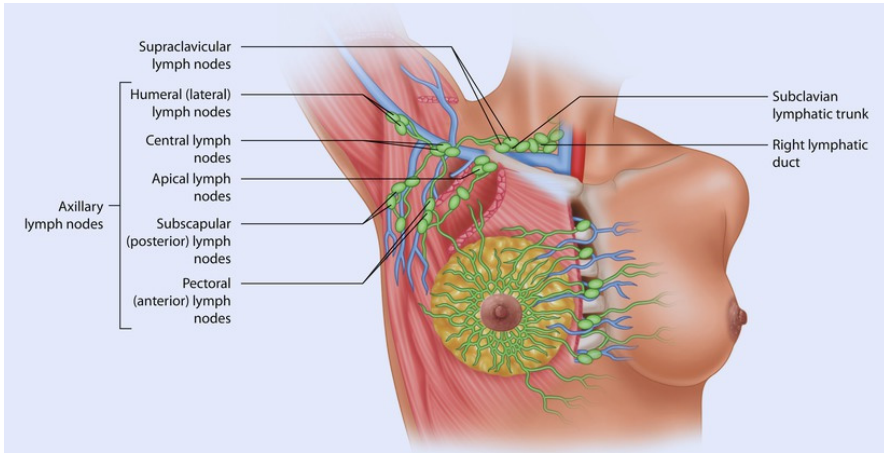


Figure 1.
Axillary lymph node groups (With permission from SpringerLink)

Axillary lymph node levels

Metastasis of breast cancer to the axillary lymph nodes is suggested to occur and progress from level to level and not as a unit [43]. There are three anatomical levels (Fig.2) of the axillary lymph nodes; [25, 43]

- level I; located inferio-lateral to the pectoralis minor muscle.
- level II; found posterior to the pectoralis minor muscle.
- level III; placed superior and medial to the pectoralis minor muscle.

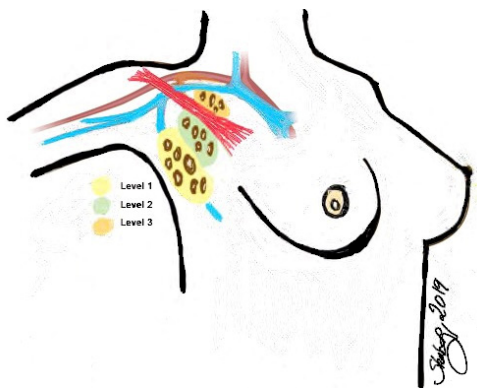


Figure 2.
Axillary lymph node levels

History and development

The first reference to breast cancer is believed to be found in the Edwin Smith Surgical Papyrus [44], which is one of the eight extant Egyptian medical papyri written in hieratic script. It dates back to (3000–2500 BC) in the ancient Egypt and was probably written by the physician-architect Imhotep, who designed the step pyramid and practised medicine in Egypt in the 30th century BC [44]. Ancient Greece and particularly Hippocrates described cases of breast cancer in detail (460–375 BC) [45]. The Roman physician Aulus Cornelius Celsus (42 BC–37AD) noted that the breasts of women were a common site of cancer. Celsus described breast cancer in his manuscript, De Medicina. Leonides, a surgeon of the Alexandrian school was perhaps the first to record that breast cancers spread to the axilla [45-47].

The existence of "Lymphatics" was first noticed by Bartholin in 1653[45]. Although Jean Louis Petit, the first president of the French academy of the surgeons (1674–1750), removed both the breast and diseased nodes in the axilla, the routine axillary lymph node removal began in the 19th century when Ernest Küster in Germany removed axillary lymph nodes, even though the nodes were not palpable and were regarded as normal [29].

W. S Halsted reported in 1894 on the breast operations performed at the Johns Hopkins Hospital from June, 1889, to January, 1894. Fifty consecutive patients were operated with radical mastectomy that included the removal of the breast, the underling pectolis muscle and axillary lymph nodes, and the wound was left opened for secondary healing [7]. D.H. Patey reported in 1948 that modified radical mastectomy i.e. mastectomy with axillary dissection but preserving the pectoralis muscle, had more advantages than radical mastectomy [48]. The gold standard of axillary dissection remained unchanged for many years until the beginning of 1970 when Bernard Fischer *et al.* [49] showed in their trial that no significant difference in the treatment failure or survival had been observed in clinically negative node patients randomly treated by conventional mastectomy with postoperative regional radiation or mastectomy followed by axillary dissection. Additionally, there were no differences between patients with clinically positive nodes managed by mastectomy or by mastectomy followed by radiation [49].

For many years, ALND has been used as a therapeutic measure, as a part of radical mastectomy, or as a separate procedure [7, 48]. However, ALND was observed to be associated with complications such as pain, lymphoedema, and neurological disabilities [50]. About 49% of women who had undergone ALND developed lymphoedema 20 years after the primary operation [51]. The axillary complication motivated the innovation of new surgical methods for management of ALN.

Axillary sampling

The era of "axillary sampling" in the 90s, where 4-5 lymph nodes were removed from the lower part of the axilla, was confirmed to be associated with a high local recurrence rate compared with ALND [52, 53]. Additionally, it was found that axillary sampling was associated with regional recurrence, distal metastases, and poor survival in node-negative breast cancer patients due to removal of an insufficient number of ALNs according to different studies [54, 55].

Sentinel node, technique and validity

The concept of the "Sentinel Node" was first used by Ernst G. *et al*. in 1960 in the management of parotid cancer [56]. The use of SNB in patients with breast cancer was launched by Giuliano *et al.* in 1991 as they performed intraoperative lymphatic mapping by injection of blue dye, with 95.6% accurately predicted nodal status in the axilla [2]. Krag *et al.* reported in 1993 in a pilot study of breast cancer patients that radio-localisation and selective resection of SN was possible [57]. Later on, lymphoscintigraphy was added pre-operatively to facilitate discovery of the location and number of SNs [2]. These two lymphatic mapping procedures constitute the basis for today´s various methods of SN identification. SNB is now the standard staging procedure and is used all over the world [58]. However, there are other tracers which can be used in SN mapping, e.g. magnetic iron oxide, indocyanine green and radiolabelled corns [59].

The SNB is based on two principles: first is the existence of lymphatic drainage to a regional lymph node, and second is the presence of the first lymph node which acts as a filter for tumour cells [60]. The sequential lymphatic dissemination and blockage of tumour cells by first draining the lymph nodes has been proved, according to Kapteijn *et al*. [60].

The validity of the SNB has been confirmed in different studies and an accuracy of 97-98% has been reported by different studies [14, 59, 61-63].

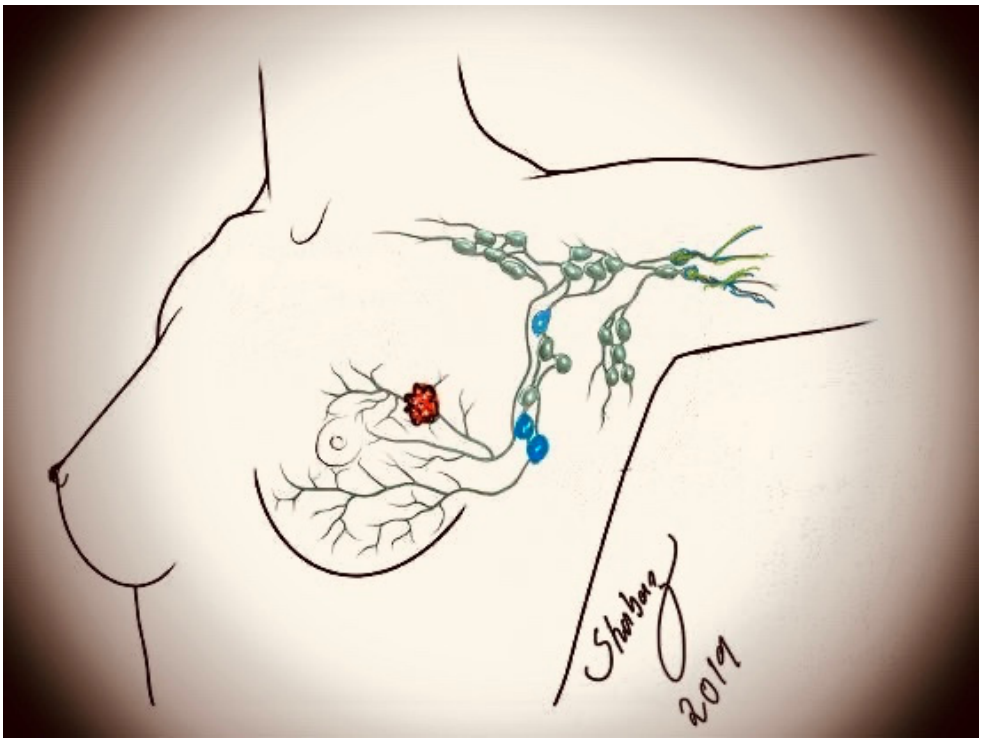


Figure 3.
Sentinel Nodes and non-Sentinel Nodes

Number of SNs removed by surgery

The concept of the SN builds upon the idea that cancer cells drain to the regional lymph node in a sequential order and in an ideal case, just one "true SN" should be identified [60].

After the launch of SNB in clinical praxis there was a controversy regarding the number of SNs to be removed during the SN procedure [64]. In the early 2000s, i.e. the beginning of the era of clinical SN implementation, there were no limitations on the number of SNs to be removed by the surgeon. The main aim of the SN procedure was to determine the pathological status of the ALN. However, studies have shown that this aim could be achieved after removal of the first SN in 91.4% of the patients and increased to 99% after removal of the second SN [64, 65].

The number of SNs which can be identified during an SN procedure depends on many factors such as tumour site, injection site, volume of the tracer used, type of trace, and the time interval between the injection and the onset of the procedure. The

experience of the surgeon who performs the operation might also affect the SN according to some studies [64-66]. However, different studies have shown that removal of four SNs is enough for assessment of axillary status and removal of more than four SNs does not lead to a better SN results or axillary staging [64, 65].

False-negative SN

The concern of the false-negative SN can be described in two different categories: false-negative results obtained as a procedure and false-negative results achieved after a frozen examination [67, 68].

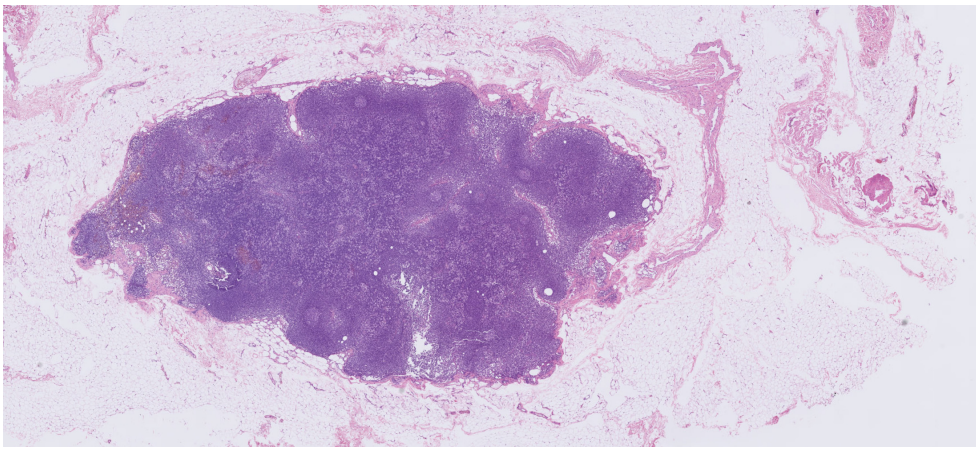
Regarding the issue as a procedure, it has been observed that the experience of the surgeon performing the operation is an important factor in minimising the false-negative results. A success rate of about 98% has been recorded if the surgeon performs more than six SN procedures per month [67]. Other factors that might be associated with the false-negative rate (FNR) results are previous surgery on the axilla, the mapping technique, and the time interval between the injection of radiolabelled tracer and the start of the operation [68].

The FNR for SNB ranges from 5% to 23% [16, 19, 69-72]. The main factors influencing the FNR are the size of metastasis (ITC and micro-metastasis) as well as the histological type of the tumour [70, 71, 73, 74]. However, late axillary nodal recurrence is rare, with a recurrence rate of (0.2- 1.6%) being reported [75-78].

Classification of SN metastasis

The metastatic deposition of cancer cells in the lymph nodes is classified in to three groups [10, 11];

1- The isolated tumour cell clusters (ITC). This is characterised by a single cancer cell, a collection of cancer cells smaller than 0.2mm or collection of less than 200 cancer cells. An ITC may be detected by immunohistochemistry (IHC) or routine histology (Fig.4). For the primary nodal (pN) category [10] only the size of the largest contiguous tumour cell cluster is used; the sum (extent) of the ITC cluster sizes is not used for primary nodal status (pN). The lymph nodes should be designated as pN0 regardless of the number of nodes containing ITCs [10, 11].



Figer 4.
Normal axillary lymph node (IHC photo by Dirk Junghans, department of pathology, CSK)

2- Micro-metastasis defined as the presence of clusters of cancer cells with a size of 0.2 mm - 2.0mm or >200 cells (Fig.5).

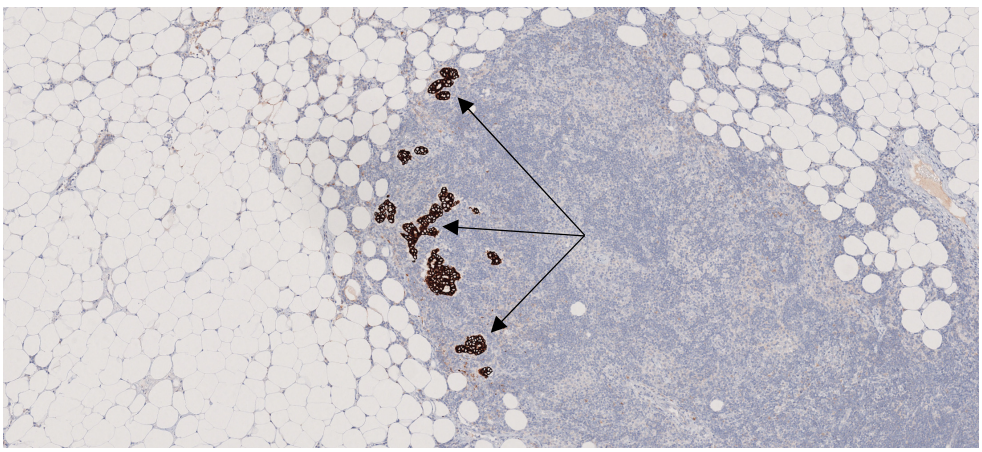


Figure 5.
Micro-metastasis in sentinel node (Photo by Dirk Junghans, department of pathology, CSK)

3- Macro-metastasis defined as clusters of cancer cells with a size >2.0 mm (Fig.6).

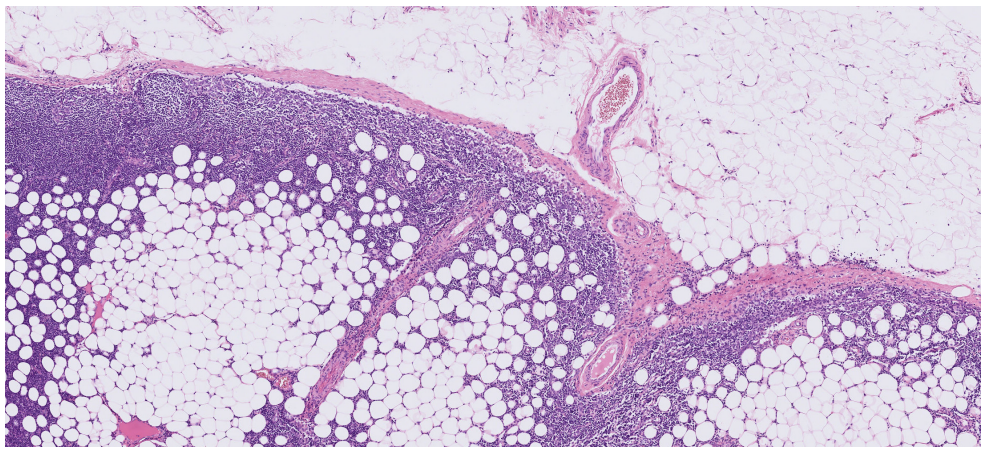


Figure 6.
Sentinel node with macro-metastasis (IHC photo by Dirk Junghans, department of pathology, CSK)

The role of lymph node metastasis in breast cancer survival and the necessity of completion ALND

For decades the prognosis of patients with invasive breast has been related to the involvement of ALN with metastasis, and it has been confirmed that an increase in the number of ALNs with metastasis is associated with failure of treatment and worse prognosis [77, 78]. A five year survival rate of >82% in patients with no involvement of ALN has been recorded, and a rate of 73% for 1-3 positive lymph nodes and about 54% for 4-6 positive ALNs and about 28% for patients with > 13 positive ALNs [77]. Although the presence of the ALN metastasis has been associated with and affects the prognostic outcome, the impact of surgical removal of ALNs on prognosis and survival among patients with a positive SN has been an ongoing debate for several decades[17, 79]. Two trials have failed to confirm a survival difference in patients with a positive SN, who were randomised to c-ALND or not [17, 79]. In the first trial, ACOSOG Z0011 they performed randomisation of patients with 1-2 positive SNs to either ALND or no axillary surgery. A 10-year follow-up showed no significant difference in the axillary recurrence rate in the two groups (86.3% and 83.6% respectively). Furthermore, no statistically significant results were observed in the disease-free survival rate among patients who underwent SNB (80.2%) compared with the patients who underwent c-ALND (78.2%). There was some criticism about the ACOSOG Z0011 as the trial only

included patients with breast tumours up to 5 cm who underwent breast-conserving surgery and all received adjuvant whole breast radiotherapy post-operatively [17, 18]. In the second trial, IBCSG 23–01, there were no statistically significant results neither. The study included patients with micro-metastasis in the SN, and the disease-free survival was 76.8% for patients who underwent SN biopsy and 74.9% for those who underwent ALND [79].

In Sweden the ongoing trial of SENOMAC is investigating the value of c-ALND in patients with a limited number of SNs with macro-metastatic involvement (maximum two) who have undergone primary breast surgery or neoadjuvant therapy [20].

Breast cancer pathology and predictive factors

Histological classification

The breast is an epithelial organ and the vast majority of the breast cancers arise from the luminal epithelial cells. Adenocarcinoma, the most common type, arises in the ducts, and ductal carcinomas account for approximately 80% of all types of breast cancer. A less common type of adenocarcinomas originates from the milk producing glands (lobular carcinoma) and accounts for about 10-15% of all types of breast cancer [1, 10, 23, 80]. This type of cancer may occur in different parts of the same breast (multifocal) or in both breasts simultaneously. They are diffuse in nature and might be difficult to detect by mammograms [80-82]. Other epithelial and non-epithelial types of breast cancer, arising from the soft tissues and stroma (5-10%), are less common e.g. mucinous, papillary, medullary, tubular, and phyllodes cancer, Paget's disease, inflammatory cancer, sarcoma and angiosarcoma [23, 80, 81].

The carcinogenesis as a process developed starts in the normal breast tissues, developing to hyperplasia or atypical hyperplasia, then converting to carcinoma *in situ* and finally to the invasive breast cancer [83]. Invasive cancer means that the cancer cells can invade and cross the cell membrane and potentially have the ability to spread to other parts and different organs of the body. In contrast, *in situ* cancer which can be ductal (DCIS) or lobular (LCIS), cannot cross the basement membrane, hence has no possibility of metastasis [81, 82].

TNM classification

The TNM (tumour, node, metastasis) classification according to the World Health Organization is composed of tumour size(T), axillary lymph node status (N) and distant metastasis status (M). It is an essential prognostic tool which guides the clinician in decision-making regarding management of the breast cancer and it is an important survival determinant in breast cancer [11, 80, 84, 85]

Histological grading

Elston and Ellis introduced the Nottingham Histological Grading in 1991 [86]. This was a revised version of histological grading published by Bloom and Richardson in 1957 [87]. This grading is based on three components: nuclear atypia, tubular formation and number of mitoses, each with a scoring table from one to three and a total scoring of three to nine for all three components together, where Grade 1 corresponds to 3-5, Grade 2 is 6-7, and Grade 3 is 8-9. The histological grading is also an important survival factor in breast cancer where the Grades 2 and 3 are associated with lower survival rate as compared with Grade 1 [86, 88].

Age and menopause status

The incidence of breast cancer increases with increasing age, and the peak incidence was in the range of 65-69 in Sweden in 2017, but after the age of 70 the incidence decreased [22, 23, 89]. More than 50% of all newly diagnosed breast cancers were in patients aged more than 60 years in 2015 [89]. Age is regarded as an independent prognostic factor [90] and breast cancer in pre-menopausal women has worse prognosis compared with older women as breast cancer in younger women is frequently associated with negative prognostic characters such as higher histological grade, node positivity, hormone-negativity and a higher proliferation rate [90-92].

Studies have shown that the age is also associated with the risk of nodal metastasis, where lymph node involvement decreases with increasing age to about 70 years and increases after the age of 70 years in patients with small breast cancer [93-95], which could probably be explained by non-compliance and comorbidity in elderly women [95]. Another possible explanation may be that women aged more than 74 years are not included in the screening mammography program [94].

Lymphovascular invasion

Lymphovascular invasion (LVI), is an independent predictor for lymph node involvement in breast cancer and it has been regarded as main route for malignant cells to enter the axillary lymph nodes [96]. LVI is defined as the presence of tumour emboli in the lymph vessels (Fig.7), lined by a single layer of endothelial cells and without the presence of red blood cells [11, 97]. During the last decade there were some studies that showed the possibility of using immunohistochemical lymphatic vessels marker might reveal the presence of LVI. Kahn *et al.* showed in their analysis that the monoclonal antibody D2-40 selectively detected lymphatic vessels in breast tissue [98, 99]. However, the use of the LVI in clinical routines is of limited value as the presence of LVI is not revealed until the final pathological report is available post-operatively.

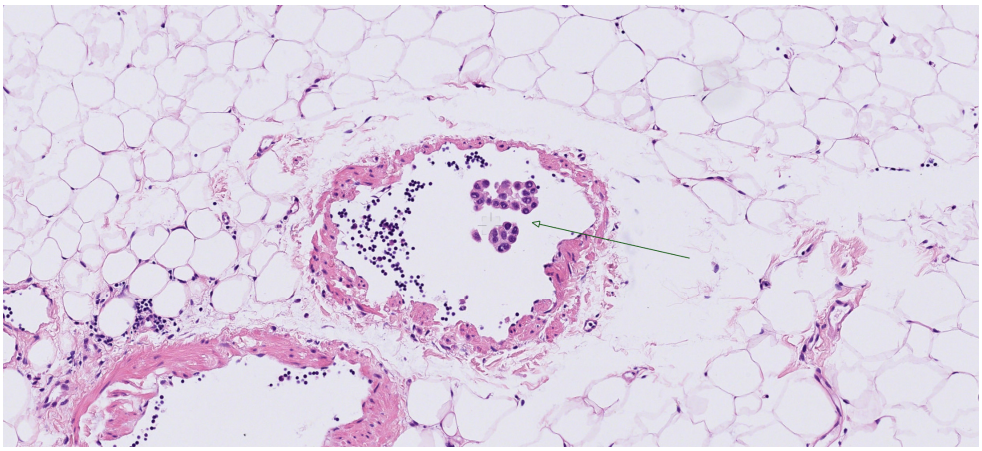


Figure 7.
Lymphovascular invasion (IHC photo by Dirk Junghans, department of pathology, CSK)

Hormone receptor status

The oestrogen receptors (ERs) and progesterone receptors (PRs) are nuclear receptor proteins. They are regarded as a prognostic and treatment predictive factors [100]. These receptors are found in approximately 75-85 % of patients with invasive breast cancer [23]. The receptor status can be analysed by IHC, where detection of the receptors is performed by using antibodies binding to their specific antigens. PRs are regulated by ERs and if an analysis reveals an ER-negative and PR-positive result the possibility of essay issues should be considered [101]. Receptor status (Fig.8) was described as positive at the study time, when these receptors present on more than 10% of the cancer cells. Positive hormonal status indicates the beneficial of the endocrine anti-hormonal treatment. There might be some variations in the positive cut-off levels and a range of 1-20% has been used [1, 102-104]. The association between the ER status and the possibility of ALN involvement has been controversial. There are studies that have confirmed a lower risk of ALN metastasis in cases with negative ER, while other studies have shown no association [4, 105].

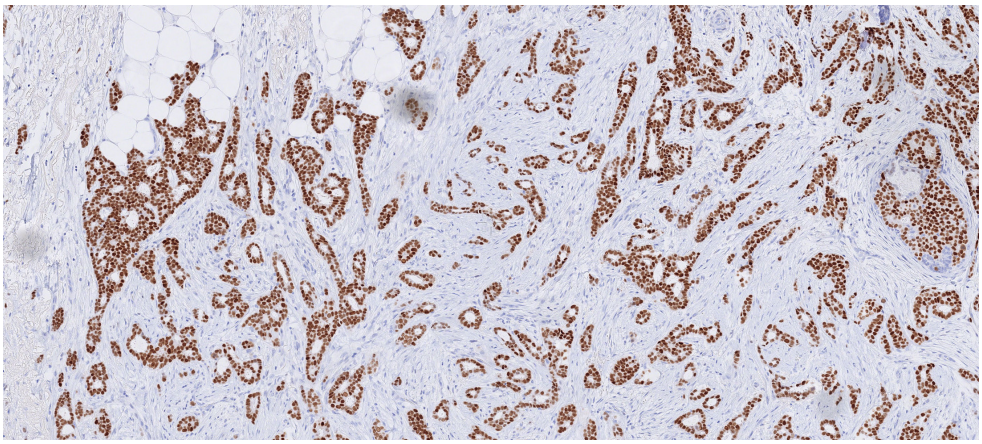


Figure 8.
Oestrogen receptor positive breast cancer (Photo by Dirk Junghans, department of pathology, CSK)

HER2 status

The Human Epidermal Growth Factor Receptor type 2 (HER2) is a tyrosine kinase receptor, and is a part of the growth factor receptors family which include HER1, HER3, HER4 and Epidermal Growth Factor Receptor (EGFR). The HER2 protein level is measured by IHC (Fig.9) and gene amplification is assessed by ISH (*In Situ* Hybridisation), using fluorescence (FISH), chromogenic (CISH) or silver enhancement (SISH) [1, 11]. IHC assesses the level of the protein expression only and four groups of patients can be identified accordingly; 0, 1+, 2+, and 3+. In Sweden all patients with 2+, or 3+ are further analysed for gene expression by gene amplification and HER2 status is regarded as positive if 2+, 3+ are amplified [1, 11].

HER2-positive breast cancer has the capacity for HER2 receptor amplification leading to an overexpression of the HER2 protein which in turn increases the angiogenesis, proliferation and invasive capability of the tumours [106-108]. Approximately 15-20% of invasive breast cancer cases are HER2 amplified [1, 23, 109, 110]. HER2 amplified breast tumours are associated with a higher risk of ALN involvement and a poor prognosis [111-113].

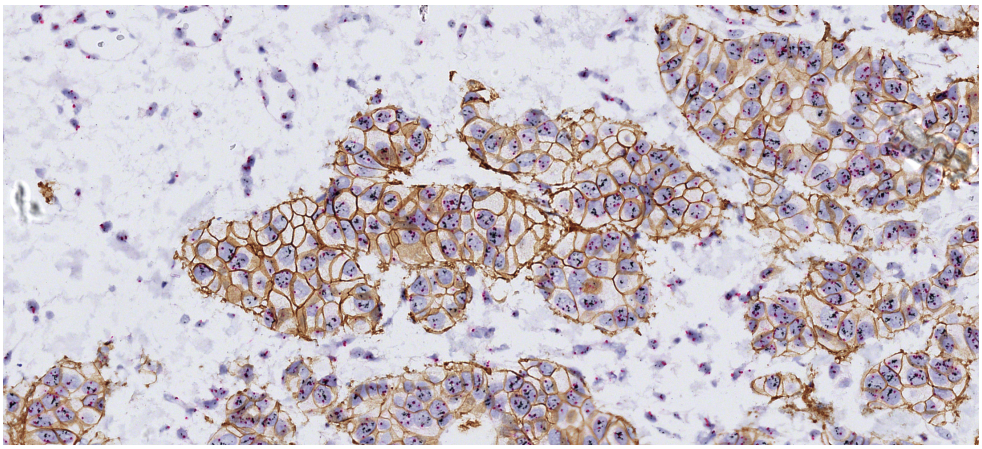


Figure 9.
HER2 3+ breast cancer (IHC photo by Dirk Junghans, department of pathology, CSK)

Multifocal breast cancer

Multifocality is defined as the presence of more than one focus of an invasive tumour in the same quadrant of the breast with the presence of normal tissue or *in situ* carcinoma in between [10, 11]. Previously, a tumour with two or more foci in different breast quadrants was called as multicentre tumour. However, both categories nowadays are regarded as one entity [11, 114]. Different analyses have shown a positive association between the presence of multifocality, tumour aggressiveness and ALN metastasis [115, 116]. The underlying biology regarding the multifocality and increased risk of lymph node metastasis is unclear [12].

Ki-67

Ki-67 is a nuclear protein which was first described first by Gerdes J. *et al.* in 1983 in Kiel [117]. The Ki-67 antibody has the ability to recognise the nuclear antigen which is expressed in proliferating cells but not in resting cells, i.e. Ki-67 can be used to evaluate the proportion of proliferating cells [117].

Ki-67 has a powerful prognostic value. A high level of Ki-67 is associated with worse disease-free or overall survival rate in breast cancer with or without ALN involvement [118, 119] and it might be associated with ALN metastasis [120]. Since 2011, the St Gallen guidelines have recommended using Ki67 to distinguish low proliferation (Luminal A-like) from high proliferation (Luminal B-like) with a cut-off level of 14% [121]. In 2013, the St Gallen recommendation for the cut-off level

was changed to 20% [104]. Still the cut-off level, the cell counts and assessment of the hotspot may vary in different centres.

In Sweden for the time being, the cut-off has been defined depending on the region and according to the recommendations of the Swedish Society of Pathology [11]. The ki-67 cut-off is described and summarised as follows in four main pathological departments in the southern Sweden;

- Lund: Low 0-14%, Intermediate 15-22%, High 23-100%.
- Malmö: Low 0-20%, Intermediate 21-30%, High 31-100%.
- Helsingborg: Low 0-14%, Intermediate 15-24%, High 25-100%.
- Kristianstad: Low 0-9%, Intermediate 10-16%, High 17-100% (Fig.10, 11).

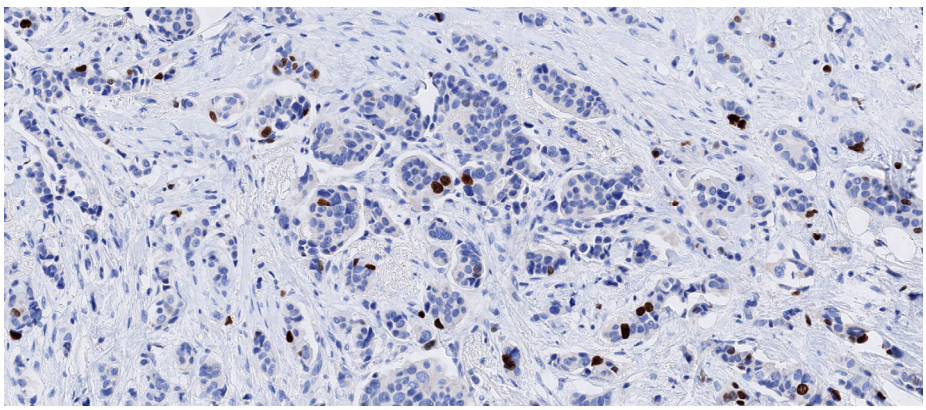


Figure 10.
Low Ki-67 (IHC photo by Dirk Junghans, department of pathology, CSK)

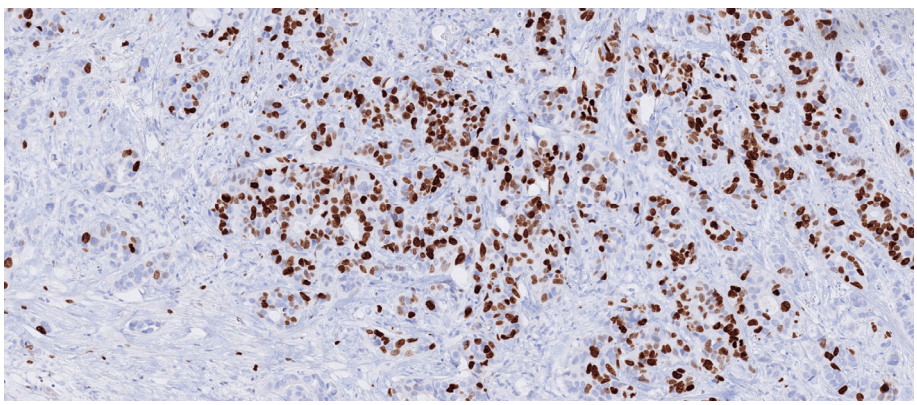


Figure 11.
High Ki-67 (IHC photo by Dirk Junghans, department of pathology, CSK)

Breast cancer diagnostics

The concept of "triple diagnostics" is the cornerstone of breast cancer diagnostics [1]. It is composed of clinical examination, radiological imaging and biopsies (fine needle and/or core biopsy). Assessment of the axilla is included in this process through clinical assessment and examination by ultrasonography. Diagnostic accuracy has been regarded as very high with this approach and a sensitivity of 100% has been reported [122]. If any of these diagnostic procedures result in cancer suspicion, further investigations are indicated such as re-biopsy, surgical biopsy, galactography, tomosynthesis, MRI, or CT [1].

Clinical breast examination and clinical axillary examination

Approximately 35-40% of breast cancer patients present with a lump in the breast [1]. Breast palpation is a simple, and brief test for clinical assessment of the breast [123]. The sensitivity is low (54%) for clinical breast examination (CBE) but the specificity is high (94%). However, a negative CBE does not exclude a breast cancer [124].

Some studies regard the CBE as an adjunct to screening mammography and might help in detection of local/regional events. However, there is still controversy about whether CBE will improve the accuracy of breast screening mammography [125, 126].

Assessment of axillary lymph nodes by clinical examination of axilla (CAE) has also been debated in studies. A study from early 1990 had recorded that the clinical examination was more sensitive than ultrasound (68% vs. 56%) [127], but with technological advances in the field of diagnostic imaging nowadays, the examination of axilla by ultrasound is now the primary imaging modality [128, 129]. However, the CAE plays an essential role in axillary staging although the accuracy is uncertain [130].

Detection mode

The majority of breast cancers are symptomless and in Sweden approximately 64% are detected by screening mammography. The symptomatic type of breast cancer is less common, where the leading symptom is "a lump in the breast" [1]. Other signs and symptoms are changes in the shape or skin of the breast, and nipple discharge or retraction. In very rare cases, breast cancer may be presented with a lump in the axilla and without any signs or symptoms in the breasts [1, 22, 23, 131].

Studies suggest that the screening mammogram may decrease the mortality rates by 5-25% [132, 133]. Furthermore, it has been shown that the possibility axillary lymph node involvement with metastasis is lower in patients with breast cancer diagnosed by screening compared with tumours not detected by screening mammography [4]. However, the advantage of the screening mammogram is still debated as breast cancer over-diagnosis predominated the early detection of small tumours and the reduction in the mortality rate might be related to the use and improvement of chemotherapy [134-136].

Treatment

Breast cancer treatment consists of local, regional and systemic approaches. The choice of the appropriate treatment is made by multidisciplinary conferences through implementation of the recommendations by the Swedish national guidelines for breast cancer care [1]. Representatives from the departments of oncology, radiology, surgery, and pathology participate in these conferences. There is a wide range of various treatment options according to the phase of the breast cancer. In general, treatment comprises of a combination of different treatment strategies involving surgery, radiotherapy, and systemic therapy.

Surgery

The majority of the patients undergo primary surgery, where the primary goal is breast conserving approach. Long-term survival after breast-conserving surgery (BCS), in combination with adjuvant radiotherapy, has been confirmed to be as effective as mastectomy [137, 138]. The choice of the surgical approach depends on many factors such as breast size, tumour size, tumour stage, tumour localisation, history of previous breast cancer, previous radiotherapy, and the patient´s requirements. Generally, primary surgery is not recommended in patients with regional or distant metastases [1]. SNB is standard for axillary status staging in all patients with invasive breast cancer.

Radiotherapy

Local radiotherapy (RT) is recommended in order to reduce the risk of local recurrence after BCS irrespective of tumour size or after mastectomy for tumours >5cm [139]. The goal is to eradicate residual cancer cells in the remaining breast tissue as a part of adjuvant therapy. It has been shown that the whole breast radiation therapy after BCS reduces the 10-year risk for local relapse by 50% compared with BCS without adjuvant RT [140, 141].

Regional RT is recommended for patients with axillary lymph node macro-metastases [142, 143]. Studies have recorded that regional RT reduced the breast cancer recurrence but no improvement in overall survival was observed [142, 143]. In general, the recurrence rate after 10 years is low (2-4%) for patients who have not received regional RT [142, 143], and the Z0011 trial [17, 18] reported a recurrence rate of <0.5% for patients with 1-2 SN metastases who received local RT. Moreover, this trial showed that the10-years overall survival for these patients

who had undergone only SNB was not inferior compared to the overall survival for patients treated with ALND [17].

The risk of developing lymphedema after RT is lower than the risk after ALND, 5% and 13 % respectively, however, the advantages and disadvantages of the RT are still being debated. [144, 145].

Systemic Therapy

Systemic therapy is recommended to eliminate the micro-metastases and to reduce the risk for relapses. This therapy is composed of endocrine therapy, chemotherapy, HER2-targeted therapy, and bisphosphonate therapy.

Chemotherapy

The goal of the chemotherapy is to eliminate the micro-metastases and to increase the survival rate [1, 146]. This type of therapy can be used as neoadjuvant (before surgery) or as adjuvant therapy (after surgery) [1]. Additionally, it can be applied in a palliative setting. The approach is poly-chemotherapy where a combination of several cytostatic drugs can be used to minimise the toxic effect and synergise the potential effects. The EBCTCG study has shown, based on the results from 40 randomized chemotherapy studies including 13000 patients, that poly-chemotherapy is more effective than mono-therapy [147].

Endocrine therapy

Two different types of anti-hormonal drugs are used as adjuvant therapy, the selective ER modulator and aromatase inhibitor (AI) [1]. In general, this therapy is recommended to all patients with positive hormonal status. An absolute risk reduction by 13% has been recorded after five years treatment with Tamoxifen and an extended treatment with five years of treatment with Letrozole leads to an additional 5% risk reduction [1, 148]. Moreover, a 10-year treatment with Tamoxifen has been shown to be associated with an additional 3% reduction in recurrence rate [1, 149, 150].

Antibody therapy

This therapy is used in breast cancer with HER2 positive status (about 10-15% of breast cancer cases) [23]. Trastuzumab is a humanised monoclonal antibody and can be used both in primary breast cancer and in cases with metastases, usually in combination with chemotherapy [149]. Data analysis involving 11991 patients has shown that Trastuzumab significantly improves overall survival and disease-free survival in HER2-positive women with early and locally advanced breast cancer [150].

Bisphosphonates

This therapy blocks the function of the osteoclast which in turn diminishes bone resorption. The therapy is applied mainly to post-menopausal women [147, 151]. In ABCSG study 3425 patients were treated with adjuvant bisphosphonate during 2006-2013, as an adjuvant to AI therapy. These patients were randomised for treatment with bisphosphonates vs. placebo and the results showed that there was a statistically significant reduction of about 1.5% (p=0,004) in the incidence of bone metastatic recurrence after 10 years [151].

Aims

The general aim of this thesis was to determine the clinicopathological predictors of axillary lymph node metastasis in primary invasive breast cancer.

The specific aims of each study were as follows:

Study I

- The first aim was to determine the accuracy of physical examination of the axilla in relation to the presence of metastases as revealed by histopathological examination.
- The second aim was to compare the tumour size in the breast, assessed by physical examination, with the tumour size according to the final histopathological report.

Study II

- To determine the clinicopathological predictors for metastases to the SN in primary invasive breast cancer.

Study III

- The first aim was to identify determinants associated with non-SNs metastases.
- The second aim was to determine the impact of the size of SN metastases and the number of SNs with macro-metastases, on non-SN involvement with metastases.

Study IV

- The main aim was to validate the performance of the SUS nomogram in an independent cohort.
- An additional aim was to assess the possibility to use the routinely collected data from a clinical registry compared with the manually retrieved medical records.

Material and Methods

Study population

Two cohorts have been used in this thesis.

1- **Study I;** included 2537 patients in Malmö, data retrieved from the SSBCG and the Regional Tumour Registry (Figure 1).

2- **Study II, III, IV;** included 3979 patients in Malmö-Lund, data recruited from the NKBC registry (Figure 2).

Study I

The Regional Tumour Registry had information about all cases with breast cancer diagnosed in Malmö 1961 to 2004. This data was retrieved in 2005. In Malmö, since 1977, patients with breast cancer have been discussed weekly at a multidisciplinary therapy conference.

In 1977, the SSBCG was established. Guidelines for treatment of breast cancer have been issued by this group. From 1981 to 2003, SSBCG had a clinical registry with a computerised database. This registry had information about age, menopausal status, tumour size by histopathological examination and hormonal receptor status, the extent of surgery and the use of adjuvant therapy. Moreover, the registry had information about the axillary lymph node status by physical examination, the final results from histopathological examination of the lymph nodes, and tumour size according to the pre-operative physical examination.

The following patients were excluded from this study:

Six patients with unknown civil registration number, 26 benign lesions, 1921 patients with history of previous breast cancer, 3326 patients diagnosed before the establishment of the clinical registry at the SSBCG in 1981, 245 patients following end of data collection into the SSBCG registry, 31 Dec 2003, 202 patients who were not registered as residents in Malmö, 481 patients treated outside Malmö, 28 breast cancers found at autopsy, and 41 patients where there was a mismatch between date of diagnosis in the Regional Tumour Registry and the SSBCG registry of more than 180 days (Fig. 12).

There were many missing cases at the beginning of the period and during the last year in the SSBCG registry as routines for collection of information in to the SSBCG registry had changed slightly over time. The final study population consisted of 2537 patients diagnosed between 1 Jan 1987 and 31 Dec 2002. All these patients had available information in the SSBCG registry, corresponding to 97%.

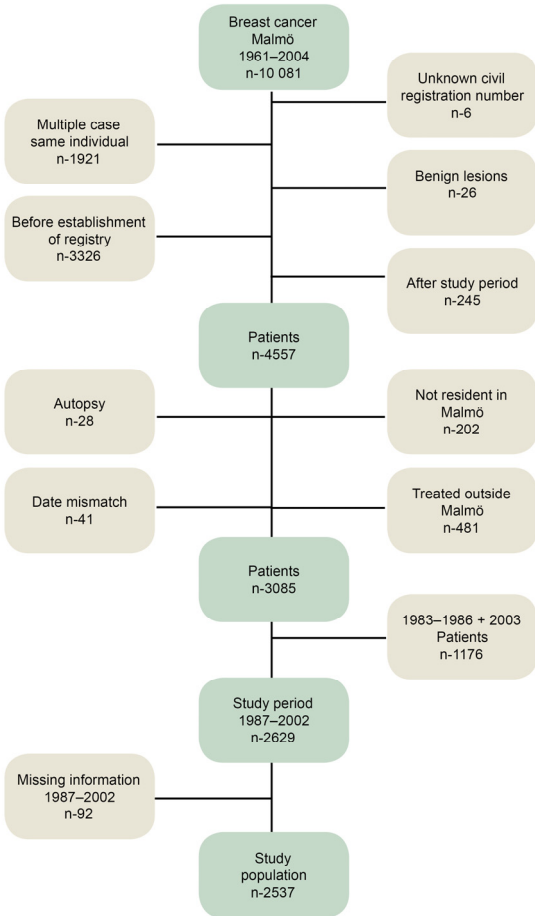


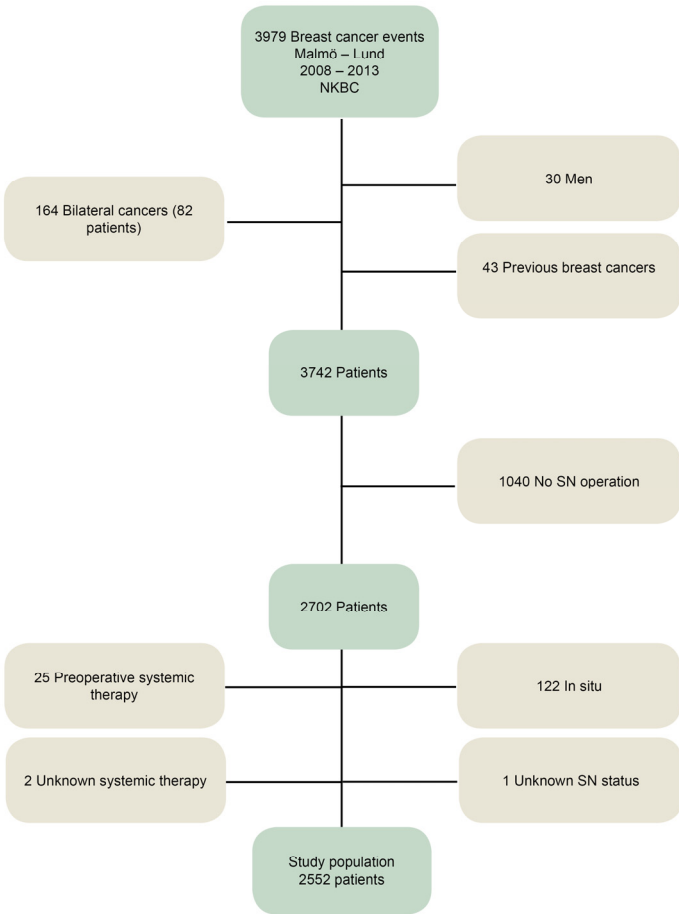
Figure 12.
Patient selection study I

Study II

This study included all patients with breast cancer in Malmö-Lund, who had undergone surgery between January 1st 2008 and December 31st 2013. A total number of 3979 cancer events were identified. Data were available in the NKBC registry. This registry manages various types of information about the cancer care as well as long-term follow up and has been in full operation since 2007. The country's regional cancer centres developed and run this registry jointly. The Regional Cancer Center in southern Sweden (RCC-Syd) manages NKBC in the southern region.

The following patients were not included in this study:

30 male patients, 82 patients with bilateral breast cancer (i.e.164 cancer events), 43 patients with a history of previous breast cancer, 1040 patients who had not undergone SNB, 122 patients with *in situ* carcinoma, 25 patients who had received systemic therapy pre-operatively, two patients with unknown status about systemic therapy, and one patient with unknown information about SN status. After exclusion of the above patients, the final study population included 2552 patients. All had undergone SNB (Fig. 13).



Figur 13.
Patient selection study II

Study III

This cohort included all women in Lund and Malmö with primary invasive breast cancer who had undergone SNB and c-ALND because of metastasis in the SN during the period of Jan 1st 2008 to Dec 31st 2013. These patients were collected from the NKBC registry and a total number of 3979 patients (cancer events) were identified.

The following patients were not included in this cohort:

122 patients with *in situ* cancer, 30 male patients. 82 patients (i.e.164 cancer events) with bilateral breast cancer, 43 patients with a history of previous breast cancer, 1040 patients who had not undergone SNB, 25 patients who had received

neoadjuvant therapy, two patients with unknown information about the systemic therapy, and one patient with unknown status regarding SN surgery.

After the above exclusions there were 1881 patients who had no metastases in SN and 671 patients with SN metastases. Out of the 671 patients there were 69 patients who had not undergone a c-ALND. The final study population consisted of the remaining 602 patients. All these patients had metastasis in the SN and had undergone c-ALND (Fig 14).

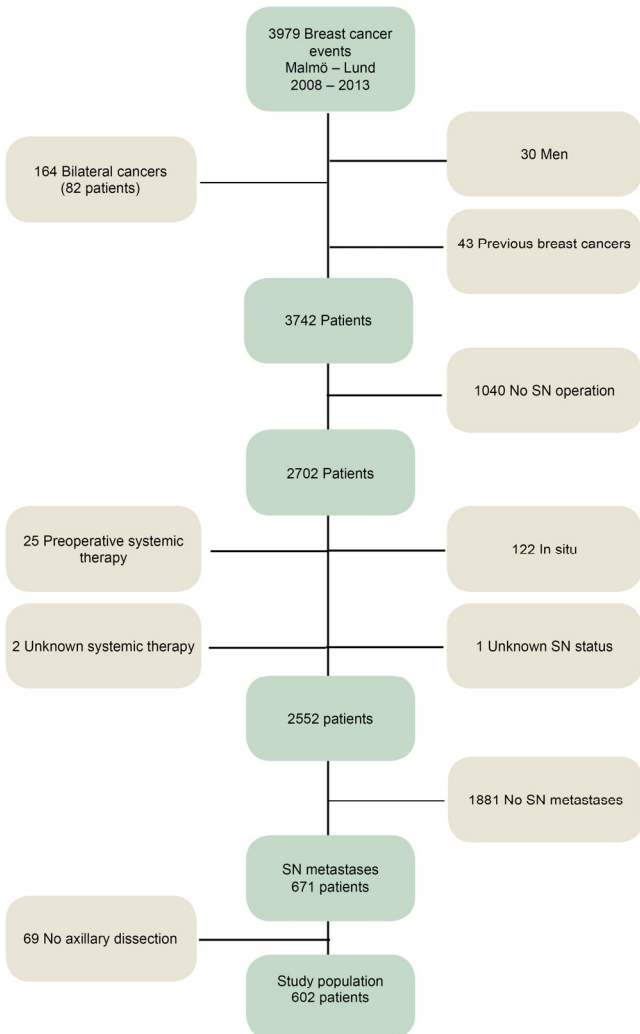


Figure 14.
Patient selection study III

Study IV

We used the same cohort as in studies I and II. We retrieved 3979 cancer events from NKBC These patients had primary invasive breast cancer and all were managed at SUS Malmö-Lund, during the period of Jan 1st 2008 to Dec 31st 2013.

The following patients were excluded from this study:

30 male patients, 126 patients with carcinoma *in situ*, 82 patients with bilateral breast cancer (164 cancer events), 43 patients with a history of previous breast cancer, 189 patients who had not undergone surgical axillary nodal staging (SN procedures), 256 patients with missing information about axillary staging status, 170 patients who had received neoadjuvant therapy, two patients with unknown status regarding systemic therapy, and 60 patients with missing axillary nodal status. The above exclusions resulted in a cohort consisting of 2939 patients. All had undergone an ALN procedure, i.e. 1801patients with SNB, 18 patients with ALN sampling and 1115 patients with ALND (Fig. 15).

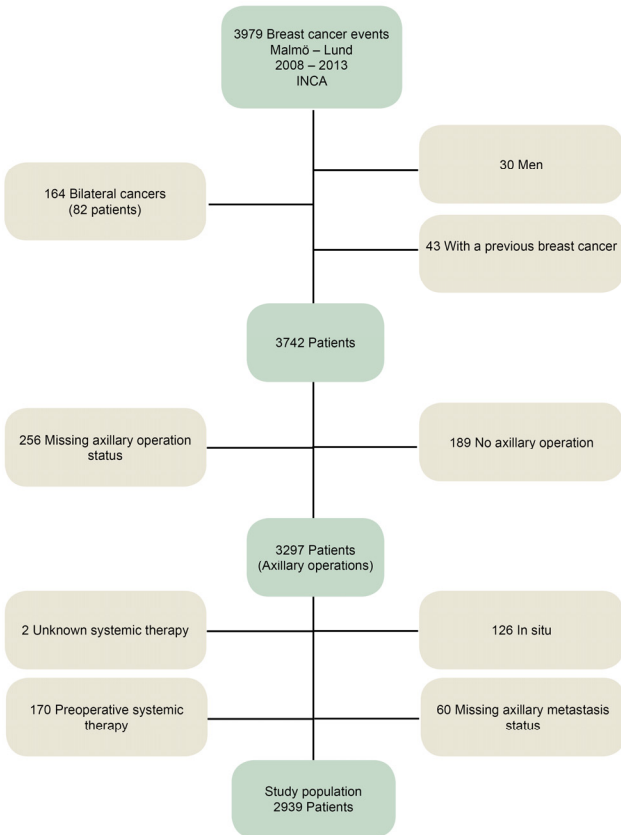


Figure 15.
Patient selection study IV

NKBC (National Quality register for Breast Cancer)

NKBC has been in full operation since 2008 [23]. The information is available on an IT platform called the Information Network for Cancer Care (INCA). All newly diagnosed primary cases of *in situ* and invasive breast cancer are reported to NKBC. The Swedish National Cancer Register contains information on the number of malignant lesions in each breast while reporting to NKBC occurs with only one malignant lesion for each breast. The coverage is almost 100%. Register data are updated twice yearly in an interactive report and are available on the NKBC web page https://statistik.incanet.se/brostcancer/ [23].

The care process is described from diagnosis to the first event of metastatic disease, recurrence (local, regional and distant) and/or death. Data about the guidelines sets by the Swedish Board of Health and Welfare (SoS), EUSOMA and EU. There is possibility of membership by patient representatives in NKBC [23]. All units that treat more than five patients annually with breast cancer are included in NKBC. Data are presented in interactive reports and are available as variables which are displayed in figures and in tables containing descriptions to assist with the interpretation of results. Quality indicators can be subdivided into structure (what is done), process (how it is done) and outcome. Data on coverage and lead-times is also recorded.

Data about the care process, systemic treatment (pre-and postoperative), radiotherapy, and endocrine therapy are reported when completed. The pathology report contains information about biopsy (pre-operative or pre-systemic treatment) or from the surgical specimen. Variables such as tumour size, tumour type, histological grade, hormonal receptor status and lymph node status are essential elements for the multidisciplinary recommendations regarding surgery, cytotoxic, endocrine therapies. Follow-up data are incomplete, which partly reflects differences in follow-up routines. Swedish national guidelines recommend yearly breast imaging for five years after diagnosis [1].

Surrogate definitions of intrinsic subtypes of breast cancer

The 12th St Gallen International Breast Cancer Conference in 2011 [121] adopted a new way of classification for breast cancer types based on the recognition of intrinsic biological subtypes. These subtypes were constructed by using clinicopathological rather than gene expression features. The purpose was mainly to facilitate the choice of appropriate systemic therapy. Five different subtypes were identified (Fig. 16) using hormonal status (oestrogen and/or progesterone), HER2 status and Ki-67 status. Luminal A and some Luminal B cancer require only endocrine therapy. Chemotherapy was considered to be indicated for most patients

with Luminal-B, HER2-positive, and TN cancer, with the addition of trastuzumab in HER2-positive cancer [121].

The 13th St Gallen International Breast Cancer Conference in 2013 [104] endorsed new evidence on aspects of therapies for early breast cancer, recommending less extensive surgery to the axilla and shorter radiation therapy. It refined its earlier classification and management of luminal cancer while retaining recommendations for the systemic adjuvant therapy of HER2-positive and TN breast cancer. The conventional clinicopathological factors provided again a surrogate subtype classification.

A cut-off level of ≥20 was recommended for high ki-67. There was no consideration of the histopathological grade of the tumour regarding the classification of the subtypes [104].This subtype of classification was used in the study IV (Fig. 16). Furthermore, the panel suggested that axillary dissection could be safely omitted in patients who had undergone BCS with one or two positive SNs with micro-metastases followed by whole breast RT [104]. ALND was also recommended for patients with three or more positive SNs or for patients with lymph node metastasis verified by biopsy before surgery [104].

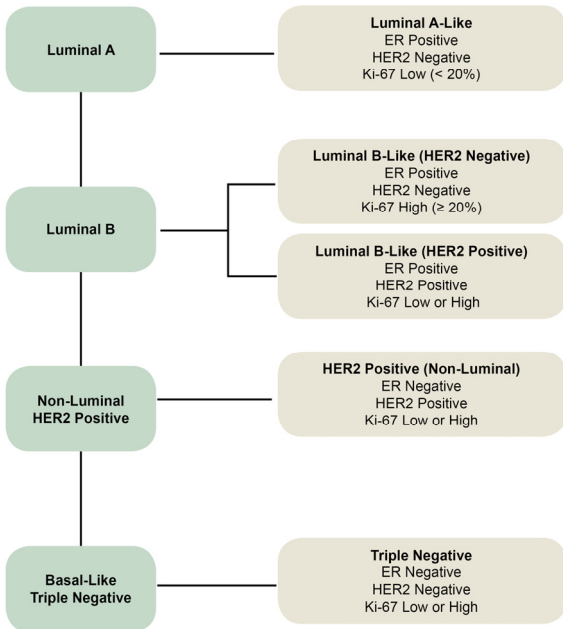


Figure 16.
Surrogate definitions of intrinsic subtypes according to St Gallen International Breast Cancer Conference 2013 [104]

Ethical Approval

Registry data might be subjected to lack of confidentiality with the risk for identification of the patient's identity and health status. This issue was managed in all studies by coding of the personal civil number for every patient and re-coding to an anonymous number. Moreover, before the launch of each study we published an announcement in the local newspaper about the aim of the study. Participation in the study was voluntary, with no obligation. It was possible for all patients to refuse participation in any study by contacting the author or co-authors. All participants who had further enquiries or who needed more information about the studies could contact the authors directly.
All studies were approved by the regional ethical review board.
Study I Dnr 615/2004.
Study II, III, IV Dnr 821/2013.

Statistical Analysis

Study I

We computed sensitivity, specificity, positive predicative value (PPV), negative predicative value (NPV), positive likelihood ratio (+LR) and negative likelihood ratio (-LR). We used on-line two-way contingency table analysis, and 95% confidence intervals (CIs), for the estimated parameters, were computed by a general method based on constant chi-square boundaries [152].

Palpable lymph nodes in axilla were regarded as positive status and axillary status was regarded as negative when no lymph nodes were detected by clinical examination. Lymph node status was regarded as positive when histopathological results showed metastasis and status was regarded as negative when histopathological examination showed no metastasis. The clinical status of the axilla was then compared with the histopathological status.

Tumour size was analysed in the same way, where tumour size > 20mm by palpation or by histopathological examination was regarded as positive status, while the status regarded as negative when the size was <20mm by palpation or histopathological examination. The tumour size by palpation was then compared to the size according to the histopathological examination.

The age group were divided into three different groups according to the menopausal status, i.e. pre-menopause (<50years), early post-menopause (≥50 - <70 years) and late post-menopause (≥70 years). Moreover, the results were calculated during two separate periods, an initial period from 1987 to 1994 and a late period of 1995 to 2002, to reveal any possible time-related variation during the study period.

Study II

For all analyses we used the Statistical Package for the Social Sciences (SPSS) program version 22.0 (SPSS Institute, Chicago, IL, USA).

First, we retrieved essential information about all the clinicopathological factors needed for the current study. These factors were age, screening, menopause status, tumour size, hormone receptor status, HER-2 status, histopathological type and grade, LVI and multifocality. Additionally, information about the size of SN metastasis was collected and we recorded SN with only ITC, as lymph nodes without metastatic involvement. Macro-metastases in the SN i.e. metastasis >2 mm and micro-metastases with a size of 0.2-2.0 mm were regarded as a positive lymph node, while all ITCs and lymph nodes without macro or micro-metastasis were regarded as negative lymph nodes.

Binary logistic regression was used to compare the association between potential predictors and metastasis in SNs. The analyses were also adjusted for all included study predictors i.e. multifocality, LVI, screening, age, menstrual status, tumour size, histopathological type and grade, HER-2 status and hormonal status for oestrogen and progesterone. ORs with 95% CIs were calculated. Analyses for centre A (Lund) and centre B (Malmö) were performed for each centre separately as well as for the two centres together.

Study III

The SPSS program version 22.0 (SPSS Institute, Chicago, IL, USA) was used in the current analysis to compare the association between the potential predictive factors and the risk for metastatic involvement of the non-SNs. All analyses were adjusted for screening, age, menstrual status, tumour size, histopathological type and grade, HER2 status, receptor status for oestrogen and progesterone, presence of multifocality and LVI. To compare associations among the number of the SNs, the size of metastases in SNs and the risk of metastases to non-SNs, binary logistic regression was used.

Statistically significant co-variates associated with metastases in non-SNs, e.g. screening, tumour types and multifocality, were included in the multivariate analysis as these analyses included a limited number of events. The analysis was also stratified for the number of SNs which had been removed by surgery. ORs with 95% CIs were calculated.

Study IV

In this study, we used SPSS version 25 for all analyses. As three major predictive factors i.e. LVI, Ki-67 and HER2 status were recorded with a high proportion of missing values, we used multiple imputation (MI). First, we identified the pattern of missing predictors in order to enter the potential variables into the model in the right order. Additionally, we included potential predictors of missingness, e.g. date of diagnosis, date of surgery and treating centres. Predictors that were included in

the multiple imputation were: mode of detection, age, menopausal status, tumour size, histological grade, ER, PR, HER2, LVI, multifocality and Ki-67 status (defined as low ≤ 20% and high >20%). We used 200 data set with 20 iterations. Linear regression was used to impute continuous variables and by applying the predictive mean matching (PMM) the categorical values were imputed.

We used the same classification, as proposed by the St. Gallen conference 2013 [104] for identification of the surrogate molecular subtypes and accordingly five subtypes were identified: Luminal A-like (LumA), Luminal B-like/HER2- negative (LumB/HER2–), Luminal B-like/HER2-positive (LumB/HER2+), HER2 positive /non-luminal (HER2+/non-luminal), and TN. The receiver operating characteristics (ROC) and the area under the curve (AUC) were calculated to achieve the discriminative performance of the nomogram for node-negative (N0) vs. node-positive (N+).

The ROC analysis was performed using the predicted probability (p) of ALN negativity – a monotone transformation of the linear predictor (LP) below. LP values for the patients in this validation cohort were calculated using the estimated regression coefficients underlying the SUS nomogram, figure 17 [21].

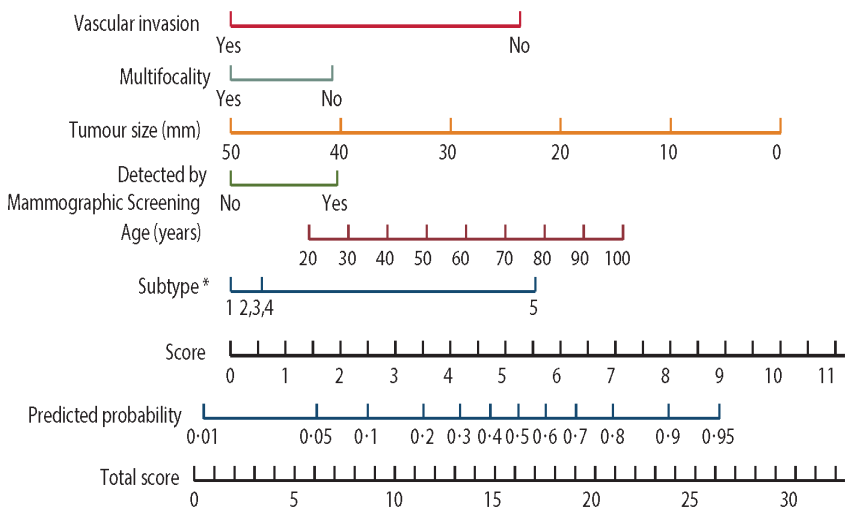


Figure 17.
The SUS Nomogram (a) N0 *versus* N+ by Dihge *et al.* [21]

The following formula was used;

LP = -1.79 + 0.17*S1 + 0.10*S2 + 0.39*S3 + 1.62*S4 + 0.021*(Age in years) + 0.56*Scr_det - 0.059*(Tumour size in mm) + 0.54*Abs_mf + 1.54*Abs_vi

where

S1 to S4 are dummy variables for four of the five St Gallen subtypes. Luminal A was chosen as the reference category.

S1 is 1 for Luminal B HER2- tumours; 0 otherwise
S2 is 1 for Luminal B HER2+ tumours; 0 otherwise
S3 is 1 for HER2+ non-luminal tumours; 0 otherwise
S4 if 1 for triple-negative tumours; 0 otherwise

Scr_det is 1 for screening detected tumours; 0 otherwise
Abs_mf is 1 if absence of multifocality; 0 otherwise
Abs_vi is 1 if absence of vascular invasion; 0 otherwise

Predictions of probability of N0 were subsequently calculated as:
p = exp (LP)/ (1+exp (LP)).

Mean and a 95% confidence interval for the AUC were calculated using the mean of all 200 estimates, and defining the 2.5 and the 97.5 percentiles by excluding the first five and the last five values of the data set including the 200 estimates. The recording methods for the HER2 status were not identical in the two centres and this issue indicated the necessity to conduct a sensitivity test which was performed by re-coding the missing HER2 amplification status into “negative” (See section statistical and methodological considerations, page 80).

The predictive probability was sub-classified into ten groups and compared with the proportion of patients with observed metastases to test the predictive accuracy of the nomogram, and finally a calibration diagram was constructed for each centre.

Main results

Study I

Out of 2537 patients there were 674 patients with ALN metastasis according to the final histopathological report but only 206 patients had a palpable lymph node in the axilla. The sensitivity was 30%, the specificity 93%, the PPV 76%, and the NPV 67%. For all age groups, during the whole study period the sensitivity was low and specificity was high (Table1).

Table 1.
Clinical axillary lymph node involvement (ALNI) compared to ALNI according to the histopathological examination. All measurements with a 95% confidence interval (CI).

Group		Histopathological ALNI		Validity measurements					
	Clinical ALNI (n)	Negative (n)	Positive (n)	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)	+LR (CI)	–LR (CI)
All	Negative	967	468	30 (28-32)	93 (92-95)	76 (71-81)	67 (66-68)	5.1 (3.9-6.6)	0.73 (0.71-0.77)
	Positive	62	206						
Age (years)									
<50	Negative	149	94	33 (28-36)	93 (89-95)	81 (70-88)	61 (58-63)	4.8 (2.6-8.9)	0.71 (0.66-0.79)
	Positive	11	47						
50-70	Negative	547	224	26 (23-28)	95 (93-96)	73 (65-80)	70 (69-71)	5.3 (3.5-8.1)	0.77 (0.74-0.82)
	Positive	28	79						
>70	Negative	271	150	34 (31-37)	92 (88-94)	77 (69-84)	64 (62-66)	4.4 (3.0-6.8)	0.70 (0.66-0.77)
	Positive	23	80						
Period									
1987-1994	Negative	497	216	32 (29-35)	93 (91-94)	73 (66-79)	69 (68-70)	4.6 (3.3-6.6)	0.72 (0.68-0.77)
	Positive	37	103						
1995-2002	Negative	470	252	29 (26-31)	94 (93-96)	80 (73-86)	74 (63-66)	5.7 (3.8-8.6)	0.74 (0.71-0.79)
	Positive	25	103						

There were 812 patients with a tumour size >20mm by histopathological examination but only 665 of these tumours were larger than 20 mm by palpation. The sensitivity was 81%, specificity was 80%, PPV was 72.0%, and NPV was 87%. Patients older than 70 years had a higher sensitivity for tumour size measurement by clinical examination whereas specificity for these patients was lower compared with the patient group younger than 70 years. Similar results were observed during the entire study period (Table 2).

Table 2.
Clinical size compared to size according to the histopathological examination. All measurements with a 95% confidence interval (CI).

Group	Clinical size (n)	Histopathological size		Validity measurements					
		<20mm (n)	>20mm (n)	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)	+LR (CI)	–LR (CI)
All	<20mm	1020	147	81 (79-83)	80 (79-81)	72 (71-74)	87 (85-88)	4.2 (3.8-4.6)	0.22 (0.20-0.26)
	>20mm	246	665						
Age (years)									
<50	<20mm	162	31	77 (72-82)	80 (76-83)	72 (67-77)	83 (80-87)	4.0 (3.0-5.0)	0.30 (0.20-0.36)
	>20mm	40	108						
50-70	<20mm	635	79	74 (70-77)	86 (85-88)	70 (66-73)	88 (87-90)	5.6 (4.7-6.6)	0.30 (0.25-0.35)
	>20mm	97	227						
>70	<20mm	223	37	89 (87-92)	67 (64-69)	75 (72-77)	85 (81-89)	2.7 (2.4-3.0)	0.15 (0.11-0.21)
	>20mm	109	330						
Period									
1987-1994	<20mm	466	62	83 (80-86)	79 (77-81)	72 (69-74)	88 (86-90)	4.0 (3.5-4.5)	0.20 (0.16-0.25)
	>20mm	122	319						
1995-2002	<20mm	554	85	80 (77-83)	81 (79-83)	73 (70-76)	85 (84-86)	4.3 (3.8-5.0)	0.24 (0.20-0.28)
	>20mm	124	346						

Study II

There were 1262 patients at centre A and 1290 patients at centre B. Out of 2552 patients at both centres there were 671 patients with SN metastasis (26.3%), distributed as 374 patients (29.6%) at centre A and 297 patients (23%) at centre B. The number of T1 tumours with positive SN was higher at centre B (80.1%) compared with centre A (67.3%) table 3, 5. Tumours detected by screening had lower risk of SN metastasis compared with tumours not diagnosed by screening (0.63; 0.51-0.80). There was a low risk of SN metastasis in cases with negative oestrogen receptor status (0.64; 0.42-0.99) when analysis was performed for both centres, but there was no statistically significant association between hormonal status and risk of SN metastasis when analysis was performed for each centre separately. There was a clear association between tumour size and risk of finding SN metastasis where T2, T3, and T4 had a higher risk of SN involvement compared with T1, with an OR of 1.84 for T2 and 2.56 for T3 and T4. Sub-classes of T1 showed the same pattern, where T1a had the lowest risk of metastasis (with an OR of 0.19 followed by T1b with an OR of 0.46) compared with T1c. The risk of SN metastasis was not high for T3 and T4 at centre A when analysis was performed separately, whereas the risk remained still high at centre B (Table 4, 5).

Rare tumour types, e.g. medullary breast cancer where observed to be associated with low risk of SN metastasis, with an OR of 0.29. Both multifocality and LVI were associated with a high risk of SN metastasis at both centres. The strongest predictive factor was observed to be LVI with an OR of 6.10 for centre A and 3.04 for centre B (Table 5).

In general, almost identical numbers of SN procedures were performed in both centres and there were no large differences in results between centre A and centre B.

Table 3.
Potential predictive factors in relation to SN status.

Determinants	Category	Total	SN Negative		SN Positive	
			N	%	N	%
Screening	No	1062	719	38.2	343	51.1
	Yes	1435	1123	59.7	312	46.5
	Unknown	55	39	2.1	16	2.4
Age	≤50	499	337	17.9	162	24.1
	51-74	1702	1290	68.6	412	61.4
	≥75	351	254	13.5	97	14.5
Menopause Status	Pre	512	348	18.5	164	24.4
	Post < 5 ys	228	168	8.9	60	8.9
	Post ≥ 5 ys	1721	1299	69.1	422	62.9
	Unknown	91	66	3.5	25	3.7
Tumour size	T1	1505	1138	60.5	367	54.7
	T2	559	346	18.4	213	31.7
	T3 & T4	25	13	0.7	12	1.8
	Unknown	463	384	20.4	79	11.8
Tumour type	Ductal	1866	1324	70.4	542	80.8
	D & L	52	35	1.9	17	2.5
	Lobular	304	216	11.5	88	13.1
	Other	330	306	16.3	24	3.6
Histological grade	I	622	488	25.9	134	20.0
	II	1112	807	42.9	305	45.5
	III	790	563	29.9	227	33.8
	Unknown	28	23	1.2	5	0.7
Estrogen receptor	Positive	2144	1538	81.8	606	90.3
	Negative	279	218	11.6	61	9.1
	Unknown	129	125	6.6	4	0.6
Progesterone receptor	Positive	1851	1320	70.2	531	79.1
	Negative	571	436	70.2	531	79.1
	Unknown	130	125	6.6	5	0.7
HER-2 status	Negative	1466	1041	55.3	423	63.0
	Positive	240	174	9.3	66	9.8
	Unknown	848	666	35.4	182	27.1
Multifocality	No	1570	1184	62.9	386	57.5
	Yes	352	208	11.1	144	21.5
	Unknown	630	489	26.0	141	21.0
Vascular invasion	No	1324	1056	56.1	268	39.9
	Yes	184	87	4.6	97	14.5
	Unknown	1044	738	39.2	306	45.6

Table 4.
Potential predictive factors and risk of SN metastases.

Determinants	Category	SN Negative	SN Positive	OR 95 % CI	OR 95 % CI*
Screening	No	719	343	1.00	1.00
	Yes	1123	312	0.59(0.49-0.70)	0.63(0.51-0.80)
	Unknown	39	16	0.86(0.48-1.57)	0.88(0.46-1.66)
Age	≤50	337	162	1.00	1.00
	51-74	1290	412	0.67(0.53-0.82)	0.92(0.63-1.37)
	≥75	254	97	0.80(0.59-1.08)	0.70(0.42-1.11)
Menopause Status	Pre	348	164	1.00	1.00
	Post < 5ys	168	60	0.76(0.53-1.08)	0.98(0.62-1.53)
	Post ≥ 5ys	1299	422	0.69(0.56-0.86)	0.82(0.56-1.22)
	Unknown	66	25	0.80(0.49-1.32)	0.89(0.50-1.53)
Tumour size	T1	1138	367	1.00	1.00
	T2	346	213	1.91(1.56–2.34)	1.84(1.47–2.33)
	T3 & T4	13	12	2.87(1.30–6.32)	2.56(1.07–6.09)
	Unknown	384	79	0.63(0.49-0.83)	0.67(0.50-0.93)
Tumour type	Ductal	1325	542	1.00	1.00
	D & L	35	17	1.19(0.66-2.13)	1.01 (0.54-1.90)
	Lobular	217	88	1.00(0.77-1.30)	0.87(0.64-1.20)
	Others	306	24	0.20(0.12-0.30)	0.29(0.18-0.46)
Histological grade	I	488	134	1.00	1.00
	II	807	305	1.37(1.09-1.73)	1.02(0.80-1.31)
	III	563	227	1.46(1.14-1.87)	1.10(0.82-1.50)
	Unknown	23	5	0.79(0.29-2.12)	1.40(0.46-4.31)
Estrogen receptor	Positive	1538	606	1.00	1.00
	Negative	218	61	0.71(0.52-0.96)	0.64(0.42-0.99)
	Unknown	125	4	0.09(0.03-0.22)	0.06(0.00-0.82)
Progesterone receptor	Positive	1320	531	1.00	1.00
	Negative	436	135	0.77(0.61-0.96)	0.78(0.56-1.07)
	Unknown	125	5	0.10(0.04-0.24)	3.80(0.30-47.42)
Her-2 status	Negative	1041	423	1.00	1.00
	Positive	174	66	0.93(0.69-1.27)	0.84(0.60-1.20)
	Unknown	666	182	0.68(0.56-0.82)	0.98(0.78-1.24)
Multifocality	No	1184	386	1.00	1.00
	Yes	208	144	2.12(1.67-2.70)	1.90(1.45-2.47)
	Unknown	489	141	0.89(0.71-1.10)	0.86(0.67-1.09)
Vascular invasion	No	1056	268	1.00	1.00
	Yes	87	97	4.40(3.20-6.04)	3.74(2.66-5.27)
	Unknown	738	306	1.63(1.36-1.98)	2.10(1.68-2.62)

Table 5.
Potential predictive factors and risk of SN metastases separately for Center A & Center B.

Determinants	Category	Center A			Center B		
		SN positive	SN negative	OR 95 % CI	SN positive	SN negative	OR 95 % CI
Screening	No	297	179	1.00	422	164	1.00
	Yes	552	179	0.62(0.46-0.86)	571	133	0.63(0.47-0.88)
	Unknown	39	16	0.82(0.42-1.60)	-	-	-
Age	≤50	157	86	1.00	180	76	1.00
	51-74	620	237	1.02(0.59-1.78)	670	175	0.82(0.48-1.44)
	≥75	111	51	0.69(0.34-1.39)	143	46	0.69(0.34-1.37)
Menopause status	Pre	167	90	1.00	181	74	1.00
	Post < 5yr	91	29	0.67(0.34-1.29)	77	31	1.32(0.70-2.50)
	Post ≥5yr	589	238	0.88(0.50-1.52)	710	184	0.77(0.43-1.34)
	Unknown	41	17	0.81(0.40-1.67)	25	8	0.87(0.34-2.16)
Tumour size	T1	394	191	1.00	744	176	1.00
	T2	136	105	1.63(1.16–2.30)	210	108	2.13(1.54–2.94)
	T3 & T4	4	7	6.28(1.50–26.40)	9	5	1.48(0.44-4.90)
	Unknown	354	71	0.60(0.42-0.84)	30	8	1.70(0.72-4.04)
Tumour type	Ductal	620	306	1.00	704	236	1.00
	D & L	25	12	0.89(0.42-1.88)	10	5	1.20(0.38-3.79)
	Lobular	80	45	0.90(0.58-1.40)	136	43	0.84(0.54-1.30)
	Others	163	11	0.23(0.11-0.50)	143	13	0.30(0.17-0.57)

Histological grade	I	218	66	1.00	270	68	1.00
	II	363	169	1.31(0.91-1.89)	444	136	0.80(0.56-1.16)
	III	290	136	1.49(0.98-2.26)	273	91	0.80(0.51-1.24)
	Unknown	17	3	1.30(0.31-5.37)	6	2	2.38(0.36-15.87)
Estrogen receptor	Positive	679	335	1.00	859	271	1.00
	Negative	98	35	0.59(0.33-1.04)	120	26	0.72(0.38-1.40)
	Unknown	111	4	0.07(0.00-1.04)	14	-	-
Progesterone receptor	Positive	578	290	1.00	742	241	1.00
	Negative	199	79	0.71(0.48-1.08)	237	56	0.79(0.50-1.23)
	Unknown	111	5	4.15(0.31-54.96)	14	-	-
HER-2	Negative	630	292	1.00	411	131	1.00
	Positive	77	44	1.04(0.66-1.64)	97	22	0.63(0.37-1.10)
	Unknown	181	38	0.93(0.59-1.50)	485	144	1.04(0.77-1.42)
Multifocality	No	452	194	1.00	732	192	1.00
	Yes	93	71	1.58(1.07-2.32)	115	73	2.21(1.50-3.23)
	Unknown	343	109	0.72(0.53-0.99)	146	32	0.88(0.56-1.40)
Vascular invasion	No	148	41	1.00	908	227	1.00
	Yes	18	34	6.10(2.98-12.50)	69	63	3.04(2.03-4.57)
	Unknown	722	299	1.64(1.10-2.44)	16	7	1.80(0.66-4.93)

Study III

This cohort included 602 patients, all operated on with c-ALND. There were 391 patients without metastasis in non-SN (65%) and 211 patients with metastasis in non-SN (35%) table 6. There was a higher risk of finding metastasis in patients with lobular type compared to ductal type tumours (1.73; 1.01-2.97). There were 11 patients who had unknown status for mode of tumour detection. These patients had high risk of non-SN metastasis (4.70; 1.36–16.19). Multifocal tumours had a high risk of metastasis compared with unifocal (2.20; 1.41-3.44) table 7. Finding macro-metastasis in SN was associated with involvement of metastasis in non-SNs. The number of SNs removed by surgeon had no impact on the presence of metastasis in the non-SNs. The number of SNs with macro-metastasis has a positive impact on finding non-SNs with metastasis (Table 8, 9). There was no correlation between other predictive factors such as hormonal status, histological grade, tumour size, LVI and the risk of non-SNs involvement (Table 7).

Table 6.
Potential determinants in relation to non-sentinel node status.

Determinants	Category	Total	Negative Non-SN		Positive Non-SN	
			N	%	N	%
Screening	No	309	197	50.4	112	53.1
	Yes	278	190	48.6	88	41.7
	Unknown	15	4	1.0	11	5.2
Age	≤50	151	103	26.3	48	22.7
	51-74	370	239	61.1	131	62.1
	≥75	81	49	12.5	32	15.2
Menopause Status	Pre	153	104	26.6	49	23.2
	Post < 5 ys	57	42	10.7	15	7.1
	Post ≥ 5 ys	370	231	59.1	139	65.9
	Unknown	22	14	3.6	8	3.8
Tumour size	T1	331	220	56.3	111	52.6
	T2	193	120	30.7	73	34.6
	T3 & T4	10	6	1.5	4	1.9
	Unknown	68	45	11.5	23	10.9
Tumour type	Ductal	490	331	84.7	159	75.4
	D & L	14	5	1.3	9	4.3
	Lobular	79	41	10.5	38	18.0
	Other	19	14	3.6	5	2.4
Histological grade	I	118	82	21.0	36	17.1
	II	272	176	45.0	96	45.5
	III	210	132	33.8	78	37.0
	Unknown	2	1	0.3	1	0.5
Estrogen receptor	Positive	545	358	91.6	187	88.6
	Negative	56	33	8.4	23	10.9
	Unknown	1	0	0.0	1	0.5
Progesterone receptor	Positive	478	319	81.6	159	75.4
	Negative	122	72	18.4	50	23.7
	Unknown	2	0	0.0	2	0.9
HER2 status	Negative	383	248	63.4	135	64.0
	Positive	61	33	8.4	28	13.3
	Unknown	158	110	28.1	48	22.7
Multifocality	No	355	247	63.2	108	51.2
	Yes	129	63	16.1	66	31.3
	Unknown	118	81	20.7	37	17.5
Vascular invasion	No	241	166	42.5	75	35.5
	Yes	91	60	15.3	31	14.7
	Unknown	270	165	42.2	105	49.8

Table 7.
Potential determinants for non-sentinel node metastases.

Determinants	Category	Negative Non-SN	Positive Non-SN	OR 95 % CI	OR 95 % CI*
Screening	No	197	112	1.00	1.00
	Yes	190	88	0.81 (0.58-1.15)	0.81 (0.54-1.21)
	Unknown	4	11	4.84 (1.50–15.55)	4.70 (1.36–16.19)
Age	≤50	103	48	1.00	1.00
	51-74	239	131	1.18 (0.79-1.76)	1.50 (0.53-2.06)
	≥75	49	32	1.40 (0.80-2.46)	1.08 (0.45-2.60)
Menopause Status	Pre	104	49	1.00	1.00
	Post < 5ys	42	15	0.76 (0.38-1.50)	0.79 (0.34-1.86)
	Post ≥ 5ys	231	139	1.28 (0.86-1.90)	1.21 (0.60-2.44)
	Unknown	14	8	1.21 (0.48-3.08)	1.45 (0.52-4.05)
Tumour size	T1	220	111	1.00	1.00
	T2	120	73	1.21 (0.83–1.74)	1.11 (0.74–1.66)
	T3 & T4	6	4	1.32 (0.36–4.78)	0.78 (0.19–3.14)
	Unknown	45	23	1.01 (0.58-1.76)	0.76 (0.40-1.44)
Tumour type	Ductal	331	159	1.00	1.00
	D & L	5	9	3.75 (1.24-11.36)	2.93 (0.92-9.37)
	Lobular	41	38	1.93 (1.19-3.12)	1.73 (1.01-2.97)
	Others	14	5	0.74 (0.26-2.10)	0.85 (0.29-2.50)
Histological grade	I	82	36	1.00	1.00
	II	176	96	1.24 (0.78-1.98)	0.88 (0.53-1.46)
	III	132	78	1.35 (0.83-2.18)	0.94 (0.54-1.65)
	Unknown	1	1	2.28 (0.14-37.43)	1.23 (0.07-21.34)
Estrogen receptor	Positive	358	187	1.00	1.00
	Negative	33	23	1.33 (0.76-2.34)	1.04 (0.47-2.34)
	Unknown	0	1	-	-
Progesterone receptor	Positive	319	159	1.00	1.00
	Negative	72	50	1.40 (0.93-2.09)	1.17 (0.66-2.07)
	Unknown	0	2	-	-
Her-2 status	Negative	248	135	1.00	1.00
	Positive	33	28	1.56 (0.90-2.69)	1.52 (0.82-2.82)
	Unknown	110	48	0.80 (0.54-1.19)	0.88 (0.55-1.39)
Multifocality	No	247	108	1.00	1.00
	Yes	63	66	2.40 (1.59-3.62)	2.20 (1.41-3.44)
	Unknown	81	37	1.04 (0.67-1.64)	0.99 (0.61-1.60)
Vascular invasion	No	166	75	1.00	1.00
	Yes	60	31	1.14 (0.68-1.91)	1.13 (0.64-1.98)
	Unknown	165	105	1.41 (0.98-2.03)	1.31 (0.86-1.99)

* Adjusted for screening, age, menopause status, tumour size, tumour type, histological grade, estrogen status, progesterone status, HER2 status, multifocality, lymphovascular invasion.

Table 8.
Number and type of metastases in sentinel node and risk of metastases in non-sentinel node.

SN	Category	Total (n)	Negative Non-SN (n)	Positive Non-SN (n)	Positive Non-SN (%)	OR (95% CI)	OR* (95% CI)
SN removed (n)	1	118	84	34	28.8	1.00	1.00
	2	208	125	83	39.9	1.64 (1.01–2.66)	1.34 (0.77-2.31)
	3	166	110	56	33.7	1.26 (0.75-2.10)	1.08 (0.61-1.93)
	4	83	56	27	32.5	1.19 (0.65-2.19)	0.96 (0.48-1.90)
	≥5	25	15	10	40.0	1.65 (0.67-4.03)	1.71 (0.65-4.53)
	Unknown	2	1	1	-	-	-
	Total	602	391	211			
Size of metastases in SN**	Micro	186	159	27	14.5	1.00	1.00
	Macro	414	232	182	43.9	4.62 (2.94–7.26)	4.91(3.01–8.05)
	Unknown	2	0	2	-	-	-
	Total	602	391	211			

*Adjusted for screening, age, menopause, tumour size, tumour type, histological grade, estrogen receptors, progesterone receptors, HER2, multifocality and lymphovascular invasion.

**If both micro- and macro-metastases, classified as macro-metastases.

Table 9.
Number of macrometastases in sentinel node and risk of metastases in non-sentinel nodes.

SN removed	Macro-metastases	Total	Negative Non-SN	Positive Non-SN	Positive Non-SN	Stratified analysis		Combined analysis	
(n)	(n)		(n)	(n)	(%)	OR (95% CI)	OR (95% CI)*	OR (95% CI)	OR (95% CI)*
1	0	47	39	8	17.0	1.00	1.00	1.00	1.00
	1	69	44	25	36.2	2.77 (1.12-6.85)	2.65 (1.05-6.66)	2.77 (1.12-6.85)	2.65 (1.05-6.66)
	Unknown	2	1	1	-	-	-	-	-
2	0	51	45	6	11.7	1.00	1.00	0.65 (0.21-2.04)	0.65 (0.21-2.07)
	1	105	60	45	42.8	5.62 (2.21-14.33)	4.83 (1.87-12.49)	3.66 (1.56-8.58)	3.09 (1.30-7.39)
	2	52	20	32	61.5	12.00 (4.33-33.23)	11.12 (3.97-31.19)	7.80 (3.03-20.04)	7.43 (2.83-19.50)
3	0	59	51	8	13.5	1.00	1.00	0.76 (0.26-2.22)	0.68 (0.23-2.02)
	1	58	39	19	32.7	3.11 (1.23-7.83)	3.68 (1.32-10.24)	2.37 (0.93-6.07)	2.15 (0.82-5.64)
	2	28	14	14	50.0	6.37 (2.23-18.23)	6.30 (1.99-19.99)	4.87 (1.69-14.10)	4.18 (1.40-12.50)
	3	21	6	15	71.4	15.94 (4.77-53.18)	16.96 (4.42-65.12)	12.19 (3.62-41.05)	10.02 (2.89-34.81)
4	0	24	21	3	12.5	1.00	1.00	0.70 (0.17-2.91)	0.57 (0.13-2.49)
	1	29	22	7	24.1	2.23 (0.51-9.77)	3.34 (0.55-20.15)	1.55 (0.50-4.85)	1.56 (0.49-4.94)
	2	11	7	4	36.3	4.00 (0.71-22.43)	9.46 (1.26-70.85)	2.79 (0.66-11.82)	2.92 (0.67-12.65)
	3	14	4	10	71.4	17.50 (3.28-93.49)	17.18 (2.34-126.2)	12.19 (3.04-48.77)	9.25 (2.22-38.53)
	4	5	2	3	60.0	-	-	-	-

* Adjusted for screening, tumour type, and multifocality.

Study IV

There were 2939 patients eligible for this study: in Lund there were 1318 patients and in Malmö 1612 patients. We found 1008 patients with axillary metastasis (34.3%) and 1931 patients without metastasis in the ALNs (65.7%). The mean age was 62.4 years and mean tumour size was 18.5 cm (Table 10). In Malmö, the AUC was 0.75 and in Lund, it was 0.73, the latter being almost identical to the original value (0.74), figure 17. This validation was conducted for the entire study period i.e. 2007-2013. The AUC value, conducted for the same period as the original study i.e. 2008-2012, was exactly identical (Table11). The calibration diagram for each centre separately showed a good agreement between the predictive probability and the observed metastases for both centres (Fig.18).

HER2 and LVI missing values were distributed differently in Malmö and Lund. In Malmö, the number of HER2-negative cases was low (39%) compared with Lund (72.5%). Moreover, the proportion of LVI cases in Malmö (84.6%) was higher compared with Lund (14.7%). The number of recorded missing LVI values in Malmö was low (2%) compared with Lund (80.6%). In general, the results were almost similar in both centres (Table 10).

Table 10.
Predictors and missing values.

Predictors	Category	Lund-Malmö		Lund		Malmö	
		n	%	n	%	n	%
Age (Year)	*Mean (Range)*	*62 (24–96)*		*62 (24–91)*		*62 (29–96)*	
Menopause status	Pre	602	20.5	268	20.3	334	20.6
	Post (6 months-5 years)	250	8.5	116	8.8	134	8.3
	Post (>5 years)	1983	67.5	873	66.2	1110	68.5
	Missing	104	3.5	61	4.6	43	2.7
Screening	No	1360	46.3	523	39.7	837	51.6
	Yes	1518	51.7	734	55.7	784	48.4
	Missing	61	2.1	61	4.6	0	0
Tumour size	T1a	121	4.1	60	4.6	61	3.8
	T1b	498	16.9	228	17.3	270	16.7
	T1c	1258	42.8	552	41.9	706	43.6
	T2	860	29.3	338	25.6	522	32.2
	T3-T4	61	2.1	22	1.7	39	2.4
	Missing	141	4.8	118	9.0	23	1.4
Histological grade	I	649	22.1	281	21.3	368	22.7
	II	1258	42.8	556	42.2	702	43.3
	III	993	33.8	457	34.7	536	33.1
	Missing	39	1.3	24	1.8	15	0.9
Estrogen receptor	Positive	2444	83.2	1062	80.6	1382	85.3
	Negative	366	12.5	146	11.1	220	13.6
	Missing	129	4.4	110	8.3	19	1.2
Progesterone receptor	Positive	2088	71.0	900	68.3	1188	73.3
	Negative	720	24.4	307	23.3	413	25.5
	Missing	131	4.5	111	8.4	20	1.2
HER2	Not amplified	1587	54.0	955	72.5	632	39.0
	Amplified	312	10.6	129	9.8	183	11.3
	Missing	1040	35.4	234	17.8	806	49.7
Ki-67	Low	82	2.8	35	2.7	47	2.9
	Medium	150	5.1	58	4.4	92	5.7
	High	233	7.9	70	5.3	163	10.1
	Missing	2474	84.2	1155	87.6	1319	81.4
Mutifocality	No	1804	61.4	679	51.5	1125	69.4
	Yes	480	16.3	180	13.7	300	18.5
	Missing	655	22.3	459	34.8	196	12.1
Vasscular invasion	No	1565	53.2	194	14.7	1371	84.6
	Yes	280	9.5	62	4.7	218	13.4
	Missing	1094	37.2	1062	80.6	32	2.0
Axillary metastasis	No	1931	65.7	856	64.9	1075	66.3
	Yes	1008	34.3	462	35.1	546	33.7
Axillary procedure	SNB	1801	61.3	833	63.2	968	59.7
	Sampling	18	0.6	12	0.9	6	0.4
	ALND	1115	37.9	471	35.2	644	39.7
	Missing	5	0.2	2	0.2	3	0.2
Total		**2939**		**1318**		**1621**	

Table 11.
AUC Lund and Malmö with 95% confidence intervals.

Centre	Period	AUC	AUC (sensitivity analysis*)
Lund – Dihge original (REF)	2009-12	0.74 (0.70 – 0.79)	-
Lund (current study – Dihge period)	2009-12	0.74 (0.73 – 0.75)	-
Lund (current study– entire period)	2008-13	0.75 (0.73 – 0.77)	0.75 (0.73 – 0.77)
Malmö (current study– entire period)	2008-13	0.73 (0.72 – 0.74)	0.73 (0.72 – 0.73)

*AUC with HER2 sensitivity analysis based on re-coded missing HER2 status to HER2 negative.

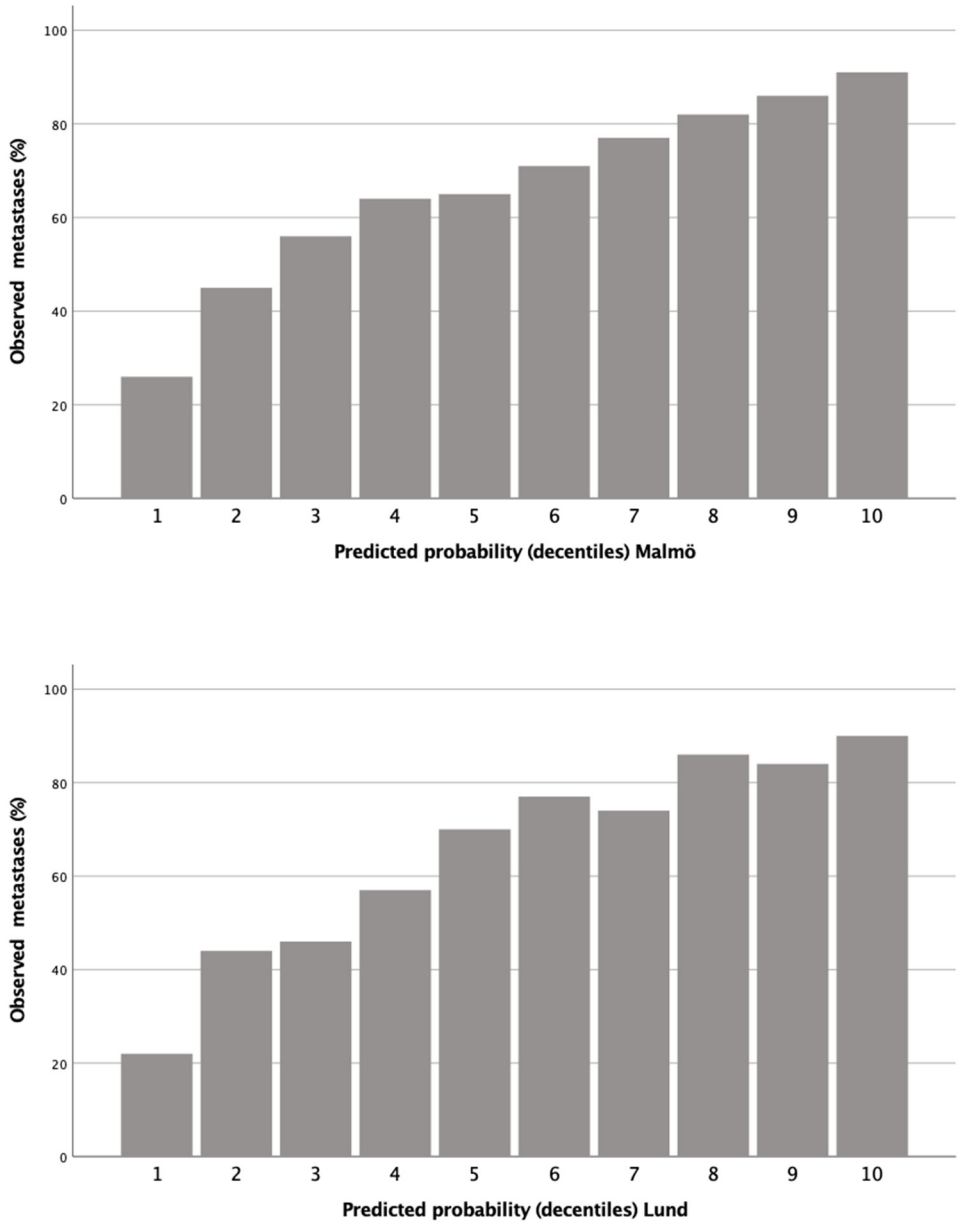


Figure 18.
Calibration diagram for Malmö and Lund.

Discussion

Study I

Out of all cases with axillary metastasis which were confirmed by histopathological examination there were only 30% were found to have palpable lymph nodes by physical examination. This result is consistent with other studies [153, 154].

Assessment of axillary lymph node status by clinical examination is difficult. This study showed a sensitivity of 30% and a specificity of 93%. This difficulty is possibly due to many factors. Normal lymph nodes may vary in consistency, shape, and there is also variation in the amount of fatty tissue they contain [155-157]. The presence of enlarged lymph nodes in the axilla might be a clinical presentation of other pathological conditions other than malignancies. Benign conditions such hidradenitis, myositis, and arthritis in the axillary region might be associated with lymphadenopathy. Performance of invasive investigations e.g. (FNB) and/or Core Biopsy (CB) might also be associated with reactive lymph adenopathy [157]. The experience of the clinician who performs the axillary examination has also been debated and it has been reported that accuracy in assessment of the axillary status by physical examination is subjected to misinterpretation [153]. In this study, we had no possibility of identifying the physician who had accomplished the physical examination as the research was performed using register data. This issue should be considered regarding the interpretation of the study results. Although the physical examination has a low sensitivity according to our study, there are still some restricted benefits and it can be regarded as a useful tool in clinical praxis. Physical examination is a simple and cheap method for assessment of the ALN status. Although there are different methods of assessing the ALN status for possible metastatic involvement, such as mammography, ultrasonography, MRI and CT, there is still a need for clinical assessment of the ALN status pre-operatively as primary physical examination is essential to determine the need for further investigations.

At the time of the study, patients with negative axillary palpation were not subjected to further investigation. Nowadays, axillary examination by ultrasonography is included in routine imaging processes and the possibility of ALN involvement with metastasis should be considered in patients with breast cancer even without physical findings in the axilla. Approximately 35-40% of patients with breast cancer presented with a lump in the breast [23]. In all these cases, clinical examination is regarded as the first step and as a cornerstone of the triple diagnostic concept for

achieving the diagnosis of breast cancer. Triple diagnostic strategy is composed of clinical examination of the breast, imaging and histopathological examination (FNB and/or CB). This study has shown that clinical examination by palpation of the mammary gland has a high specificity for tumours larger than 20 mm in early pre-menopausal women.

The indication for the use of neoadjuvant chemotherapy has been changed during the last decade, and according to the Swedish National Guidelines [1] for breast cancer a tumour size >20 mm in combination with other tumour characteristics such as high ki-67, TN, HER2 positive tumours and axillary involvement, are regarded as possible indications for neoadjuvant chemotherapy [1]. However, the tumour size is usually estimated by using a combination of clinical assessment and imaging modalities such as mammography and ultrasonography which give a better estimation. Moreover, the tumour size is an important factor in cases where BCS is used, where a sufficient tumour-free margin is essential. Overestimation of the tumour size was observed in our study in women aged >70 years. A possible explanation is the density of the breast in post-menopausal women, which is usually low compared with pre-menopausal women. This may lead to a false-overestimation by the clinician. Other causes might be the presence of oedema and hematoma secondary to biopsy. Additionally, inclusion of skin and surrounding tissues in palpated tumour might give the illusion of a larger tumour size than actual size [158]. The estimated tumour size in the current study was obtained through the registry data and it is unclear which method was used to estimate the tumour size clinically.

In conclusion, axillary metastases estimated by clinical examination were associated with a large proportion of false-positive and false-negative results. Tumour size estimated by clinical examination was associated with both under- and overestimation.

Study II

We have analysed the clinicopathological predictors for SN metastases in primary invasive breast cancer in this study and we found that multifocal tumours, tumours with LVI, and tumours larger than 20mm (T2-T4) had a higher risk of SN metastases. Tumours detected by screening mammography and tumours with negative oestrogen receptor status were associated with a low risk of SN involvement.

The presence or absence of metastatic involvement of the ALNs is an important prognostic factor. ALN status predicts clinical outcome and determines the extent of the adjuvant/neoadjuvant therapy in primary invasive breast cancer [2, 3]. However, there is no benefit of SNB in cases with no metastatic involvement. Many breast cancer cases are detected nowadays at an early stage with no metastasis in

the SN, and an incidence of approximately 30% has been reported for cases with SN metastasis [159].

SNB is associated with fewer complications compared with ALND [2, 3, 9]; however, the SN-negative patients gain no benefit from SNB. Identification of this group of patients is difficult but it has been suggested that the use of clinicopathological predictors might facilitate this identification [159]. In this study, we found that the tumour size was a powerful predictor of the SN metastasis. The risk of finding metastasis in the SN was high in tumours measuring more than 20 mm. This result is consistent with other studies [21, 159, 160]. Moreover, we observed that the risk of SN metastasis was low in medullary cancer, which is a rare type of breast cancer. However, there was a poor statistical power for all rare tumours, and we merged these rare types in to one sub-group in this study. The LVI has been suggested to be the most powerful predictor of SN metastasis [21, 161, 162].This was also observed in our study with LVI and, in order of significance, LVI was followed by tumour size and multifocality. The usefulness of LVI in clinical praxis is of limited value because the LVI is not available routinely; however, the use of the immunohistochemical lymphatic vessels marker might reveal the presence of LVI pre-operatively [96].

In Sweden approximately 64% of breast cancers are detected by screening mammogram [23]. Imaging in mammography screening has been improved, and new techniques make breast cancer easier to detect at early stage and at smaller sizes [163]. In the current study we found that there was a lower risk of SN metastasis in breast cancer detected by screening mammography. This might be due to many factors but one explanation is the possibility of detecting T1 breast cancer with a size less than 10mm.

We found in this study that the risk of SN metastases was low in tumours with negative hormonal status for oestrogen (0.64; 0.42– 0.99). The association between the ER status and the possibility of ALN involvement has been controversial. There are studies that have confirmed a lower risk of ALN metastasis in cases with negative ER while other studies have shown no association [105, 164, 165]. However, negative oestrogen receptor status and association with SN metastasis have not been established and is still not clear as compared with receptor-positive tumours [153, 164-166].

In conclusion, multifocality and LVI were powerful predictive factors for SN metastasis. Breast cancer detected by screening mammography and tumours with negative ER status were less likely to be associated with SN involvement. Tumours larger than 20 mm had a high risk of metastasis to SN.

Study III

This study showed that there was no non-SN involvement in 65% of patients who underwent c-ALND because of SN metastases. Multifocal cancer and lobular cancer

were associated with a high risk of non-SNs metastases. The presence of macro-metastases in SN was associated with higher risk of metastases to non-SNs. The total number of SNs removed by surgery had no impact on finding metastases in non-SNs. The number of SNs with macro-metastases was also associated with a higher risk of finding non-SN metastases. There was a positive association between the number of SNs with macro-metastases and the possibility of non-SN metastases, regardless of the number of SNs removed by surgery.

During the last decade the necessity of the c-ALND has been questioned as the majority of these patients have no non-SN involvement and the impact of survival might not be affected by omitting c-ALND [18]. We consider that our analysis has a strong statistical power as it includes 602 patients from a non-selected population-based cohort of consecutive cases. These patients had essential data available in the NKBC registry [23]. To omit c-ALND there is need for accurate identification of low risk patients. c-ALND has been debated. The value of c-ALND, even if there are metastases in the SN, has been questioned [17, 20]. In the Z0011 randomised trial from the American College of Surgeons Oncology Group (ACOSOG) [17] there were comparison between the ALND and avoidance of axillary surgery in patients with a maximum of two SNs with metastases. The results showed that there was no negative impact on survival for patients where an ALND had been performed [17].

In our study there was a positive association between the type of cancer and the possibility of ALN metastasis. The lobular type had a higher risk of non-SN metastasis compared with the ductal type. This might suggest that omission of ALND in cases with lobular cancer might require consideration. It is unclear why the lobular type has a greater tendency for metastasis to ALN but it has been reported that immune response, cell adhesion, and cell invasion associated with loss of E-Cadherin in the extra cellular space, as well as the differences in gene expression between lobular and ductal type might be possible explanations for lymph node metastasis in lobular breast cancer [167].

According to our study, there was a positive association between multifocal tumours and risk of ALN involvement but the underlying pathology was unclear. A possible explanation for this association might be the presence of more lobular cancer in multifocal tumours compared with unifocal tumours [115].We found in this study that the total number of SNs excised had no impact on finding metastases in non-SNs. Moreover, there was a higher risk of finding non-SN involvement in the presence of macro-metastases in SNs compared with presence of micro-metastases in SNs. Elisabeth *et al.* and Hwang *et al.* have recorded that the size of metastases in the SN was the most important predicting factor for the presence of metastasis in non-SN [168, 169]. In our study, the size of metastases in SNs was associated with the risk of non-SN metastases in our study. We found that regardless of the total number of SNs excised during operation, the number of SNs with macro-metastases

was associated with the risk of non-SN involvement. The same observation was recorded by Dingemans *et al.* [170].

We conclude that multifocal tumours and lobular breast cancer had a high risk of non-SN metastasis. There was a high risk of metastasis to non-SN in cases of macro-metastases in SNs. The total number of SNs excised by the surgeon had no impact on diagnosis of metastasis in non-SN. Additionally, there was a positive association between the risk of non-SN metastasis and the number of SNs with macro-metastasis, regardless of the number of SNs removed by the surgeon.

Study IV

In this study, our validation was almost identical to the original results for the same period and the SUS nomogram performed well with a good prediction of metastases in the SN.

The necessity of performing axillary surgery has been debated during the last decade [17, 20]. Approximately 65% of selected patients with breast cancer have disease-free ALNs [4, 12]. To facilitate the selection of theses patient groups i.e. patients with disease-free SNs, different nomograms have been developed and used to help in decision-making on whether or not to omit axillary surgery.

The MSKCC nomogram was developed at the Memorial Sloan-Kettering Cancer Center (New York, NY) [171]. This nomogram was created by Bevilacqua *et al.* and is available as a web page. According to their study, which included 3786 patients, there was a positive association between LVI, multifocality, age, tumour size and ALN metastasis. The AUC achieved by using this nomogram was 0.754 [171].

Dihge *et al*. showed that tumours detected by screening mammography, absence of multifocality and LVI, increasing age, and TN tumours were associated with non-metastatic axillary involvement. The AUC value for the SUS nomogram was 0.74 [21].

In general, the predictors used in different nomograms are almost similar. However, there are variations and controversies in different studies regarding the predictors used for detection of SN metastasis, e.g. tumour location [171, 172]. Zian Zhang *et al*. showed that ALN metastases were less common in the inner lower quadrant of the breast [172] while Bevilacqua *et al.*[171] reported that tumours located in the upper inner quadrant of the breast were less often associated with axillary metastasis. This might suggest that the usefulness of tumour location is still unclear and in the SUS nomogram tumour location was not used as a possible predictor.

The usefulness of nomograms has been debated in different studies due to factors associated with reporting, statistical methods and study design[173]. In a systematic review of methodological conduct and reporting performed by Collins *et al.,* 120 models were evaluated and they observed that an important measure of prediction

e.g. calibration, was not included in most analyses [173]. Our calibration for the SUS nomogram showed a good agreement between predicted and observed values.

We conclude that the SUS nomogram showed a good prediction of the axillary lymph node metastasis and performed adequately in an independent cohort. Additionally, we observed that routine data from the NKBC registry was as useful as manually retrieved clinical records.

Statistical and methodological considerations

Study I

All cases with breast cancer diagnosed in Malmö from 1961 to 2004 were recorded in the Regional Tumour Registry. This data was retrieved in 2005. In 1977, the SSBCG was established. Guidelines for treatment of breast cancer were issued by this group. Routinely, all patients with breast cancer were discussed at a weekly multidisciplinary conference. From 1981 to 2003, SSBCG had a clinical registry with a computerised database.

Information based on register data needs periodic validation. Parkin and Bray [174, 175] proposed a validation strategy of cancer registry data in which four quality aspects were suggested, namely completeness, timeliness, comparability and validity [174]. The completeness of available data in the current study regarded to be fair good as almost all patients had available information in the SSBCG registry, corresponding to 97%. However, a limitation was the accuracy of the main variable used in the study i.e. tumour size by clinical examination. TNM classification was used for tumour size measurement but the actual method of estimation used by the clinician was not recorded in the registry. Additionally, the shrinkage effect of the formalin, which was used for preservation of the specimens post-operatively, had not been considered in the size estimation by the pathologist. Studies have considered different aspects of this issue, but the majority of the studies support shrinkage of free tumour margin only with no influence on the tumour size [176-178]. Furthermore, the assessment of axillary status for the presence or absence of palpable lymph nodes might be associated with false-positive and false-negative results because the results of the physical examination might depend on the individual assessment by the physician. There was no information in the registry on whether the axillary physical status was checked and confirmed by one clinician or more.

Study II, III, IV

In general, the quality of the NKBC register is regarded as very high, with periodic validation control of data recording [23, 179]. However, there are some considerations and issues regarding these studies. In study II, the final study

population was composed of 2552 patients. The original cohort included 3979 patients. There were 1040 patients which corresponded to 22.8%, who did not undergo SNB. These patients were excluded in this study, even though 48% of this group had T2 tumours. A possible explanation might be that indications and routines for SNB were different at the time of the study in that all cases with T2 larger than 30mm or multifocal tumours underwent ALND directly. Now, this indication is no longer valid.

The indication for c-ALND, at the time of study III, was the presence of metastases in the SNs (macro and/or micro); however, there was consideration about 69 women who had a positive SNB but did not undergo a c-ALND. We had no information in the registry on why these women did not undergo SNB.

Another potential issue was the availability of information about different clinicopathological predictors used in the current study pre-operatively e.g. LVI and multifocality. Information about these predictors is usually available post-operatively after the final pathological results are obtained. This may limit the value of these predictors pre-operatively. Furthermore, there was no information on internal mammary lymph nodes in the NKBC data base, which can be regarded as an important missing predictor since previous studies have shown that the internal mammary lymph node status is an independent prognostic factor [180-182]. However, information about intramammary lymph nodes has no impact on treatment and is not used in clinical practice.

In study IV, there was also consideration of the availability of two essential predictors pre-operatively. This issue is described in the study III (see study III considerations above).

The major issue in the study IV was missingness in the original data base. There were three predictors for ALN metastases (LVI, Ki-67 and HER2 status) were recorded with a high proportion of missing values. There was no active check for completeness during the data collection from the NKBC registry during the study period and we observed a relatively large proportion of missing values for some essential variables. Multifocality information was missing for 22.3% of all patients, HER2 status for 35.4%, LVI for 37.2% and Ki-67 for 84.2%. However, we found that a possible solution for this issue was the implementation of multiple imputation, where 200 data sets with 20 iterations were applied [183, 184].

Furthermore, there was an issue with HER2 status. HER-2 amplification status was the only available HER2 status in the NKBC registry during the study period. The proportions of non-amplified and “missing” were different in each centre, although in general, the proportions of amplified tumours were almost identical in both centres. HER2 status might have been recorded in different ways at each centre, probably because the routines for registration had been applied in a different manner. In Malmö, the FISH test was not applied for cases with HER2 0, HER2 1+, and HER2 status might have been classified as missing. As a possible solution we

performed a sensitivity analysis and re-coded missing HER2 values as non-amplified, and the results were conformed to be consistent with the original analysis after this sensitivity test (Table 11).

Conclusions

Study I

- Axillary metastases estimated by clinical examination were associated with a large proportion of false-positive and false-negative results.
- Tumour size estimated by clinical examination was associated with under- and over-estimation.

Study II

- Tumours detected by screening mammography were less likely to be associated with metastasis to SNs. Negative oestrogen receptor status was associated with a lower risk for SN metastasis. Tumour size more than 20 mm had a higher risk of metastasis to SNs. Multifocality and LVI were strong predictive factors for SN metastasis.

Study III

- Lobular breast cancer and multifocal tumours had a high risk of non-SN metastasis.
- The presence of macro-metastases in SNs was associated with a high risk of finding metastases in non-SNs. The number of SNs with macro-metastasis, regardless of the number of SNs removed by surgery, increased the risk of metastatic involvement of non-SNs. The total number of SNs removed by surgery had no impact on diagnosis of metastasis in non-SN

Study IV

- The SUS nomogram showed a good prediction of the SN metastasis.
- The SUS nomogram performed adequately in an independent cohort, and routine data from the NKBC was as useful as manually retrieved clinical records.

Future perspectives and clinical implications

Axillary lymph node status is an important factor in the management of patients with primary invasive breast cancer. The results of this thesis indicate that it is possible to predict metastatic involvement of SN by using different clinicopathological factors.

Mode of detection, tumour size, oestrogen hormonal status, LVI, multifocality and size of metastasis in the lymph node were shown to have a positive association with the presence or absence of axillary metastasis. Historically the ALND has been used as therapeutic measure as a separate procedure or as a part of radical mastectomy [7, 48]. It is well known now that ALND is associated with complications such as lymphoedema, pain and neurological dysfunctions [50]. Approximately 49% of women who underwent ALND developed lymphoedema 20 years after the primary operation [51]. In Sweden it has been reported that 15-20% of patients who underwent ALND and adjuvant radiotherapy had a higher incidence of developing swelling in the operated arm. Moreover, they had a decreased long-term health-related quality of life [9, 185]. SNB has been used as a standard procedure in staging ALN status since the early 2000s. The procedure has been proved to be safe and accurate with a very low (< 7%) FNR [186]. Since the launch of SNB there has been an obvious reduction in the incidence of complications secondary to ALN surgery [9, 185]. However, studies in this thesis have confirmed that > 65% of patients undergoing SNB and/or c-ALND have a disease-free ALN status. Different analyses have shown that there is no contribution to a better survival after extensive ALN surgery in selected patients with low risk for metastatic involvement of ALN [4, 12, 17, 18]. That is why the axillary surgical de-escalation and/or no axillary surgery should be considered in the future. However, the identification of this low risk group of patients is challenging, complicated and may be regarded as the main obstacle in this process.

Although the results of this thesis might facilitate the identification of low-risk patients by using the clinicopathological predictors or by conducting a nomogram with predictive performance for N0 vs. N+, there is still a need for call of further investigations to facilitate definitive identification of this group of patients. A recent analysis in Sweden has reported that it is possible to identify predictors of ALN involvement by using clinicopathological factors, gene expression analyses, and

mixed factors (both clinicopathological factors and gene expression) [187]. It has been concluded that clinicopathological factors and mixed factors of ALN involvement have comparable accuracy but the mixed predictors identified more node-negative patients. The study has suggested that the use of mixed predictors might reduce the SNB rate for patients with low risk for ALN involvement [187]. Furthermore, there is an ongoing SENOMAC study where patients with macro-metastases (1-2 SNs) are randomised to ALND or no ALND. This study is planned to include 3700 patients in different countries in Europe simultaneously for a period of about seven years [20].

In conclusion, there is still a need for SNB in all patients with clinically node-negative primary invasive breast cancer, and performance of the ALND is necessary for all patients with clinical node-positive or metastatic involvement of more than two SNs with macro-metastases.

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Study I

Errata

A reference was unfortunately omitted in the discussion.

Now reads:

Normal lymph nodes vary widely in size, consistency, and fat content [3,16]. Lymphadenopathy is an element of many nonmalignant diseases, and reactive adenopathy may not be distinguishable from metastasis [16].

Should read:

Normal lymph nodes vary widely in size, consistency, and fat content [2,3,16]. Lymphadenopathy is an element of many nonmalignant diseases, and reactive adenopathy may not be distinguishable from metastasis [2,16].

The error above does not affect our results or conclusions.

The publishing journal have been informed.

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Clinical Assessment of Axillary Lymph Nodes and Tumor Size in Breast Cancer Compared with Histopathological Examination: A Population-Based Analysis of 2,537 Women

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Abstract

Background The clinical assessment of axillary lymph nodes status and tumor size is important for the management of patients with breast cancer. The first goal of this study was to determine the accuracy of axillary lymph node status in relation to the presence of metastases as revealed by histopathological examination. The second goal was to compare the tumor size as assessed by physical examination, with the size obtained by histopathological examination.

Methods This study was based on a consecutive series of 2,537 patients diagnosed with breast cancer in Malmö, Sweden, between 1987 and 2002. These patients had available information in the South Swedish Breast Cancer Group registry, corresponding to 97 %. The axillary lymph nodes status was compared with the results of the histopathological examination for the presence of metastases. Tumor size by physical examination was compared with the tumor size after histopathological examination.

Results There were 674 women with axillary lymph nodes metastases according to histological examination; only 206 of these cases had palpable lymph nodes at clinical examination. The sensitivity was 30 % and the specificity 93 %. There were 812 tumors measured to be larger than 20 mm according to histopathological examination, but only 665 of these tumors were considered larger than 20 mm by clinical examination. This corresponded to a sensitivity of 81 % and a specificity of 80 %.

Conclusions We conclude that the possibility of axillary metastases estimated by clinical examination is subjected to a large proportion of false-positive and false-negative results. Similarly, tumor size estimated by clinical examination is subject to under- and overestimation in comparison to histopathological examination.

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Introduction

Clinical assessment of axillary lymph nodes status is an important factor in planning of the surgical strategy in patients with breast cancer [1–4]. The likelihood of axillary lymph node metastases as determined by clinical examination before histological examination is difficult to predict [3, 5–7]. Clinically palpable axillary lymph nodes are widely considered as a contraindication to the sentinel lymph node procedure [2, 6]; as a consequence, a number of patients without regional disease are undergoing axillary dissection with subsequent potential complications [8].

Preoperative assessment of tumor size in breast cancer also is an important key factor in deciding the appropriate treatment according to current guidelines for the management of breast cancer [9]. Tumor size may be estimated using different modalities before surgery, but clinical assessment by palpation remains the first and easiest way to estimate tumor size. There may be a considerable difference between the estimated tumor sizes preoperatively and after histological examination [1, 10, 11].

This study was based on a consecutive series of 2,537 patients diagnosed with breast cancer in Malmö, Sweden, between 1987 and 2002.

The goal of the present study was to determine the accuracy of clinically assessed axillary lymph node status in relation to the presence of metastases as revealed by

histopathological examination postoperatively. An additional goal was to compare the preoperative tumor size as assessed by physical examination, with the size obtained by histopathological examination.

Materials and methods

Patient registry

The South Swedish Breast Cancer Group (SSBCG), which was established in 1977, has issued guidelines for treatment of patients with breast cancer [12]. SSBCG set up a clinical registry in 1981, which continued until 2003. In Malmö since 1977, each patient with breast cancer is reviewed and discussed at a weekly breast cancer conference at which there are representatives from the Departments of oncology, radiology, surgery, plastic surgery, and pathology.

Decision about the management, i.e., extent of surgery and the use of adjuvant therapy, primarily depends on tumor size, lymph node status, hormone receptor status, age, and menopausal status. All of this information was entered into the register run by the SSBCG.

Data about the axillary lymph node status by physical examination were collected together with the results obtained by histopathological examination of these lymph nodes. Information also was collected on tumor size according to the physical examination before the surgery and after the histopathological examination, TNM, type of surgery, and adjuvant treatment.

All of the information was already available at a computerized database at The South Sweden Regional Tumour Registry.

Study population

All cases with breast cancer diagnosed in Malmö, or registered as residents in Malmö, between 1961 and 2004 were retrieved from The Regional Tumour Registry during the autumn of 2005.

After excluding cases with unknown civil registration number ($n = 6$), multiple cases in the same individual ($n = 1{,}921$), benign lesions ($n = 26$), cases diagnosed before the establishment of the clinical registry at the SSBCG in 1981 ($n = 3{,}326$), and following end of data collection into the SSBCG registry, December 31, 2003 ($n = 245$), 4,557 cases remained. Of them, 202 were not registered as residents in Malmö, 481 had been treated outside Malmö, and 28 were found at autopsy. An additional 41 cases had a mismatch between date of diagnosis in The Regional Tumour Registry and the SSBCG registry of more than 180 days. This left 3,805 cases. Routines for collection of information in to the SSBCG registry had changed slightly over time, with many missing cases in the beginning of the period and during the last year the SSBCG registry was run. The final cohort consisted of cases diagnosed between January 1, 1987 and December 31, 2002, in all 2,629 individuals. Of these 2,629 women, 2,537 individuals had available information in the SSBCG registry, corresponding to 97 %.

Statistical methods

Axillary lymph node status by physical examination was regarded as positive in case of palpable lymph nodes and as negative in case of nonpalpable lymph nodes. The axillary status was compared with the results of the histological examination for the presence of metastases. Axillary lymph nodes with metastases were regarded as positive and those without metastases as negative.

The patients were divided into two groups according to tumor size: those with tumors <20 mm, and those with tumors >20 mm. This choice was made according to the TNM classification [13, 14]. Tumor size by physical examination preoperatively was compared with the size of the tumor after histological examination.

A "positive test" for axillary lymph node status was palpable lymph nodes and for tumor size it was a tumor perceived as larger than 20 mm. Sensitivity, specificity, positive predicative value (PPV), negative predicative value (NPV), positive likelihood ratio (+LR), negative likelihood ratio (−LR), and 95 % confidence intervals (CI) were calculated. Confidence intervals for the estimated parameters were computed by a general method (based on constant χ^2 boundaries) [15].

Comparisons were made in two different periods: 1987–1994 and 1995–2002. Patients were further divided into three age groups. One group assumed to be mainly premenopausal age <50 years, a second group assumed to be mostly postmenopausal aged 50–70 years, i.e., women invited to the mammography screening program, and a third group of postmenopausal women aged >70 years.

Results

There were 674 women with axillary lymph nodes metastases according to histological examination; only 206 of these cases had palpable lymph nodes at clinical examination. The sensitivity was 30 %, the specificity 93 %, the PPV 76 %, and the NPV 67 % (Table 1). Sensitivity was low and specificity was high in all age groups. The +LR was 5.1 and −LR was 0.73. No large differences were noticed in relation to different time periods (Table 1).

According to histopathological examination, there were 812 tumors measured to be larger than 20 mm, but only 665 of these tumors were considered larger than 20 mm by

Table 1 Clinical axillary lymph node involvement (ALNI) compared with ALNI according to the histopathological examination

Group	Clinical ALNI (n)	Histopathological ALNI		Validity measurements					
		Negative (*n*)	Positive (*n*)	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)	+LR (CI)	–LR (CI)
All	Negative	967	468	30 (28–32)	93 (92–95)	76 (71–81)	67 (66–68)	5.1 (3.9–6.6)	0.73 (0.71–0.77)
	Positive	62	206						
Age (yr)									
<50	Negative	149	94	33 (28–36)	93 (89–95)	81 (70–88)	61 (58–63)	4.8 (2.6–8.9)	0.71 (0.66–0.79)
	Positive	11	47						
50–70	Negative	547	224	26 (23–28)	95 (93–96)	73 (65–80)	70 (69–71)	5.3 (3.5–8.1)	0.77 (0.74–0.82)
	Positive	28	79						
>70	Negative	271	150	34 (31-37)	92 (88-94)	77 (69–84)	64 (62–66)	4.4 (3.0–6.8)	0.70 (0.66–0.77)
	Positive	23	80						
Period									
1987–1994	Negative	497	216	32 (29–35)	93 (91–94)	73 (66–79)	69 (68–70)	4.6 (3.3–6.6)	0.72 (0.68–0.77)
	Positive	37	103						
1995–2002	Negative	470	252	29 (26–31)	94 (93–96)	80 (73–86)	74 (63–66)	5.7 (3.8–8.6)	0.74 (0.71–0.79)
	Positive	25	103						

All measurements with a 95 % confidence interval (CI)

clinical examination. This corresponded to a sensitivity of 81 %, a specificity of 80 %, a PPV of 72.0 %, and a NPV of 87 %. Sensitivity related to the preoperative diagnosis of tumors larger than 20 mm was considerably higher for women older than age 70 years, whereas this group had a lower specificity concerning the detection of tumors larger than 20 mm. The +LR was 4.2 and the −LR was 0.22. All results were similar in relation to different time periods (Table 2).

Discussion

Among women with palpable lymph nodes, 24 % had no lymph node metastases, and in women with no palpable lymph nodes 32 % had lymph node metastases according to the histopathological examination. This suggests large difficulties in the clinical estimation of the axillary lymph nodes status.

Patients with clinically suspicious axillary nodes comprise a variety of findings. Normal lymph nodes vary widely in size, consistency, and fat content [3, 16]. Lymphadenopathy is an element of many nonmalignant diseases, and reactive adenopathy may not be distinguishable from metastasis [16].

Clinically positive axillary lymph nodes are usually considered as a sign of regional metastases, whereas their absence is regarded as a good prognostic factor [17]. However, several previous studies have shown that clinical examination of axillary lymph nodes and estimation of suspicious metastases by palpation is an inaccurate way of assessment even when the examination is performed by an experienced surgeon [2, 18]. Lanng et al. [6] showed in a study involving 301 patients that even if the examination was performed by a specialist breast surgeon, the examination had little value. When the surgeons considered the axilla to be normal, they were wrong in 44 % of cases. Other studies have reported similar results, e.g., Voogd et al. [18] who showed in a population-based study involving 5,123 patients that 34 % of patients who were known to have nonpalpable lymph nodes before surgery had positive lymph nodes at pathological examination after axillary dissection.

Tumor size by palpation had a high specificity concerning the detection of tumors larger than 20 mm in premenopausal women, whereas it had a low specificity in postmenopausal women where overestimation of tumor size at palpation was most common.

Tumor size estimated by physical examination was used in this analysis, although the palpated tumor size is usually used in decision making along with radiological size. This is particularly the case when dealing with tumors larger than 40 mm where neoadjuvant therapy might be the primary choice of treatment [12]. In addition, the estimated tumor size is an important factor peroperatively in cases of partial mastectomy and breast conservative surgery to achieve sufficient macroscopic marginal.

There are different ways to estimate tumor size; physical examination, mammography, and ultrasonography are common methods, and many studies have indicated that measurement by

Table 2 Clinical size compared with size according to the histopathological examination

Group		Histopathological size		Validity measurements					
	Clinical size (n)	<20 mm (*n*)	>20 mm (*n*)	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)	+LR (CI)	–LR (CI)
All	<20 mm	1,020	147	81 (79–83)	80 (79–81)	72 (71–74)	87 (85–88)	4.2 (3.8–4.6)	0.22 (0.2–0.26)
	>20 mm	246	665						
Age (yr)									
<50	<20 mm	162	31	77 (72–82)	80 (76–83)	72 (67–77)	83 (80–87)	4.0 (3.0–5.0)	0.3 (0.2–0.36)
	>20 mm	40	108						
50–70	<20 mm	635	79	74 (70–77)	86 (85–88)	70 (66–73)	88 (87–90)	5.6 (4.7–6.6)	0.3 (0.25–0.35)
	>20 mm	97	227						
>70	<20 mm	223	37	89 (87–92)	67 (64–69)	75 (72–77)	85 (81–89)	2.7 (2.4–3.0)	0.15 (0.11–0.21)
	>20 mm	109	330						
Period									
1987–1994	<20 mm	466	62	83 (80–86)	79 (77–81)	72 (69–74)	88 (86–90)	4.0 (3.5–4.5)	0.2 (0.16–0.25)
	>20 mm	122	319						
1995–2002	<20 mm	554	85	80 (77–83)	81 (79–83)	73 (70–76)	85 (84–86)	4.3 (3.8–5.0)	0.24 (0.2–0.28)
	>20 mm	124	346						

All measurements with a 95 % confidence interval (CI)

ultrasonography is the most accurate way [9, 19–21], e.g., Hieken et al. [20] who showed in a study that included 180 patients with invasive breast cancer that ultrasonography is more accurate than mammography in assessing breast cancer size. Moreover, Shoma et al. [19] showed in a study involving 162 patients that it was common to overestimate the tumor size during clinical examination.

Overestimation of the tumor size may be due to several reasons. Local bleeding and increased inflammatory reaction/edema after biopsy could result in an overestimation. In addition, the physical palpation includes not only the tumor but also the surrounding tissue and the skin, which in turn might increase the estimated tumor size [3, 10].

Another explanation could be that breast specimens undergo shrinkage after histological fixation; Docquier et al. [21] and Yeap et al. [22] suggested that breast specimens undergo shrinkage after histological fixation, losing more than a third of their original closest free margin, whereas the tumor itself does not shrink substantially.

The strengths of the present study include the size of the sample: more than 2,500 patients with breast cancer. The patient cohort was a population-based consecutive series, and there was no selection, in terms of tumor stage or other reasons, to or from Malmö University Hospital. Validity of the diagnosis was probably very high as cases were identified from two sources: The Regional Cancer Registry and the clinical registry run by the SSBCG. The histopathological assessment was performed at one department of a limited number of pathologists, several of them working in the department for decades. Similarly, all preoperative examinations were performed in the same surgical department.

Our study shows that estimation of suspicious regional metastases by clinical examination is very difficult, and finding palpable lymph nodes during clinical examination in patients with breast cancer does not necessarily mean regional metastases of breast cancer. This is of great importance, because palpable lymph nodes are widely considered a contraindication for performing the sentinel node procedure [2, 6], which may save these patients from an unnecessary axillary lymph node dissection [23]. On the other hand, the absence of palpable lymph nodes in the axilla does not exclude metastases. There are different ways to assess the axillary lymph node status before surgery, i.e., physical examination, radiological examination (ultrasonography, mammography, CT, PET-CT, and MRI), and needle biopsies. The present study indicates the need for such additional examinations to improve accuracy of the preoperative assessment of axillary lymph node status.

The result of our study also indicate that whenever the preoperative tumor size at physical examination is used as decision-making value in choosing the appropriate management of patients with breast cancer, there is a clear risk of over- and underestimation of tumor size, and additional measurement by help of other modalities must be taken in consideration.

We conclude that the possibility of axillary metastases estimated by clinical examination is subject to a large proportion of false-positive and false-negative results. Similarly, tumor size estimated by clinical examination is subject to a considerable misclassification with both under- and overestimation compared with histopathological results.

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Study II

Majid et al. World Journal of Surgical Oncology (2018) 16:54
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World Journal of Surgical Oncology

RESEARCH Open Access

Predictive factors for sentinel node metastases in primary invasive breast cancer: a population-based cohort study of 2552 consecutive patients

Shabaz Majid[1,2*], Lisa Rydén[3,4] and Jonas Manjer[2,3]

Abstract

Background: Axillary lymph node status is one of the most important prognostic factors for breast cancer. The aim of this study was to determine predictive factors for metastasis to sentinel node (SN) in primary invasive breast cancer.

Method: This is a study of 3979 patients with primary breast cancer during 2008–2013 in Malmö and Lund scheduled for surgery and included in the information retrieved from Information Network for Cancer Care (INCA). The final study population included 2552 patients with primary invasive breast cancer. The risk of metastases to SN were examined in relation to potential clinicopathological factors such as age, screening mammography, tumor size, tumor type, histological grade, estrogen status, progesterone status, Her-2 status, multifocality, and lymphovascular invasion. Binary logistic regression was used; adjusted analyses yielded odds ratio (OR) with 95% confidence interval.

Results: Tumors detected by mammography screening were less likely to be associated with metastases to SN compared to those not found by mammography screening (0.63; 0.51–0.80). Negative hormonal status for estrogen associated with lower risk for SN metastases compared to tumor with positive estrogen status (0.64; 0.42–0.99). Tumors with a size more than 20 mm had higher risk to metastasize to SN (1.84; 1.47–2.33) compared to tumors less than 20 mm. Multifocality (1.90; 1.45–2.47) and lymphovascular invasion (3.74; 2.66–5.27) were also strong predictive factors for SN metastases.

Conclusion: SN metastasis is less likely to occur in women with invasive breast cancer diagnosed by screening mammogram. Tumors with negative estrogen status are associated with low risk for SN metastases. Tumors larger than 20 mm, multifocality, or lymphovascular invasion are also factors associated with high risk for SN metastases.

Keywords: Invasive breast cancer, Predictive factors, Sentinel node metastases

Background

Axillary lymph node status is still one of the most important prognostic factors for predicting clinical outcome in invasive breast cancer [1, 2], and it also determines the extent of axillary surgery and adjuvant/systemic therapy. Recently, the value of an axillary clearance when metastatic spread is found has been questioned [3, 4]. Indeed, it may be questioned if staging is necessary in all cases, e.g., even in patients where the risk of metastatic spread is very low. However, this demands that low-risk groups can be accurately identified [5].

Physical examination is a poor predictor of axillary lymph node metastasis [6], and evaluation of the axilla by ultrasound has been shown to be unreliable [7].

Sentinel node biopsy (SNB) has been used since the late 1990s to evaluate the axillary lymph node status [1]. The sentinel lymph node is defined as the first lymph nodes to which cancer cells are most likely to spread from the primary tumor. SNB has minimized the need for axillary lymph node dissection (ALND) dramatically which in turn decreases the subsequent complications

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after ALND such as lymphedema, chronic pain, and neurological disabilities [8, 9]. Many studies have confirmed that SNB is technically feasible, safe, and associated with fewer complications as compared with ALND [1, 2, 9]. However, the SNB as a process is time consuming and resource intensive and, in the majority of patients, SNs are without metastases, and although the SNB is associated with fewer complications, there is still a risk to develop disabilities postoperatively [9, 10]. The proportion of T1 invasive breast carcinomas is increasing due to factors such as better diagnostic methods and public screening programs, and the role of SNB and ALND in these patients has been questioned [3, 11, 12].

The aim of this study was to define clinical and pathological factors that predict patients who are likely to be node-positive and thus to have the possibility for better planning of surgical or systemic therapy. Moreover, the information can enable the identification of patients with a high probability of node-negative tumor where the SN procedure may possibly be omitted.

Methods

The background population consists of all cases of breast cancer among women in Lund and Malmö operated on between January 2008 and December 2013. Every patient was identified by a 12-digit civil registration number which is unique for every Swedish citizen. All patients operated on because of breast cancer were included, and a total number of 3979 cases (cancer events) were identified. The indication for SNB has been changed over the time; in the early beginning of 2000s, the SNB procedure was performed only when tumor size was smaller than 30 mm and all cases with tumor size larger than 30 mm as well as multifocal tumors underwent ALND directly in both centers.

The following patients were excluded: 30 male patients, 82 cases with bilateral breast cancer (that is, 164 cancer events), 43 cases with previous breast cancer, and 1040 cases who were not operated on with SNB; 122 cases were diagnosed with in situ breast cancer and 25 patients who had received systemic therapy preoperatively. In two patients, it was not known if they had received systemic therapy, and one patient had unknown information about SN status. Regarding those cases not operated on with SNB (1040 patients), there were 599 who underwent an axillary dissection; 191 women had neither underwent SNB nor an ALND, and information on axillary surgery was missing in the remaining 250 patients.

In the 1040 excluded patients, 48.5% had tumors that were stage T2 or above; among ALND operated cases, this percentage was 35.3%; and in women with neither SNB nor ALND was 1.25%. The corresponding percentage in our study population of 2552 patients was 22.8%.

Furthermore, among all 1040 excluded patients, 18.7% had a multifocal tumor. In ALND cases, this proportion was 16.7%; in patients with no surgery in the axilla, it was 1.8%; and in our study population, it was 13.8%. Following these exclusions, the final study population included 2552 patients (Fig. 1).

In Sweden, a nationwide database for breast cancer is available on an IT platform called The Information Network for Cancer Care (INCA). INCA manages various information about cancer care as well as long-term follow-up. It is run and developed jointly by the country's regional cancer centers. INCA has been in full operation since 2007. The Regional Cancer Center in Southern Sweden (RCC-Syd) is the main center in the southern area which manages this registry.

In Malmö and Lund at Skåne University Hospital, every patient with breast cancer is reviewed and discussed before and after surgery at a weekly multidisciplinary breast cancer conference at which there are representatives from the departments of oncology, radiology, surgery, and pathology. A special registration form designed by INCA is available, and this form is filled in by a surgeon in cooperation with a secretary who is specifically employed for this reason and who is responsible for entering the data into the platform. The present study was approved by the Ethic Committee at Lund University, Lund, Sweden (LU-Dnr 2013/821).

All information used in the present study such as screening, age, menopause status, tumor size, histopathological type and grade, receptor status, Her-2, multifocality, and lymphovascular invasion as well as information about SNB, i.e., type and size of metastases, were retrieved from INCA.

All women in the background population aged 40–74 years are invited to the public mammography screening. Mode of detection was recorded as screening-detected yes/no. Menopause status was defined as premenopausal or postmenopausal.

Postmenopausal women were subdivided as to when they had their last menstruation, 6 months to 5 years ago or more than 5 years after menopause. Tumor size was defined according to the TNM classification, T1 tumor ≤ 20 mm, T2 tumor 21–50 mm, and T3–T4 > 50 mm. [13]. The T1 tumors were further classified to subgroups T1a 1–5 mm, T1b 6–10 mm, and T1c 11–20 mm. The histopathological types were classified according to the WHO classification system; this system describes mainly six different histopathological types of invasive cancer [13–15]. We merged this into four different groups, i.e., ductal, lobular, combined ductal with lobular, and other rare types. Histological grade was defined according to the Nottingham histological grading (NHG) [16]. Multifocal tumors were defined as two or more tumors with normal tissue or in situ tumors at a distance of at least 20 mm. Lymphovascular invasion is defined

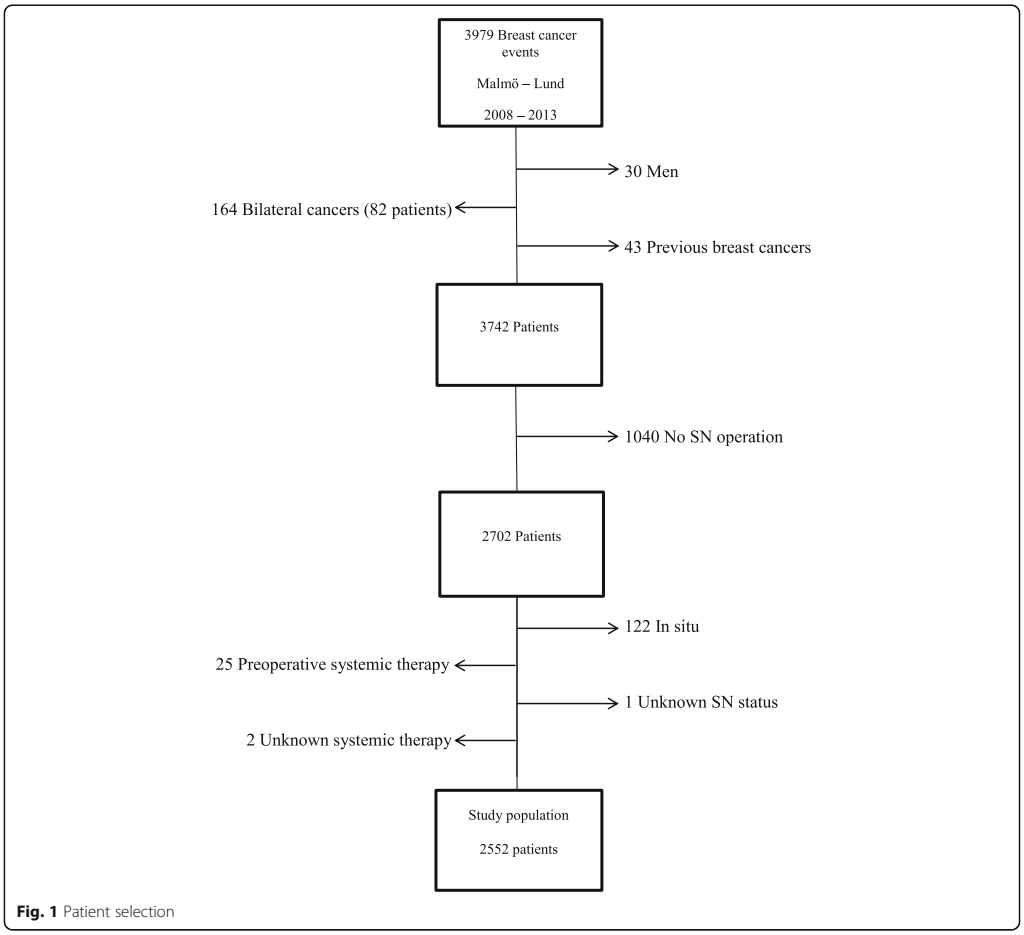

Fig. 1 Patient selection

as tumor cells in vascular spaces, tumor cells in underlying endothelium of vascular channels, and tumor cells invading through a vessel wall and endothelium [17]. Receptor status for both estrogen and progesterone was measured by immunohistochemistry (IHC). Receptor percentage more than 10% was regarded as positive and those with 10% or less as negative [18]. Her-2 protein was analyzed with IHC, and test results were reported as 0, 1+, 2+, or 3+. Fluorescent in situ hybridization (FISH) uses fluorescent pieces of DNA that specifically stick to copies of the HER2 gene in cells. In all cases, IHC test was used first and then completed by FISH in certain cases where Her-2 was 2+ or 3+. Her-2 status was classified as negative when Her-2 IHC = 0–1+ and 2+ in non-amplified tumors. Her-2 was regarded as positive if Her-2 was classified as 2+ or 3+ and amplified by FISH [19]

Metastases in SN were classified as macrometastases when the size was > 2 mm and regarded as micrometastases when the size was 0.2–2.0 mm. All metastases with a size less than 0.2 mm were regarded as sub-micrometastases. Lymph nodes with only sub-micrometastases also referred to as isolated tumor cells (ITCs) were regarded as without metastases in the present analysis [18].

Lymph nodes with macro- or micrometastases were regarded as positive and those without metastases as negative. To compare the association between potential predictive factors and metastases in SN, binary logistic regression was used and all analyses were adjusted for all included factors, i.e., screening, age, tumor size, menstrual status, histopathological type and grade, receptor

status for estrogen and progesterone, Her-2 status, presence of multifocality, and presence of vascular invasion. This yielded odds ratios (ORs) with 95% confidence intervals (CIs). Main analyses were performed for the two centers together, center A (Lund) and center B (Malmö). Moreover, the analyses were also performed for each center separately. Statistical Package for the Social Sciences (SPSS) program version 22.0 (SPSS Institute, Chicago, IL, USA) was used for all analyses.

Results

There were in total 671 patients with SN metastases (26.3%), three hundred seventy four patients (29.6%) in center A and 297 patients (23.0%) in center B. Tumors detected by mammography screening were less likely to be associated with SN metastases compared with those not found by screening mammography (0.63; 0.51–0.80), Tables 1 and 2. A tumor with a size more than 20 mm, i.e., T2, T3, and T4, had higher possibility to metastasize to the SN compared to tumors less than 20 mm (T1): 1.84 and 1.47–2.33 for T2 and 2.56 and 1.07–6.09 for T3 and T4. An additional analysis showed that T1a tumors had the lowest risk for SN metastases (0.19; 0.09–0.40) followed by T1b (0.46; 0.34–0.63) compared with T1c.

A negative association with SN metastases was seen in cases with negative hormonal status for estrogen (0.64; 0.42–0.99). Multifocal tumors (1.90; 1.45–2.47) or tumors with vascular invasion (3.74; 2.66–5.27) had higher risk of SN metastases. Tumor types other than ductal and lobular, i.e., medullary and other rare types, were associated with low risk for SN metastases (0.29; 0.18–0.46). Adjusted analyses were similar to crude values except for histological grade II and III where crude analyses were associated with higher risk for SN metastases, but this was not observed with adjusted analyses. Overall, there were no large differences in results between the two centers; however, the risk of metastases to SN in all T3 and T4 cases were not high in center B while in center A there was a high risk of SN metastases in T3 and T4 cases (Table 3). In all analyses, there was no statistical significance in different histological grades in both centers, and hormonal status was not statistically significant when we analyzed each center separately. These analyses included few cases and CI was relatively wide.

Table 1 Potential predictive factors in relation to SN status

Determinants	Category	Total	SN negative		SN positive	
			N	%	*N*	%
Screening	No	1062	719	38.2	343	51.1
	Yes	1435	1123	59.7	312	46.5
	Unknown	55	39	2.1	16	2.4
Age	≤ 50	499	337	17.9	162	24.1
	51–74	1702	1290	68.6	412	61.4
	≥75	351	254	13.5	97	14.5
Menopause status	Pre	512	348	18.5	164	24.4
	Post < 5 years	228	168	8.9	60	8.9
	Post ≥ 5 years	1721	1299	69.1	422	62.9
	Unknown	91	66	3.5	25	3.7
Tumor size	T1	1505	1138	60.5	367	54.7
	T2	559	346	18.4	213	31.7
	T3 and T4	25	13	0.7	12	1.8
	Unknown	463	384	20.4	79	11.8
Tumor type	Ductal	1866	1324	70.4	542	80.8
	D and L	52	35	1.9	17	2.5
	Lobular	304	216	11.5	88	13.1
	Other	330	306	16.3	24	3.6
Histological grade	I	622	488	25.9	134	20.0
	II	1112	807	42.9	305	45.5
	III	790	563	29.9	227	33.8
	Unknown	28	23	1.2	5	0.7
Estrogen receptor	Positive	2144	1538	81.8	606	90.3
	Negative	279	218	11.6	61	9.1
	Unknown	129	125	6.6	4	0.6
Progesterone receptor	Positive	1851	1320	70.2	531	79.1
	Negative	571	436	70.2	531	79.1
	Unknown	130	125	6.6	5	0.7
HER-2 status	Negative	1466	1041	55.3	423	63.0
	Positive	240	174	9.3	66	9.8
	Unknown	848	666	35.4	182	27.1
Multifocality	No	1570	1184	62.9	386	57.5
	Yes	352	208	11.1	144	21.5
	Unknown	630	489	26.0	141	21.0
Vascular invasion	No	1324	1056	56.1	268	39.9
	Yes	184	87	4.6	97	14.5
	Unknown	1044	738	39.2	306	45.6

Discussion

In this study, we identified predictive factors for SN metastases by analyzing clinical and pathological characteristics of the tumors in patients with primary invasive breast cancer, and we found that SN metastasis is less likely to occur in women diagnosed by screening mammography. Tumors with negative estrogen status were associated with low risk of SN metastases. Tumors with a size more than 20 mm, multifocality, or lymphovascular invasion had more risk for SN metastases.

The strengths of the present study include the size of the sample, where 3979 patients with breast cancer were included from a non-selected population-based cohort of consecutive cases. Those patients who did

Table 2 Potential predictive factors and risk of SN metastases

Determinants	Category	SN negative	SN positive	OR 95% CI	OR 95% CI[a]
Screening	No	719	343	1.00	1.00
	Yes	1123	312	0.59(0.49–0.70)	0.63(0.51–0.80)
	Unknown	39	16	0.86(0.48–1.57)	0.88(0.46–1.66)
Age	≤ 50	337	162	1.00	1.00
	51–74	1290	412	0.67(0.53–0.82)	0.92(0.63–1.37)
	≥ 75	254	97	0.80(0.59–1.08)	0.70(0.42–1.11)
Menopause Status	Pre	348	164	1.00	1.00
	Post < 5 years	168	60	0.76(0.53–1.08)	0.98(0.62–1.53)
	Post ≥ 5years	1299	422	0.69(0.56–0.86)	0.82(0.56–1.22)
	Unknown	66	25	0.80(0.49–1.32)	0.89(0.50–1.53)
Tumor size	T1	1138	367	1.00	1.00
	T2	346	213	1.91(1.56–2.34)	1.84(1.47–2.33)
	T3 and T4	13	12	2.87(1.30–6.32)	2.56(1.07–6.09)
	Unknown	384	79	0.63(0.49–0.83)	0.67(0.50–0.93)
Tumor type	Ductal	1325	542	1.00	1.00
	D and L	35	17	1.19(0.66–2.13)	1.01 (0.54–1.90)
	Lobular	217	88	1.00(0.77–1.30)	0.87(0.64–1.20)
	Others	306	24	0.20(0.12–0.30)	0.29(0.18–0.46)
Histological grade	I	488	134	1.00	1.00
	II	807	305	1.37(1.09–1.73)	1.02(0.80–1.31)
	III	563	227	1.46(1.14–1.87)	1.10(0.82–1.50)
	Unknown	23	5	0.79(0.29–2.12)	1.40(0.46–4.31)
Estrogen receptor	Positive	1538	606	1.00	1.00
	Negative	218	61	0.71(0.52–0.96)	0.64(0.42–0.99)
	Unknown	125	4	0.09(0.03–0.22)	0.06(0.00–0.82)
Progesterone receptor	Positive	1320	531	1.00	1.00
	Negative	436	135	0.77(0.61–0.96)	0.78(0.56–1.07)
	Unknown	125	5	0.10(0.04–0.24)	3.80(0.30–47.42)
Her-2 status	Negative	1041	423	1.00	1.00
	Positive	174	66	0.93(0.69–1.27)	0.84(0.60–1.20)
	Unknown	666	182	0.68(0.56–0.82)	0.98(0.78–1.24)
Multifocality	No	1184	386	1.00	1.00
	Yes	208	144	2.12(1.67–2.70)	1.90(1.45–2.47)
	Unknown	489	141	0.89(0.71–1.10)	0.86(0.67–1.09)
Vascular invasion	No	1056	268	1.00	1.00
	Yes	87	97	4.40(3.20–6.04)	3.74(2.66–5.27)
	Unknown	738	306	1.63(1.36–1.98)	2.10(1.68–2.62)

[a]Adjusted including screening, age, menopause status, tumor size, tumor type, histological grade, estrogen status, progesterone status, Her-2 status, multifocality, and vascular invasion

not undergo SNB procedure and were excluded in final study population, they were mainly divided into two groups, carcinoma in situ and advanced invasive tumors where majority were T2 tumors. The reliability of collected data and accuracy of registration might be questioned; however, the quality of the INCA registry is regarded as very high with periodic validation control of data recording [20].

Assessment of axillary lymph status is essential because it predicts the clinical outcome and it also determines the extent of axillary surgery and adjuvant/systemic therapy. Node-negative patients do not benefit from axillary surgery,

Table 3 Potential predictive factors and risk of SN metastases separately for center A and center B

Determinants	Category	Center A			Center B		
		SN positive	SN negative	OR 95% CI	SN positive	SN negative	OR 95% CI
Screening	No	297	179	1.00	422	164	1.00
	Yes	552	179	0.62(0.46–0.86)	571	133	0.63(0.47–0.88)
	Unknown	39	16	0.82(0.42–1.60)	–	–	–
Age	≤ 50	157	86	1.00	180	76	1.00
	51–74	620	237	1.02(0.59–1.78)	670	175	0.82(0.48–1.44)
	≥ 75	111	51	0.69(0.34–1.39)	143	46	0.69(0.34–1.37)
Menopause status	Pre	167	90	1.00	181	74	1.00
	Post < 5 years	91	29	0.67(0.34–1.29)	77	31	1.32(0.70–2.50)
	Post ≥ 5 years	589	238	0.88(0.50–1.52)	710	184	0.77(0.43–1.34)
	Unknown	41	17	0.81(0.40–1.67)	25	8	0.87(0.34–2.16)
Tumor size	T1	394	191	1.00	744	176	1.00
	T2	136	105	1.63(1.16–2.30)	210	108	2.13(1.54–2.94)
	T3 and T4	4	7	6.28(1.50–26.40)	9	5	1.48(0.44–4.90)
	Unknown	354	71	0.60(0.42–0.84)	30	8	1.70(0.72–4.04)
Tumor type	Ductal	620	306	1.00	704	236	1.00
	D and L	25	12	0.89(0.42–1.88)	10	5	1.20(0.38–3.79)
	Lobular	80	45	0.90(0.58–1.40)	136	43	0.84(0.54–1.30)
	Others	163	11	0.23(0.11–0.50)	143	13	0.30(0.17–0.57)
Histological grade	I	218	66	1.00	270	68	1.00
	II	363	169	1.31(0.91–1.89)	444	136	0.80(0.56–1.16)
	III	290	136	1.49(0.98–2.26)	273	91	0.80(0.51–1.24)
	Unknown	17	3	1.30(0.31–5.37)	6	2	2.38(0.36–15.87)
Estrogen receptor	Positive	679	335	1.00	859	271	1.00
	Negative	98	35	0.59(0.33–1.04)	120	26	0.72(0.38–1.40)
	Unknown	111	4	0.07(0.00–1.04)	14	–	–
Progesterone receptor	Positive	578	290	1.00	742	241	1.00
	Negative	199	79	0.71(0.48–1.08)	237	56	0.79(0.50–1.23)
	Unknown	111	5	4.15(0.31–54.96)	14	–	–
HER-2	Negative	630	292	1.00	411	131	1.00
	Positive	77	44	1.04(0.66–1.64)	97	22	0.63(0.37–1.10)
	Unknown	181	38	0.93(0.59–1.50)	485	144	1.04(0.77–1.42)
Multifocality	No	452	194	1.00	732	192	1.00
	Yes	93	71	1.58(1.07–2.32)	115	73	2.21(1.50–3.23)
	Unknown	343	109	0.72(0.53–0.99)	146	32	0.88(0.56–1.40)
Vascular invasion	No	148	41	1.00	908	227	1.00
	Yes	18	34	6.10(2.98–12.50)	69	63	3.04(2.03–4.57)
	Unknown	722	299	1.64(1.10–2.44)	16	7	1.80(0.66–4.93)

and they may suffer from complications regardless of the type of surgery performed, i.e., SNB or ALND [9]. However, the incidence of SN metastasis has been reported to be 33.2% in invasive breast cancer [21].

SNB has been used as standard method for the assessment of axillary status since the early 2000s, and usually, a SNB will be followed by an axillary dissection in case of SN metastases, but for the last 5 years and in recent publications, ALND has been questioned in patients with metastatic SN due to the encouraging survival results for patient not undergoing axillary surgery [22]. This has led to calls for more conservative management

of the axilla in early breast cancer, and there is still continued debate about the role of axillary dissection in this patient population [23].

In this study, we observed that tumor size is an independent predictive factor for positive SN status, where SN metastases were observed in 367 patients with T1 (24.3%). Capdet et al. showed in a study involving 1416 patients that SN metastases were detected in 368 patients (26%) with T1 cancer, and young age, tumor size and location, histological type, histological grade, and lymph vascular invasion appeared to be significant risk factors of SN involvement [24]. Viale et al. showed in a study involving more than 4000 patients that tumor size and peritumoral vascular invasion emerged as the most powerful independent predictors for SN metastases [21].

In our study, the risk of SN metastases was not influenced by histological grade (Table 2); other studies have shown that the risk of SN metastases increased not only depending on tumor size but also on the histological grade and the patient age. Mustafa et al. showed in a study involving more than 2000 patients with T1 tumors that histological grades II–III in women before the age of 40 years had higher incidence of sentinel node involvement compared with histological grade I [25].

We observed in this study that the risk of SN involvement was low in tumors of rare type, e.g., medullary breast cancer. However, all rare tumors were merged in one sub-group in this study as these types were rare and separate analyses were difficult due to poor statistical power (Table 2).

We observed in our study that the strongest independent predictor of SN involvement was lymphovascular invasion (3.74; 2.66–5.27) followed by, in order of significance, size of the tumor (2.56; 1.07–6.09) and multifocality (1.90; 1.45–2.47), while Gajdos et al. showed in a study which involved 850 consecutive patients who underwent ALND for T1 breast cancer that axillary lymph node metastases were most significantly related to lymphatic invasion in the primary tumor, followed by tumor size and patient age [26]. Yoshihara et al. has showed in their evaluation of 1300 patients that lymphovascular invasion and tumor size emerged as the most powerful independent predictors of ALN metastases, followed by the location of the tumor in the breast and the presence of multiple foci [27]. However, the usefulness of lymphovascular involvement in decision making before surgery is of limited clinical value as this factor is not known until the final pathological report is available.

Mammography screening for breast cancer becomes more prevalent; improvements in imaging and new techniques make breast tumors easier to be found at smaller sizes than before [28]. In this study, we observed that breast cancer which is detected by mammography screening had lower risk for metastatic involvement of the sentinel nodes. This is probably due to many different factors but most possibly because of early detection of invasive tumors with small size less than 10 mm which is in turn associated with lower risk for SN metastases.

The possibility of metastatic involvement of SN in breast cancer with negative hormonal status particularly estrogen receptor status has not been established clearly compared with receptor-positive tumor. Our findings indicate that the risk of SN metastases is low in tumors with negative hormonal status for estrogen (0.64; 0.42–0.99). Mattes et al. observed in their study including 7274 patients with T1–T3 infiltrating ductal cancer that HR−/HER2− cancers had a significantly lower risk (OR 0.686) of nodal positivity than the HR+/HER2− subtype [29]. Similarly has Ugras et al. showed in their study involving 11,596 patients that nodal metastases were least frequent in triple negative (TN) cancers compared with other subtypes [30].

The results of this study showed that it is possible to identify patients with invasive breast cancer with a high risk of metastatic involvement of the sentinel nodes. This knowledge is useful in clinical practice and it might help in order to improve planning for surgical or systemic therapy. Furthermore, this study might help in identifying patients with a high probability of node-negative tumor where the SN procedure may possibly be omitted, although it is still very difficult to identify and select cases defiantly as the most powerful predictors for metastases to SN according to many studies are those which are available after histopathological examination such as lymphovascular invasion.

Conclusions

We conclude that SN metastasis is less likely to occur in women with invasive breast cancer diagnosed by screening mammogram and in tumors with negative estrogen status. Tumors larger than 20 mm, multifocality, or lymphovascular invasion are also factors associated with higher risk for SN metastases.

Abbreviations

ALND: Axillary lymph node dissection; CI: Confidence interval; FISH: Fluorescent in situ hybridization; HER2: Human epidermal growth factor receptor 2; HR: Hormone receptor; IHC: Immunohistochemistry; INCA: Information Network for Cancer Care; ITC: Isolated tumor cell; LU-Dnr: Lund document number; NHG: Nottingham histological grading; OR: Odds ratio; RCC-Syd: Regional Cancer Center in Southern Sweden; SN: Sentinel node; SNB: Sentinel node biopsy; SPSS: Statistical Package for the Social Sciences; TN: Triple negative; TNM: Tumor lymph node metastasis; WHO: World Health Organization

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Availability of data and materials
The data that support the findings of this study are available from INCA but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of INCA.

Authors' contributions
All authors were involved in drafting the manuscript or revising it critically for important intellectual content and approved the final manuscript.

Ethics approval and consent to participate
The present study was approved by the Ethic Committee at Lund University, Lund, Sweden. (LU-Dnr 2013/821).

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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Study III

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Determinants for non-sentinel node metastases in primary invasive breast cancer: a population-based cohort study of 602 consecutive patients with sentinel node metastases

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Abstract

Background: Sentinel node biopsy (SNB) is the standard procedure for axillary staging in patients with clinically lymph node negative invasive breast cancer. Completion axillary lymph node dissection (c-ALND) may not be necessary for all patients as a significant number of patients have no further metastases in non-sentinel nodes (non-SN) and c-ALND may not improve survival. The first aim of our study is to identify clinicopathological determinants associated with non-SN metastases. The second aim is to determine the impact of the number of sentinel node (SN) with macro-metastases and the type of SN metastases on metastatic involvement in non-SN.

Methods: This is a retrospective study of 602 patients with primary invasive breast cancer operated on with SNB and c-ALND in Lund and Malmö during 2008–2013. All these patients had micro- and/or macro-metastases in SNs. Information was retrieved from the national Information Network for Cancer Care (INCA). The risk of metastases to non-SNs were analyzed in relation to clinicopathological determinants such as age, screening mammography, tumour size, tumour type, histological grade, estrogen status, progesterone status, HER2 status, multifocality and lymphovascular invasion. Additionally, we compared the association between the number of the SN and the type of metastases in SN with the risk of metastases to non-SNs. Binary logistic regression was used, yielding odds ratios (OR) with 95% confidence intervals (CI).

Results: We found that 211 patients (35%) had metastases in non-SNs and 391 patients (65%) had no metastases in non-SNs. Lobular type (18%) of breast cancer (1.73; 1.0 1-2.97) and multifocal (31.3%) tumours (2.20; 1.41–3.44) had a high risk of non-SNs metastases. As compared to only micro-metastases, the presence of macro-metastases in SNs was associated with a high risk of metastases to non-SNs (4.91; 3.01–8.05). The number of SN with macro-metastases, regardless of the number of SNs removed by surgery, increases the risk of finding non-SNs with metastases. The total number of SN removed by surgery had no impact on diagnosis of metastases in non-SNs. No statistically significant associations were observed regarding other studied determinants.

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Conclusion: We conclude in the present study that lobular cancer and multifocal tumours were associated with a high risk of non-SN involvement. The presence of the macro-metastases in SNs and the number of SN with macro-metastases has a positive association with presence of metastases in non-SNs. The total number of SNs removed by surgery had no impact on finding metastases in non-SNs. These factors may be valuable considering whether or not to omit c-ALND.

Keywords: Invasive breast cancer, Sentinel node metastases, Non-sentinel node metastases, Determinants, Completion axillary lymph node dissection

Background

Sentinel node biopsy (SNB) is the standard procedure for axillary staging in patients with clinically lymph node negative breast cancer. Completion axillary lymph node dissection (c-ALND) has traditionally been performed at many breast centres when the final pathological report reveals macro-metastases in 1–2 sentinel lymph nodes [1, 2]. However different studies, including Z0011 trial from American College of Surgeons Oncology Group (ACOSOG), show that c-ALND is not contributed to better survival [3, 4]. Still axillary lymph node status remains one of the most important and powerful prognostic factor in invasive breast cancer as it predicts clinical outcome and is an indication for systemic therapy [5, 6].

SN metastases are classified according to the size of metastases; isolated tumour cells (ITC < 0.2 mm), micro-metastases (0. 2-2.0 mm) and macro-metastases (> 2.0 mm) [7]. In our department, and still in many other centers, the main indication to perform a c-ALND is involvement of SN with macro-metastases. The presence of ITC or micro-metastases is no longer an indication to perform a c-ALND when the patient is planned to undergo radiation therapy, e.g. in breast conserving surgery [8].

There are many benefits of SNB such as avoidance of unnecessary ALND in patients with no axillary metastases. Most of the complications associated with ALND might also occur after SNB. However, the risk of developing bleeding or infection post operatively is less likely to occur following SNB as compared to ALND. Moreover, the incidence of developing pain, sensory disorder or lymph oedema in the upper arm is very low after SNB [9]. Still the SNB as a procedure is time consuming and needs resources.

It is still unknown if c-ALND is necessary to be performed in all cases with metastatic involvement of SN and the possibility of omitting c-ALND has been discussed in several studies, as the risk of metastases to non-SNs may be low and the impact of an ALND on survival is not clear [3, 4].

The first aim of our study is to identify clinicopathological determinants associated with metastases to non-SNs in patients with metastases in SNs. The second aim is to determine the impact of the number of SN with macro-metastases and the type of SN metastases on metastatic involvement in non-SNs.

Methods

The Information Networks for Cancer (INCA) is a nationwide database for breast cancer in Sweden which is available on an IT platform. This registry collects information about the cancer care and manages long term follow up. The center in Southern Sweden which manages the registry is the Regional Cancer Center in Southern Sweden (RCC-Syd). By law, all cancer diagnoses have to be reported to the Swedish Cancer Registry and this routine is implemented through INCA.

In this study we included all women operated on because of breast cancer in Lund and Malmö during the period of January, 1st 2008 to December, 31st 2013. They were identified using the clinical registry INCA, and a total number of 3979 cases with breast cancer were found using the unique twelve-digit Swedish civil registration number.

We excluded the following patients from the main study population; 43 cases with previous breast cancer, 122 cases with in situ breast cancer, 82 cases with bilateral breast cancer, 1040 cases who were not operated on with SNB and 25 patients who had received neoadjuvant systemic therapy. There were two patients who had unknown information about the systemic therapy and one patient had unknown status about SN surgery, finally all 30 male patients were excluded in this analysis. Among all patients in the study population there were 1881 patients who had no metastases in SN. We identify totally 671 cases with SN metastases including 69 women who did not undergo a subsequent ALND. The final study population following these exclusions resulted in 602 cases with metastases in SN and all these cases were operated on with c-ALND (Fig. 1).

This study was approved by the regional ethical review board of Lund University (reference 2013/821).

All included patients in this study have been reviewed and discussed at a multidisciplinary breast cancer conference (surgery, radiology, oncology and pathology) at Skåne University Hospital in Malmö and Lund. INCA has a unique and specially designed registration form and all available information about every breast cancer case transfers to the INCA platform. In the present study we retrieved information from INCA about SLNB

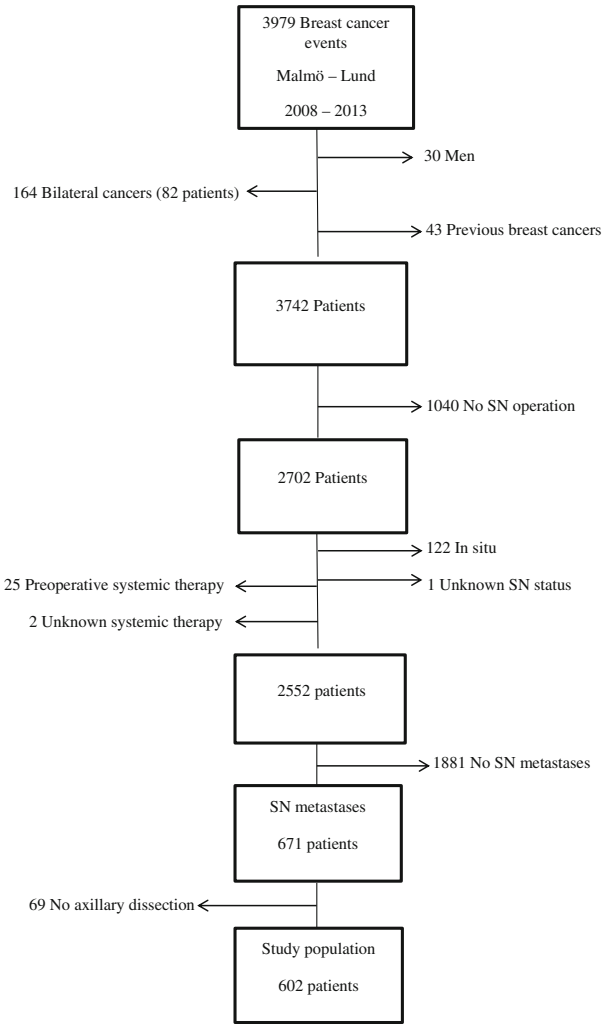

Fig. 1 Patient selection

and c-ALND, i.e. number of lymph nodes removed, type of metastases as well as information about histopathological type and grade, receptor status, HER2 status, tumour size, multifocality, lymphovascular invasion, age and menopause status.

The mode of cancer detection was recorded as screening-detected vs. not detected by screening mammography. We identify menopause status as pre- or post-menopausal. The post-menopausal women were further more sub-classified according to their last menstruation, i.e. 6 months to 5 years or more than 5 years after menopause.

WHO-classification system has been used to identify the histopathological types, accordingly six types of invasive cancer were identified [10–12]. Furthermore these six types were merged into four different groups i.e. ductal, lobular, combined ductal with lobular and other rare types. Nottingham histological grading score (NHG) was used to define the histological grades [13, 14]. TNM classifications were used to define the tumour size. T1

tumour ≤ 20 mm, T2 tumour 21-50 mm and T3-T4 > 50 mm [10]. Lymphovascular invasion was defined according to the Swedish society of pathology (KVAST) classification, i.e. invasion of vessel wall, underlying endothelium or vascular spaces by tumour cells.Two or more tumours with normal tissue and/or in situ tumours at a distance of 20 mm were regarded as multifocal tumours [15]. Estrogen and progesterone were measured by immunohistochemistry (IHC), and a positive receptor status was identified when the receptor percentage was more than 10% while receptor status was regarded as negative when the percentage was less than 10% [7]. IHC was used to analyze HER2 protein and test results 1+, 2+, or 3+ were reported. IHC test was completed by Fluorescent In Situ Hybridization (FISH) in cases where HER2 was 2+ or 3+. HER2 status was classified as negative when HER2 IHC =0–1, 2+ or 3+ in non-amplified tumours. All cases of HER2 2+ or 3+, which were amplified by FISH, regarded as positive HER2 tumours [16].

In the present analysis metastases in non-SNs were regarded as macro metastases when the size was > 2 mm and as micro-metastases when the size was 0. 2-2.0 mm. Metastases with a size less than 0.2 mm were regarded as isolated tumour cells (ITC). All ITC regarded as no metastases according to international guideline for lymph node metastases [7]. Non-SNs with macro- or micro-metastases were regarded as positive, and those without metastases as negative.

We used binary logistic regression to compare the association between different determinants and metastases in non-SNs. We adjusted all analyses for all studied determinants i.e. histopathological type and grade, presence of multifocality, presence of lymphovascular invasion, receptor status for estrogen and progesterone, HER2 status, tumour size, menstrual status, screening and age. Binary logistic regression was also used to compare the association between the number of the SN, the type of metastases in SN and the risk of metastases to non-SNs. These analyses included a limited number of events and only a selected set of co-variates were included in the multivariate analysis, i.e. those statistically significantly associated with metastases in non-SNs (screening, tumour types and multifocality). This analysis was also stratified for the number of SLNs which had been removed. Odds ratios (OR) with 95% confidence intervals (CI) were analyzed. For all analyses we used the Statistical Package for the Social Sciences (SPSS) program version 22.0 (SPSS Institute, Chicago, IL, USA).

Results

Out of the 602 patients operated on with c-ALND, 211 patients (35%) had metastases in non-SNs and 391 patients (65%) had no metastases in non-SNs, Table 1. There was a high risk of metastases to non-SNs in women with lobular type tumour (18%) compared with ductal type tumours (1.73; 1.01–2.97). Multifocal tumour (31.3%) were also associated with a high risk of non-SN metastases compared with unifocal tumours (2.20; 1.41–3.44), Table 2. There was a high risk for non-SN metastases in 11 patients with unknown status for mammography screening as mode of detection, compared to those patients who were not diagnosed by screening mammography (4.70; 1.36–16.19), Table 2. There were no other statistically significant associations between all other studied determinants and involvement of non-SNs, Table 2.

The total number of SNs removed by surgery had no clear impact on finding metastases in non-SNs, Table 3. The presence of macro-metastases in SN was associated with a high risk of metastases to non-SNs compared with presence of only micro-metastases in SNs (4.91; 3.01–8.05), Table 3. Stratified analysis showed that the number of SNs with macro-metastases, regardless the number of SNs removed by surgery, increases the risk of finding non-SNs with metastases. Combined analysis using one SN with only micro-metastases as reference showed a positive correlation between the number of SNs with macro-metastases and the possibility of non-SN involvement with metastases, Table 4.

Discussion

The present registry-based study showed that 65% of patients, who underwent c-ALND because of SN metastases, have no further additional non-SN involvement. Lobular types (18%) and multifocal tumours (31.3%) were associated with a high risk of non-SN metastases. The total number of SN removed by surgery had no impact on finding metastases in non-SNs. On the contrary the presence of macro-metastases in SNs contributed with higher risk of metastases to non-SNs. The number of SN with macro-metastases is also associated with the higher risk of finding non-SNs with metastases. Furthermore there was a positive association between the number of SN with macro-metastases and the probability of non-SNs involvement with metastases regardless the number of SNs removed by surgery.

Axillary lymph node status is an important factor in managing patients with primary breast cancer. SNB is the standard method for staging, however the value of the c-ALND has been questioned during the last decade as the majority of these patients have disease-free non-SNs and omitting c-ALND probably has no impact on survival [3].

This analysis included 602 patients from a non-selected population-based cohort of consecutive cases with essential data available from the main breast cancer registry in southern Sweden (INCA) which is a strength of our study. A limitation is however, that we had no information on why 69 women who had a positive SNB did not undergo a c-ALND. Furthermore, analysis based on the data collected from a registry and the reliability of collected data

Table 1 Potential determinants in relation to non-sentinel node status

Determinants	Category	Total	Negative Non-SN		Positive Non-SN	
			N	%	N	%
Screening	No	309	197	50.4	112	53.1
	Yes	278	190	48.6	88	41.7
	Unknown	15	4	1.0	11	5.2
Age	≤50	151	103	26.3	48	22.7
	51–74	370	239	61.1	131	62.1
	≥75	81	49	12.5	32	15.2
Menopause Status	Pre	153	104	26.6	49	23.2
	Post < 5 ys	57	42	10.7	15	7.1
	Post ≥5 ys	370	231	59.1	139	65.9
	Unknown	22	14	3.6	8	3.8
Tumour size	T1	331	220	56.3	111	52.6
	T2	193	120	30.7	73	34.6
	T3 & T4	10	6	1.5	4	1.9
	Unknown	68	45	11.5	23	10.9
Tumour type	Ductal	490	331	84.7	159	75.4
	D & L	14	5	1.3	9	4.3
	Lobular	79	41	10.5	38	18.0
	Other	19	14	3.6	5	2.4
Histological grade	I	118	82	21.0	36	17.1
	II	272	176	45.0	96	45.5
	III	210	132	33.8	78	37.0
	Unknown	2	1	0.3	1	0.5
Estrogen receptor	Positive	545	358	91.6	187	88.6
	Negative	56	33	8.4	23	10.9
	Unknown	1	0	0.0	1	0.5
Progesterone receptor	Positive	478	319	81.6	159	75.4
	Negative	122	72	18.4	50	23.7
	Unknown	2	0	0.0	2	0.9
HER2 status	Negative	383	248	63.4	135	64.0
	Positive	61	33	8.4	28	13.3
	Unknown	158	110	28.1	48	22.7
Multifocality	No	355	247	63.2	108	51.2
	Yes	129	63	16.1	66	31.3
	Unknown	118	81	20.7	37	17.5
Vascular invasion	No	241	166	42.5	75	35.5
	Yes	91	60	15.3	31	14.7
	Unknown	270	165	42.2	105	49.8

might be questioned, however the quality of the INCA registry is regarded as very high with periodic validation control [17]. A potential problem is, however, that the availability of information about different clinicopathological determinants used in the present study might be limited or unavailable preoperatively, before the final pathological results are available, and this may limit the pre-operative value of these determinants. Previous studies have suggested that the internal mammary lymph node status is an independent prognostic factor. A limitation of the present analysis is that there was no information on internal mammary lymph nodes in the INCA data base. However, this

Table 2 Potential determinants for non-sentinel node metastases

Determinants	Category	Negative Non-SN	Positive Non-SN	OR 95% CI	OR 95% CI[a]
Screening	No	197	112	1.00	1.00
	Yes	190	88	0.81 (0.58–1.15)	0.81 (0.54–1.21)
	Unknown	4	11	4.84 (1.50–15.55)	4.70 (1.36–16.19)
Age	≤50	103	48	1.00	1.00
	51–74	239	131	1.18 (0.79–1.76)	1.50 (0.53–2.06)
	≥75	49	32	1.40 (0.80–2.46)	1.08 (0.45–2.60)
Menopause Status	Pre	104	49	1.00	1.00
	Post <5ys	42	15	0.76 (0.38–1.50)	0.79 (0.34–1.86)
	Post ≥5ys	231	139	1.28 (0.86–1.90)	1.21 (0.60–2.44)
	Unknown	14	8	1.21 (0.48–3.08)	1.45 (0.52–4.05)
Tumour size	T1	220	111	1.00	1.00
	T2	120	73	1.21 (0.83–1.74)	1.11 (0.74–1.66)
	T3 & T4	6	4	1.32 (0.36–4.78)	0.78 (0.19–3.14)
	Unknown	45	23	1.01 (0.58–1.76)	0.76 (0.40–1.44)
Tumour type	Ductal	331	159	1.00	1.00
	D & L	5	9	3.75 (1.24–11.36)	2.93 (0.92–9.37)
	Lobular	41	38	1.93 (1. 19-3.12)	1.73 (1.01–2.97)
	Others	14	5	0.74 (0. 26-2.10)	0.85 (0. 29-2.50)
Histological grade	I	82	36	1.00	1.00
	II	176	96	1.24 (0.78–1.98)	0.88 (0.53–1.46)
	III	132	78	1.35 (0.83–2.18)	0.94 (0.54–1.65)
	Unknown	1	1	2.28 (0. 14-37.43)	1.23 (0.07–21.34)
Estrogen receptor	Positive	358	187	1.00	1.00
	Negative	33	23	1.33 (0.76–2.34)	1.04 (0.47–2.34)
	Unknown	0	1	–	–
Progesterone receptor	Positive	319	159	1.00	1.00
	Negative	72	50	1.40 (0.93–2.09)	1.17 (0.66–2.07)
	Unknown	0	2	–	–
Her-2 status	Negative	248	135	1.00	1.00
	Positive	33	28	1.56 (0.90–2.69)	1.52 (0.82–2.82)
	Unknown	110	48	0.80 (0.54–1.19)	0.88 (0.55–1.39)
Multifocality	No	247	108	1.00	1.00
	Yes	63	66	2.40 (1.59–3.62)	2.20 (1.41–3.44)
	Unknown	81	37	1.04 (0.67–1.64)	0.99 (0.61–1.60)
Vascular invasion	No	166	75	1.00	1.00
	Yes	60	31	1.14 (0.68–1.91)	1.13 (0.64–1.98)
	Unknown	165	105	1.41 (0.98–2.03)	1.31 (0.86–1.99)

[a]Adjusted for screening, age, menopause status, tumour size, tumour type, histological grade, estrogen status, progesterone status, HER2 status, multifocality, lymphovascular invasion

information is not used in clinical practice and currently has no impact on treatment.

In the present analysis we found that 65% of patients, underwent c-ALND because of SN metastases, have no further additional non-SN metastases, this may suggest the possibility of omitting ALND in certain cases with SN metastases but this demands accurate identification of low risk patients. Different studies have questioned the value of c-ALND even if there are metastases in the SN. The Z0011 randomized trial from the American College of surgeons

Table 3 Number and type of metastases in sentinel node and risk of metastases in non-sentinel node

SN	Category	Total (n)	Negative Non-SN (n)	Positive Non-SN (n)	Positive Non-SN (%)	OR (95% CI)	OR[a] (95% CI)
SN removed (n)	1	118	84	34	28.8	1.00	1.00
	2	208	125	83	39.9	1.64 (1.01–2.66)	1.34 (0.77–2.31)
	3	166	110	56	33.7	1.26 (0.75–2.10)	1.08 (0.61–1.93)
	4	83	56	27	32.5	1.19 (0.65–2.19)	0.96 (0.48–1.90)
	≥5	25	15	10	40.0	1.65 (0.67–4.03)	1.71 (0.65–4.53)
	Unknown	2	1	1	–	–	–
	Total	602	391	211			
Type of metastases in SN[b]	Micro	186	159	27	14.5	1.00	1.00
	Macro	414	232	182	43.9	4.62 (2.94–7.26)	4.91 (3.01–8.05)
	Unknown	2	0	2	–	–	–
	Total	602	391	211			

[a]Adjusted for screening, age, menopause, tumour size, tumour type, histological grade, estrogen receptors, progesterone receptors, HER2, multifocality and lymphovascular invasion
[b]If both micro- and macro-metastases, classified as macro-metastases

Oncology Group (ACOSOG) compared ALND versus no axillary surgery in patients with a maximum of two SNs with metastases, and the study supported the view that there is no negative impact on survival for patients where an ALND is omitted [4].

Our study showed that there was a high risk of metastases to non-SNs in patients with lobular type compared with ductal type tumours. Adachi Y. et al. showed in their study including 3771 patients that 31 cases with lobular type (18%) had more non-SN metastases than 457 (21%) cases with ductal type and lobular cancer was an important factor for the prediction of non-SN positivity in cases with macro-metastases in SNs. Adachi Y. et al. thus suggested that omitting c-ALND for lobular type with positive SNs requires more consideration [18]. Previous studies showed that loss of E-Cadherin in the extra cellular space and the differences in gene expression between lobular and ductal cancers are associated with immune response, cell invasion and cell adhesion which might be a possible reason for metastatic involvement of lymph nodes in lobular type of breast cancer [19].

Table 4 Number of macrometastases in sentinel node and risk of metastases in non-sentinel nodes

SN removed	Macro- metastases	Total	Negative Non-SN	Positive Non-SN	Positive Non-SN	Stratified analysis		Combined analysis	
(n)	(n)		(n)	(n)	(%)	OR (95% CI)	OR (95% CI)[a]	OR (95% CI)	OR (95% CI)[a]
1	0	47	39	8	17.0	1.00	1.00	1.00	1.00
	1	69	44	25	36.2	2.77 (1. 12-6.85)	2.65 (1.05–6.66)	2.77 (1. 12-6.85)	2.65 (1.05–6.66)
	Unknown	2	1	1	–	–	–	–	–
2	0	51	45	6	11.7	1.00	1.00	0.65 (0. 21-2.04)	0.65 (0. 21-2.07)
	1	105	60	45	42.8	5.62 (2. 21-14.33)	4.83 (1.87–12.49)	3.66 (1.56–8.58)	3.09 (1.30–7.39)
	2	52	20	32	61.5	12.00 (4.33–33.23)	11.12 (3.97–31.19)	7.80 (3.03–20.04)	7.43 (2.83–19.50)
3	0	59	51	8	13.5	1.00	1.00	0.76 (0. 26-2.22)	0.68 (0. 23-2.02)
	1	58	39	19	32.7	3.11 (1. 23-7.83)	3.68 (1.32–10.24)	2.37 (0.93–6.07)	2.15 (0.82–5.64)
	2	28	14	14	50.0	6.37 (2. 23-18.23)	6.30 (1.99–19.99)	4.87 (1.69–14.10)	4.18 (1.40–12.50)
	3	21	6	15	71.4	15.94 (4.77–53.18)	16.96 (4.42–65.12)	12.19 (3.62–41.05)	10.02 (2.89–34.81)
4	0	24	21	3	12.5	1.00	1.00	0.70 (0. 17-2.91)	0.57 (0. 13-2.49)
	1	29	22	7	24.1	2.23 (0.51–9.77)	3.34 (0.55–20.15)	1.55 (0.50–4.85)	1.56 (0.49–4.94)
	2	11	7	4	36.3	4.00 (0.71–22.43)	9.46 (1. 26-70.85)	2.79 (0.66–11.82)	2.92 (0.67–12.65)
	3	14	4	10	71.4	17.50 (3. 28-93.49)	17.18 (2.34–126.2)	12.19 (3.04–48.77)	9.25 (2. 22-38.53)
	4	5	2	3	60.0	–	–	–	–

Stratified analysis; comparisons within groups defined by number of removed SNs. Combined analysis; all groups compared using one SN with only micro-metastases as reference
[a]Stratified and combined analysis adjusted for screening, tumour type, and multifocality

We observed in our study that mode of detection (screening mammography vs not) had no clear impact on finding non-SN metastases. Tvedskov et al. showed in their study involving 995 patients, registered in the Danish Breast Cancer Cooperative Group (DBCG) Database, that there was no large difference in the risk of non-SN metastases between patients with clinically detected and screening detected cancers with micro-metastases or ITC in the SN [20]. In our study there was a high risk for non-SN metastases in 11 patients with unknown status for mode of detection. This may be a chance finding, but we choose to include this variable in the multivariate analyses for type and number of SN metastases.

In this cohort we observed that multifocal tumour were associated with high risk of non-SN involvement with metastases. Similarly Cabioglu et al. found in their study including 1322 patients with invasive breast cancer that multifocal tumour had more potentials of metastases to axillary lymph nodes compared with unifocal invasive tumour, regardless of primary tumour size. It is unclear with underlying biology regarding the multifocality and increased risk of lymph node involvement but the aggressiveness of multifocal tumours has been proposed as underlying cause in some studies, another proposed theory is finding higher proportion of lobular type in multifocal tumours compared with unifocal tumour. Furthermore using the largest diameter or the combined diameter of the multifocal tumors, as the size of the tumour, has been proposed as a possible explanation [21].

There were no statistically significant findings for other determinants included in this study i.e. age, menopause status, tumour size, histological grade, estrogen status, progesterone status, HER2 status, lymphovascular invasion. Y. Andersson et al. showed in their analysis that tumour size and histological grade were significantly associated with non-SN status [22]. Dighe L. et al. showed in their study that tumour size and vascular invasion were strongly associated with the metastatic involvement of SN, and they created a nomogram that facilitate preoperative decision-making regarding the extent of axillary surgery [23]. The use of nomograms has also been suggested by others, and some are available as a web-based tool [24]. A metanalysis performed by van la Parra RF. et al. included data from 56 candidate studies showed that eight different variables possibly related to high risk of finding non-SN metastases. These 8 individual characteristics were; size of metastases in the SN, extracapsular extension in the SN, number of the positive SN, number of the negative SN, tumour size, ratio of positive sentinel nodes, lymphovascular invasion in the primary tumour and method of detection, all these predictors were associated with high risk of finding metastases in non-SNs [25].

In this analysis we observed that the total number of SN removed by surgery has no impact on finding metastases in non-SNs, while the type of metastases in SN is an important predictor for non-SN metastases where presence of macro-metastases in SN strongly contributed with a high risk of finding additional non-SN involvement with metastases compared with presence of micro-metastases in SN. Van den Hoven I. et al. showed in their analysis including 513 patients with positive SN underwent c-ALND at 10 participating hospitals that the presence of negative SN as well as continuous size of the largest SLN metastases are strong predictors for the presence of metastases in the non-SNs [26]. Similarly, Elisabeth A. Mittendorf et al. and Hwang RF. et al. have observed in their studies that the size of metastases in the SN was the most important predicting variable for the presence of additional non-SN involvement [27, 28].

We also found that not only the type of metastases has a positive association with the risk of non-SN metastases but the number of SN with macro-metastases was associated with the risk of metastases in non-SNs regardless of the total number of SNs removed at surgery. Combined analysis, using one SN with only micro-metastases as a reference, showed a positive correlation between the number of SN with macro-metastases and the risk of non-SN involvement with metastases. Siem A. Dingemans et al. showed in their analysis that in patients with macro-metastases in SNs, tumor size larger than 2 cm, extranodal growth, and non-negative SNs are predictors of non-SN involvement [29].

The present study provides evidence that clinicopathological determinants such as lobular type or multifocality as well as the type of SN metastases and the number of the SN with macro-metastases may possibly be used as supporting tools in evaluating the risk of lymphatic spread to the non-SNs and may help clinician in taking final decision before performing c-ALND, however the benefit of the c-ALND, even when there are macro-metastases in the non-SNs, is not clear and an accurate identification of the low risk patients who may possibly omit c-ALND is still difficult.

Conclusion

We conclude that lobular cancer and multifocal tumours are associated with a high risk of non-SN involvement. The presence of the macro-metastases in SNs, vs. only micro-metastases, and the number of SN with macro-metastases has a positive association with metastases in non-SNs. These factors may be valuable considering whether or not to omit c-ALND.

Abbreviations

ACOSOG: American College of Surgeons Oncology Group; ALND: Axillary Lymph Node Dissection; c-ALND: Completion Axillary Dissection; CI: Confidence Interval; FISH: Fluorescent in situ hybridization; HER2: Human Epidermal Growth Factor Receptor 2; IHC: Immunohistochemistry; INCA: Information Network for Cancer Care; ITC: Isolated Tumour Cell; KVAST: Swedish society of pathology; NHG: Nottingham Histological Grading;

non-SN: Non-Sentinel Node; OR: Odds Ratio; RCC-Syd: Regional Cancer Center in southern Sweden; SN: Sentinel Node; SNB: Sentinel Node Biopsy; SNM: Sentinel Node Metastases; SPSS: Statistical Package for the Social Sciences; TNM: Tumour Lymph Node Metastasis; WHO: World Health Organization

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Authors' contributions
All authors took part in the concept and design of the study (SM, LR, JM). SM acquired the data. Statistical analysis was performed by SM in collaboration with JM. All authors took part in interpretation and SM drafted the manuscript which was revised by LR and JM. All authors (SM, LR, JM) approved the final manuscript and take responsibility for all.

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Availability of data and materials
The data that support the findings of this study are available from INCA but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of INCA.

Ethics approval and consent to participate
The present study was approved by the regional ethical review board of Lund University (reference 2013/821). All former patients were informed through adds in local newspapers, according to the instructions of the ethical review board, about the study and that they could at any time ask the researchers to omit their data from the analyses.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Breast cancer and axillary lymph node status

Shabaz Majid is a consultant surgeon currently working at the Breast Unit, Kristianstad Central Hospital (CSK). His clinical interest is oncoplastic surgery of the breast and his field of research is breast cancer.

Axillary lymph node status is an important factor in management of patients with invasive breast cancer. Moreover, it guides further axillary surgery and adjuvant/neoadjuvant therapy. Nowadays, the majority of patients with breast cancer are diagnosed at an early stage and 65% of all cases have no axillary lymph node metastases (low risk patients). These patients have no benefit of axillary surgery. That is why de-escalation surgery and/or no axillary surgery may be considered in the future in order to avoid surgical complication. This thesis presents the association between primary invasive breast cancer and axillary lymph node status. Potential pre-operative clinicopathological predictors for presence or absence of axillary lymph node metastases are studied. The implementation of these predictors in clinical praxis might facilitate the identification of low risk patients.

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