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Lymph node status after sentinel node biopsy followed by axillary lymph node dissection among breast cancer patients: a population-based study

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List of abbreviations

SN	Sentinel node
NSN	Non-sentinel node
SNB	Sentinel node biopsy
ALND	Axillary lymph node dissection
LNM	Lymph node metastasis
ER	Estrogen receptor
PR	Progesterone receptor
HER2	Human epidermal growth factor receptor 2
Ki67	Antigen associated with cellular proliferation
BRE-grade	Bloom-Richardson-Elston grading system. I-III with III being the most aggressive

Abstract

Background: Axillary lymph node dissection (ALND) for breast cancer patients is performed if there are metastases in sentinel nodes (SNs). However, ALND does not seem to provide a therapeutic benefit for breast cancer patients with few involved SNs and the current literature indicates that patients with <3 positive SNs are overtreated if ALND is performed. Luminal A-like tumors are recommended chemotherapy if ≥ 4 lymph node metastases (LNMs) are present. The extent to which patients with luminal A-like tumors with ≥ 4 LNMs could be potentially undertreated with adjuvant chemotherapy if the indication for ALND is set to >2 positive SNs is unclear.

Aim: To quantify the group of luminal A patients with ≥ 4 LNMs who risk being undertreated with adjuvant chemotherapy and to investigate if the ratio between positive SNs and the total amount of examined SNs can provide additional information of the axillary tumor burden.

Material and methods: Patients in Region Västra Götaland with clinically node-negative breast cancer who had undergone sentinel node biopsy (SNB) followed by ALND were analyzed and the SNB findings were compared to the results from the ALND. The potentially undertreated luminal A group was quantified. The ratio of positive to total SNs and its ability to describe the axillary tumor burden was evaluated using Pearson's chi-square test.

Results and conclusion: 16 (17.6%) of the 91 patients with luminal A-like tumors had ≥ 4 LNMs and 2 of these had >2 positive SNs leaving 14 patients (15.4%) with <3 positive SNs and ≥ 4 LNMs. The ratio of positive SNs to the total amount of SNs examined was significant in a multivariable analysis for predicting ≥ 4 LNMs among luminal A-like patients ($p=0.027$). This was also true for all subtypes combined ($p<0.001$). Our results indicate that about one sixth of patients with luminal A-like tumors may be undertreated with adjuvant chemotherapy due to a falsely low axillary staging if the indication for ALND is set to >2 positive SN. The SN ratio could be effective at identifying many of these patients.

Lymph node status after sentinel node biopsy followed by axillary lymph node dissection: a population-based study

Background

Breast cancer is the most common form of cancer among women and approximately one tenth of women are affected during their lifetime. A sentinel node biopsy (SNB) is a well-established procedure which provides reliable prognostic information at the cost of low morbidity. The definition of a sentinel node (SN) is a lymph node that receives lymphatic drainage directly from a primary tumor. In Sweden, patients with SN positive breast cancer are recommended an axillary lymph node dissection (ALND) due to the risk of tumor engagement of additional lymph nodes, i.e. non-sentinel nodes (NSNs). ALND is associated with increased morbidity from early and late complications. Examples of early complications are nerve damage, bleeding, fat necrosis and seroma. Late complications include lymphedema, decreased arm mobility and chronic pain. If ALND provides no benefit, it should therefore be avoided. Around 80% of patients show no further lymph node metastases (LNMs) following the ALND [1].

Studies that have investigated the survival rate of ALND compared to only SNB among breast cancer patients with 1-2 positive SNs and with a clinical stage of T1-T2N0M0 have not found a difference in survival [2, 3]. In the ACOSOG Z0011 study, SNB positive patients were randomized to no further surgery or ALND. The patients were treated with partial mastectomy followed by breast radiation therapy and systemic treatment. Only SNB compared to SNB + ALND resulted in similar locoregional control and no significant difference in survival rate was observed. Long term follow-up 10 years later support the initial findings [4]. An objection to the generalizability of the study is that the patients generally had

a favorable prognosis with a median number of 1 LNM. Another limitation is that the study accrued only half of the originally planned number of patients which resulted in low statistical power of the study.

The prevalence of >2 positive SNs or extracapsular growth seems to be able to identify patients with a high risk of additional NSN metastases (72%) while the risk for patients with 1-2 positive SNs is much lower [3]. The current literature therefore suggests that many patients are overtreated when they undergo ALND based on 1-2 positive SNs and it has become a widespread standard to avoid ALND in such cases.

Breast cancer is classified into different subtypes based on the immunohistochemical profile which is determined by hormone receptor expression (estrogen receptor and progesterone receptor), HER2-expression, grade of proliferation (measured by the Ki67 proliferation marker) and differentiation. Luminal A is the subtype with the best prognosis. It is characterized by a low proliferation rate, a high expression of hormone receptors and a low expression of the HER2 receptor. Luminal B is hormone receptor positive but is characterized by a higher proliferation rate (Ki67) than Luminal A. The HER2-positive tumors are characterized by amplification of the HER2-receptor gene and can be subdivided into HER2/luminal (hormone receptor positive) and HER2/non-luminal (hormone receptor negative). The triple negative tumors are negative for hormone receptors and HER2. Having a low proliferation rate makes the Luminal A subtype less responsive to chemotherapy and several studies have questioned the benefit of chemotherapy in this patient group [5-7]. There is an inverse relationship between chemotherapy responsiveness and estrogen receptor expression as the latter serves as an indirect measure of other biomarkers related to tumor growth [8-10]. However, having ≥ 4 LNMs is strongly prognostic of a more advanced and aggressive disease among ER positive, HER2 negative breast cancer patients with macrometastases in SN [11, 12]. Patients with ≥ 4 involved lymph nodes at the time of

diagnosis also have a less favorable prognosis and a more aggressive breast cancer phenotype [13]. There are studies which have shown that high-risk hormone receptor positive breast cancer patients with ≥ 4 LNMs benefit from chemotherapy [14-18] although there are also studies which have failed to demonstrate this specifically for luminal A patients [5, 6]. A limitation of the studies that did not show a therapeutic benefit for chemotherapy for luminal A patients is that they did not primarily focus on patients with a high axillary tumor burden of ≥ 4 LNMs.

ALND does not exclusively serve a therapeutically function as it also provides important prognostic information. The current Swedish guidelines recommend adjuvant chemotherapy to all lymph node positive patients except for patients with luminal A-like tumors with < 4 LNMs for whom adjuvant chemotherapy can be omitted [19]. This means that the ALND provides crucial information for luminal A-like breast cancer patients who have < 3 positive SNs but ≥ 4 total LNMs (SNs + non-SNs).

Purpose

To identify the proportion of patients with a luminal A-like tumor with a clinically negative axillary status and < 3 positive SNs who after ALND have a total of ≥ 4 LNMs and therefore would have received a different systemic treatment recommendation if the ALND would not have been performed. The aim of this study is to quantify this potentially undertreated group.

A secondary objective was to describe the accuracy of the SNB to predict a high axillary tumor burden (a total of ≥ 4 LNM) depending on the ratio of positive/examined SNs. We see no reason to believe that SNB predictions would vary depending on subtype and will therefore include all subtypes to achieve a high enough power.

Material and methods

Hypothesis

5-10% of luminal A-like patients will be undertreated with adjuvant chemotherapy if the indication for ALND is set to >2 positive SNs.

Study population

The study is population-based and includes data from 370 patients who were registered as having undergone sentinel node biopsy (SNB) followed by axillary lymph node dissection (ALND) in Region Västra Götaland between 2014-2016. The data was obtained from Regionalt Cancercentrum Väst which is a register containing information of cancer patients regarding the number of positive SNs (micrometastases and macrometastases) and the number of LNMs from the ALND, the date of the breast cancer diagnosis, the responsible hospital and the social identity number for each patient. All registered patients were reviewed in the medical record system and further information was thus collected regarding immunohistochemistry (ER, PR, Ki67, HER2 and BRE grade), tumor size, radicality of surgery, other cancer diseases, axillary lymph node status (clinically and via ultrasound) and recurrence or death caused by the breast cancer. Only patients who had undergone SNB + ALND and who fulfilled the inclusion criteria and whose tumor could be classified were analyzed. Patients who had undergone ALND despite having a negative SNB were excluded. Patients with insufficient data for tumor classification, patients with clinically positive axilla and patients whose surgery was not radical were also excluded (see figure 2). SN was defined as blue and/or hot nodes in the dissected tissue and remaining axilla, as well as any palpable nodes within the sampled tissue. The luminal tumors were then classified in luminal A-like and luminal B-like according to the algorithm proposed by Maisonneuve et al using INCA data with lab specific Ki-67 cutoffs (figure 1) [20]. The sub-group of patients with luminal A-like tumors were analyzed regarding results from SNB and ALND. The dates of the latest mammographic follow-ups were obtained through medical records and recurrences

(locoregional or distal) were noted. The amount of recurrences per 1000 person years were calculated.

Statistical methods

The ratio of positive SNs compared to the total amount of examined SNs and its ability to predict a tumor burden of ≥ 4 LNMs was analyzed using a binary logistic regression analysis which also included other known risk factors for having ≥ 4 LNMs such as SN macrometastases, tumor size and grade. A p-value of < 0.05 was considered significant. The analysis was performed for the luminal A-like group and for all subtypes combined to achieve a high enough power.

The study was approved by the Regional Ethical Review Board in Göteborg (reference number: 145-18). No consent was needed from the patients according to the Declaration of Helsinki.

Results

Ninety-eight patients did not meet the inclusion criteria and were excluded (figure 1). In total, 272 patients were included in the study. Patient characteristics can be seen in table 1. Ninety-one of the included patients had a luminal A-like tumor which accounts for 38.2% of the patients meeting the inclusion criteria. Two of the patients were men and the patients were born between 1924-1983. Ten of the patients had been treated for a contralateral breast cancer.

The amount of positive SNs after exclusion varied between 1-4 and the total amount of LNMs after ALND varied between 1-23. The distribution of LNMs according to subtype can be seen in table 2. Out of the 34 excluded patients with micrometastases, one patient (luminal B-like) had ≥ 4 LNMs. The HER2/luminal and HER2/non-luminal groups were small and will

henceforth be referred to as HER2-positive. The relationship between the amount of positive SNs in the range of 1-3 and the amount of LNMs for all subtypes can be seen in table 3.

Sixteen of the Luminal A patients (17.6%) had ≥ 4 LNMs. Of these, 2 had > 2 positive SNs. Hence, 15.4% of luminal A patients had < 3 positive SNs but ≥ 4 LNMs. In total, 43 patients (18.1% of all patients) had ≥ 4 LNMs and 40 of these (93.0% of the patients with ≥ 4 LNMs) had < 3 positive SNs.

When combining all subtypes, the number of SNs removed varied between 1-8 and the mean value was 1,79 (Table 1). In the group with 1/1 positive SN (130 patients), 24 (18.5%) had ≥ 4 LNMs. In the group with 1/2 positive SNs (45 patients), 1 patient (2,2%) had ≥ 4 LNMs. In the group with one positive SN and a ratio of $\leq 1/3$ (27 patients), no patients had ≥ 4 LNMs. Among the 20 patients with 2/2 positive SNs, 14 patients (70%) had ≥ 4 LNMs and in the group with 2 positive SNs and a ratio of $\leq 2/3$, 1 out of 10 patients had ≥ 4 LNMs (10.0%). In the multivariable analysis, the ability of the SN ratio to predict ≥ 4 LNMs was significant ($p=0.001$). The amount of SNs was also significant ($p=0.00001$) but the grade or tumor size were not significant. The predictive probability of having ≥ 4 LNMs (y axis) based on the ratio of positive SNs among patients with 1 and 2 positive SNs (x axis) is illustrated in figure 3.

Most luminal A-like patients had 1/1 positive SN and 16.3% of these had ≥ 4 LNMs (table 5). The only patients in the group with only one positive SN and ≥ 4 LNMs were the ones where only one SN had been removed (resulting in a ratio of 1/1). In the group with 2/2 positive SNs, 71.4% had ≥ 4 LNMs. In the multivariable analysis for the luminal A-like group, the SN ratio was significant in predicting ≥ 4 LNMs ($p=0.027$). Other variables that were significant were SN macrometastases ($p=0.002$) and tumor size ($p=0.02$) but grade was not significant.

The frequency of recurrences can be seen in table 6. Follow-up data from 8 patients, of which 6 were luminal B-like, 1 was luminal A-like and 1 was triple-negative, was missing (mostly due to the breast cancer diagnosis being very recent and therefore not enough time had passed for a follow-up). The mean follow-up time was 25.2 months. Fourteen patients had recurrences during the short follow-up period. The sum of the days from diagnosis to the latest negative mammography and the days until the 16 recurrences had occurred respectively was equal to 176 743 which translates to 484 years. This amounts to 1 recurrence per 30 person years and 33 recurrences per 1000 person years. The frequency of recurrences was higher among patients with HER2/luminal (8.3%) and triple-negative (9.6%) breast cancers compared to luminal A-like (4.4%) and luminal B-like (6.8%).

Discussion

Our results suggest that around one in six patients with a luminal A-like tumor may be undertreated with chemotherapy if ALND is not performed. If the indication for ALND is set to >2 positive SNs, 2 of the 16 patients with a luminal A-like tumor with ≥ 4 LNMs would have been identified, leaving 15.4% undertreated with systemic therapy.

The amount of LNMs provides valuable prognostic information for breast cancer patients [21]. SNB serves as a surrogate marker for LNMs and spares many patients unnecessary morbidity from ALND. Multiple studies have shown that many patients are still being surgically overtreated when the indication for ALND is set to ≥ 1 positive SNs as it does not seem to provide a locoregional therapeutic benefit among patients with 1-2 positive SNs [2-4]. There is reason to believe that the guidelines will evolve towards a more restrictive approach regarding ALND during the coming years. However, ALND does provide valuable

prognostic information, especially for luminal A-like patients where findings of ≥ 4 LNMs have been shown to predict an aggressive course and a potential therapeutic benefit of adjuvant chemotherapy [14] although there also are studies which have failed to demonstrate this [5]. According to the current guidelines where adjuvant chemotherapy is indicated for patients with a luminal A-like tumor with ≥ 4 LNMs, the results of the ALND can change the treatment recommendations regarding chemotherapy which makes an accurate surrogate marker crucial in order to treat this subgroup of patients optimally[19].

SNB results in a false negative SN in 10% of the cases [22-24] and in the majority of cases with a solitary positive SN there are no additional LNMs [1]. Factors that predict tumor engagement of NSNs are the number of positive SNs, ≥ 3 SNs removed, macrometastases in SNs, a larger percentage of the SN occupied by metastases, lymphovascular invasion, tumor size, extracapsular growth, higher histologic grade and estrogen receptor negativity [1, 25, 26]. Our findings support that the number of positive SNs and the ratio between positive SNs and the amount of examined SNs can significantly predict a high axillary tumor burden. This was true for the luminal A-like group ($p= 0.029$) and for all subtypes combined ($p<0.0001$). Tumor size was a significant predictor of ≥ 4 LNMs among patients with a luminal A-like tumor ($p= 0.02$) but it did not remain significant when analyzing all subtypes together. The majority (54.6%) of the patients in our study had 1/1 positive SN and 54.6% of these had no additional LNMs which indicates a bad specificity and a significant overtreatment in the majority of cases when the indication for ALND is set to ≥ 1 positive SNs. Sixteen of the luminal A-like patients (17.6%) in our study had ≥ 4 LNMs but only 2 of them had >2 positive SNs which gives the algorithm used in the ACOSOG Z0011 trial, if applied to this material, a sensitivity of 15.4% in identifying patients with luminal A-like tumors who potentially could benefit from chemotherapy. Hence, a total of 15.4% of the luminal A-like patients in this

study would have been undertreated according to the current guidelines if the indication for ALND is set to >2 positive SNs. The same pattern of specificity and sensitivity of SNB for identifying patients with ≥ 4 LNMs could be seen when all subtypes were combined.

When analyzing all subtypes, 50% of the patients in the group with 2 positive SNs ($n=30$) had ≥ 4 LNMs and the ACOSOG Z0011 algorithm if applied to these patients, justifying ALND at >2 positive SNs, seems to be inaccurate at effectively identifying patients with a high axillary tumor burden. A total of 14 of these 15 patients (93.3%) had 2/2 positive SNs suggesting that removing more SNs could have identified some of these as having >2 positive SNs. Only 2.2% of the patients with 1/2 positive SNs had ≥ 4 LNMs compared to the group of 1/1 positive SN where 18.5% had ≥ 4 LNMs. As many as 70% of the patients with 2/2 positive SNs had ≥ 4 LNMs which suggests that removing 2 SNs instead of 1 could provide valuable predictions of the axillary tumor burden. None of the patients with $\leq 1/3$ positive SNs ($n=27$) had ≥ 4 LNMs. Our results suggest that the amount of positive SNs in the range of 1-2 alone is bad at predicting a high axillary tumor burden. However, the relationship between the amount of positive SNs and the number of SNs removed seems to provide accurate predictions additional to the amount of positive SNs. These results are in line with findings from previous studies [25, 27, 28]. A low SN ratio among patients with 1-2 positive SNs seems to reliably predict a low axillary tumor burden. Our results therefore suggest that removing multiple SNs is not only important in order to minimize the false negative rate [29] but also to provide an accurate method of distinguishing patients with <4 LNMs, which is especially important for luminal A patients for whom adjuvant chemotherapy is not indicated.

Other studies have shown that the rate of false negative SNBs decreases as more SNs are removed and that the first positive SN almost always is found among the first 3-4 removed which means that removing 3-4 SNs provides a superb sensitivity if the indication for ALND

is ≥ 1 positive SNs [29, 30]. It has been shown that the most radioactive SN is not necessarily the SN most likely to contain metastases which could be explained by variations in the size of the lymph node, the number of afferent lymphatic channels and the fact that SNs contaminated by large metastases might not be able to absorb the radioactive isotope as well [29, 31-33]. Removing >4 SNs is associated with increased seroma formation and infections and it also increases costs and the duration of the surgery [30, 34]. If luminal A-like patients with ≥ 4 LNMs benefit from chemotherapy and the indication for ALND is set to >2 positive SNs, our findings suggest that it is important to remove at least 3-4 SNs if possible.

The amount of removed SNs varied between 1-8 with a mean value of 1,79 which is lower than what is optimal to prevent false negative rates and removing few SNs reduces the reliability of the method. The procedure of localizing the SNs might have varied between different hospitals. Among patients with fewer removed SNs, it could have been evident to the surgeon that they contained metastases providing an indication for ALND according to the Swedish guidelines and making it unnecessary to remove further SNs. Perhaps the surgeon would have identified and removed more SNs if the indication for ALND had been >2 positive SNs. In the study conducted by Dengel et al, the SNB + ALND group (having >2 positive SNs) had a mean of 5 SNs removed compared to 3 in the SNB only group. The authors speculate that this might be due to the surgeon finding abnormal lymph nodes intraoperatively and having to document >2 positive SNs in order to be able to perform the ALND[3]. Our results are in line with previous studies that suggest that many patients are overtreated surgically. It is important that different hospitals use a similar SNB method to make it possible to establish a universal algorithm which effectively identifies patients with a high enough axillary tumor burden to justify an ALND. Differences in the amount of

identified SNs could also be due to different lymphovascular anatomy or variations in distribution of the radioactive isotope.

There are several strengths and limitations to the study. It is a population based study with a clear purpose that could objectively be answered. Also, the data received from Regionalt Cancercentrum Väst was crosschecked by going through the medical records and the data used was reliable. However, it was a relatively small population group and larger studies are needed to confirm the results. There is also the possibility that the methodology of the SNB differed between different hospitals which could make the findings regarding the SN ratio hard to generalize. Lastly, the group that could benefit from more accurate SN predictions is the luminal A-like group because of the potential change in treatment recommendations and it would therefore have been preferential to have analyzed the SN ratio on a large enough luminal A-like group. Unfortunately, we had to include the other subtypes as well in order to achieve a high enough power but we see no reason for the SN predictions to vary depending on subtype.

Conclusion

Our results suggest that around 15,6% of luminal A-like patients potentially may be undertreated with adjuvant chemotherapy due to a false low axillary staging if the indication for ALND is set to >2 positive SNs. We hypothesize that many of these patients can be identified by removing 4 SNs as we found a significant relationship between the ratio of positive SNs compared to the total amount of examined SNs and the total number of LNMs.

Populärvetenskaplig sammanfattning på svenska

Bröstcancer drabbar ungefär var åttonde kvinna. Prognosen vid bröstcancer beror till stor del på hur stor spridning sjukdomen har fått. Ett avgörande mått på detta är hur många lymfkörtlar i armhålan som innehåller metastaser. Lymfkörtlar dränerar lymfa från primärtumören och vid många cancrar är lymfkörtlarna den första vävnaden som tumören sprider sig till.

Vid diagnostiserad bröstcancer utförs en så kallad portvaktskörtelundersökning för att få information om tumörens spridning till lymfkörtlar i armhålan. Informationen används vid ställningstagande till utförandet av en axillutrymning (alla lymfkörtlar i armhålan tas bort) som komplement till det kirurgiska borttagandet av själva tumören. I dagsläget i Sverige är en axillutrymning indicerad vid positiv portvaktskörtelundersökning där minst 1 lymfkörtel av samtliga undersökta lymfkörtlar visar cancerceller. Studier har på senare år antytt att många av dessa patienter överbehandlas och att det alltså inte finns någon överlevnadsvinst i att ta bort alla lymfkörtlar genom en axillutrymning om bara ett fåtal lymfkörtlar är drabbade av metastaser. En gräns på 3 eller fler positiva portvaktskörtlar som indikation på att utföra axillutrymning har föreslagits vara mer rimlig.

Luminal A är subgruppen med bäst prognos och den karakteriseras av att den uttrycker hormonreceptorer varför den behandlas med hormonreceptorblockerare som komplement till kirurgi. Enligt det nationella vårdprogrammet rekommenderas tillägg av cytostatika vid spridning till 4 eller fler lymfkörtlar i armhålan. Ifall indikationen för axillutrymning höjs till minst 3 positiva portvaktskörtlar kommer subgruppen av luminal A-patienter med 2 eller färre positiva portvaktskörtlar men med 4 eller fler totala lymfkörtelmetastaser att underbehandlas med cytostatika enligt rådande rekommendationer i det nationella vårdprogrammet. Syftet med studien var att kvantifiera denna grupp.

Vi fann att cirka en sjättedel av alla patienter med luminal A hade 4 eller fler lymfkörtelmetastaser och alltså kvalificerade för rekommendation av cytostatikabehandling. Totalt var det 15.6% av luminal A-patienter som hade 2 eller färre positiva portvaktskörtlar men 4 eller fler totala lymfkörtelmetastaser och detta är alltså andelen som riskerar att bli underbehandlade.

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Tables and figures

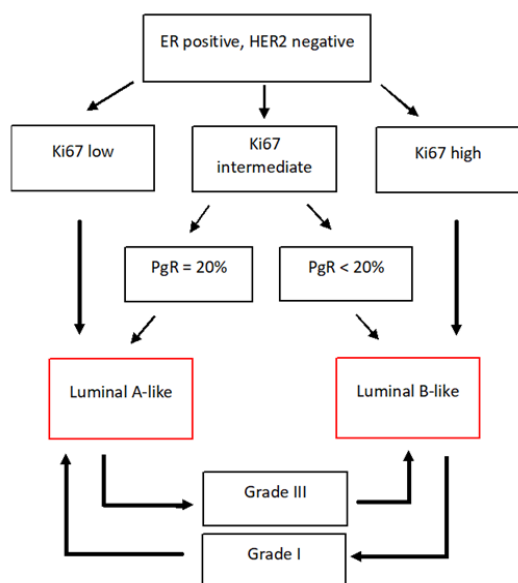


Figure 1: Classification of luminal tumors

Classification of hormone positive breast cancers. Luminal A has the best prognosis and is characterized by a low proliferation rate (low Ki67 marker) and high hormone receptor expression (ER= estrogen receptor, PR= progesterone receptor). Grade is determined by tumor differentiation and a higher grade corresponds with a less favorable prognosis.

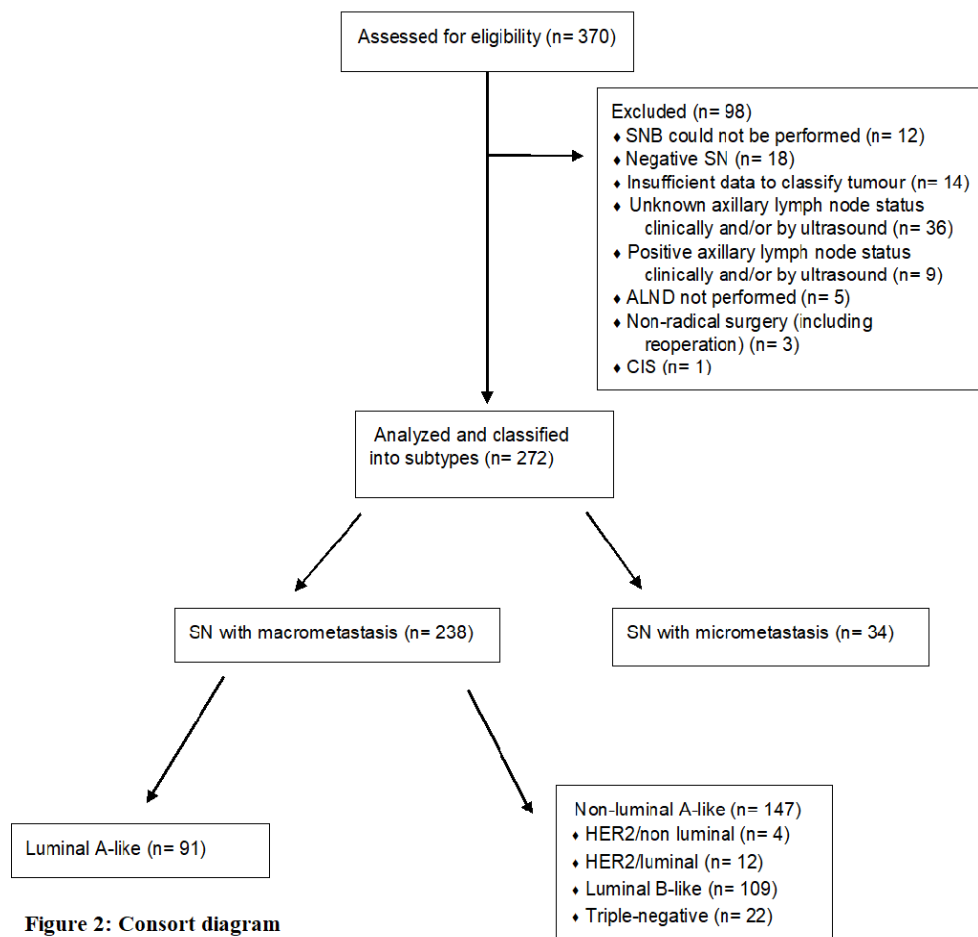
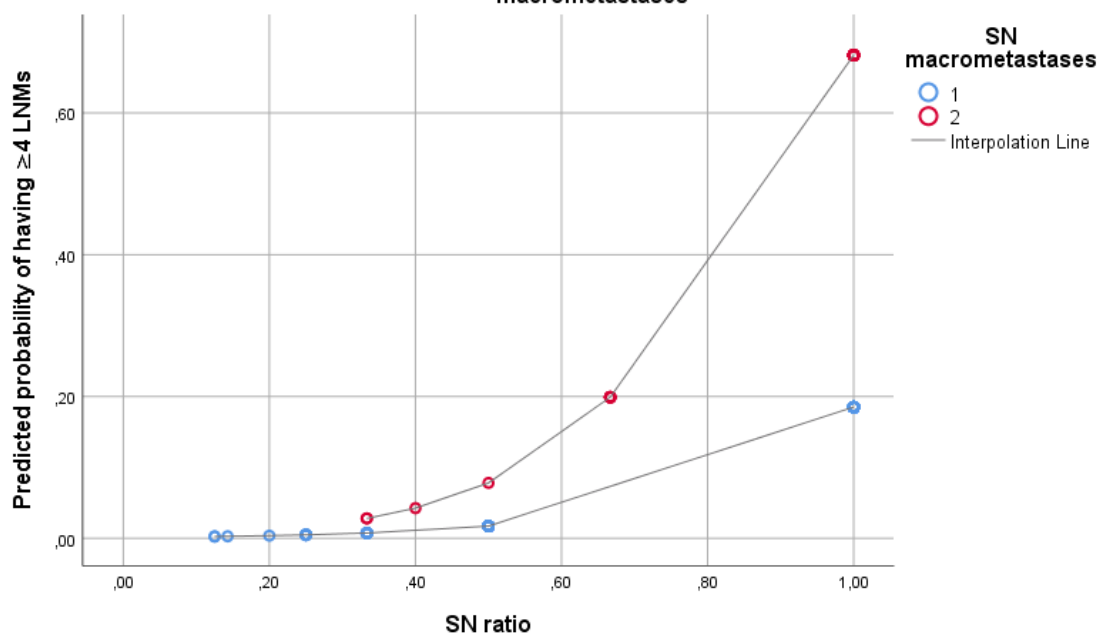


Figure 2: Consort diagram

Consort diagram for patients analyzed. SNB= sentinel node biopsy. SN= sentinel node. ALND= axillary lymph node dissection. CIS= cancer in situ (not invasive cancer). Macrometastasis= metastasis measuring >0.2 mm. Micrometastasis= metastasis measuring <0.2 mm.

Figure 3: Predicted probability of having ≥ 4 LNMs based on the SN ratio and the number of SN macrometastases



The likelihood of having ≥ 4 LNMs decreases with a decreased SN ratio.

Y axis represents the probability of having 4 or more lymph node metastases. X axis represents the sentinel node ratio (metastasized sentinel nodes divided by total amount of examined sentinel nodes). Blue line represents patients with 1 positive macrometastasis in sentinel node. Red line represents patients with 2 macrometastases in sentinel nodes. The likelihood of having 4 or more metastases increases with an increasing sentinel node ratio (ie a larger proportion of the examined sentinel nodes with metastases).

Table 1: Patient characteristics

Variables	Luminal A-like (n=91)	Luminal B-like (n=109)	HER2/luminal (n=12)	HER2/non luminal (n=4)	Triple-negative (n=22)
Mean age (mean \pm SD)	66.3 (\pm 10.53)	63.2 (\pm 12.64)	58.6 (\pm 10.4)	65.5 (\pm 7.05)	61.8 (\pm 15.8)
Breast tumor size mm (median \pm SD)	17(\pm 23.41)	24 (\pm 16.94)	20 (\pm 21.19)	13 (\pm 7.94)	26.5 (\pm 28.02)
Number of SNs removed (mean \pm SD)	1.80 (\pm 1.29)	1.72 (\pm 1.07)	1.58 (\pm 1.0)	1.25 (\pm 0.50)	2.23 (\pm 1.41)
SN macrometastases (mean \pm SD)	1.18 (\pm 0.44)	1.14 (\pm 0.42)	1.08 (\pm 0.29)	1 (\pm 0)	1.55 (\pm 0.86)
Total number of lymph nodes examined after ALND (mean \pm SD)	14.2 (\pm 5.94)	13.94 (\pm 5.89)	11.08 (\pm 4.30)	9.50 (\pm 2.89)	15.18 (\pm 5.53)

Total number of lymph node metastases (mean \pm SD)	2.35 (\pm 2.40)	2.79 (\pm 3.32)	2.0 (\pm 1.76)	1.5 (\pm 1.0)	4.05 (\pm 5.53)
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Patient characteristics according to subtype. Most of the patients had a hormone receptor positive tumor (Luminal A or Luminal B). SN= sentinel node. ALND= axillary lymph node dissection.

Table 2: lymph node status after ALND according to subtype

Subtype	<4 lymph node metastases (n)	\geq 4 lymph node metastases (n)
Luminal A-like (n=91)	75 (82.4%)	16 (17.6%)
Luminal B-like (n=109)	87 (79.8%)	22 (20.2%)
HER2/luminal (n=12)	11 (91.7%)	1 (8.3%)
HER2/non luminal (n=4)	4 (100%)	0 (0%)
Triple-negative (n=22)	16 (72.7%)	6 (27.3%)

Most of the patients had less than 4 lymph node metastases in all subgroups. More than one sixth of patients with Luminal A-like tumors had 4 or more lymph node metastases and would be recommended adjuvant chemotherapy. ALND= axillary lymph node dissection.

Table 3: Relationship between the axillary lymph node tumor burden and the amount of positive SNs

Subtype	Lymph node metastases	1 positive SN	2 positive SNs	3 positive SNs
Luminal A (n= 91)	<4 (n= 75)	69 (92.0%)	6 (8.0%)	0 (0%)
	\geq 4 (n= 16)	8 (50.0%)	6 (37.5%)	2 (12.5%)
Luminal B (n=108)	<4 (n= 87)	82 (94.3%)	5 (5.7%)	0 (0%)
	\geq 4 (n= 21)	14 (66.7%)	7 (33.3%)	0 (0%)
HER2-positive (n=16)	<4 (n= 15)	14 (93.3%)	1 (6.7%)	0 (0%)
	\geq 4 (n= 1)	1 (100%)	0 (0%)	0 (0%)
Triple negative (n= 21)	<4 (n= 16)	12 (75.0%)	3 (18.8%)	1 (6.2%)
	\geq 4 (n= 5)	2 (40.0%)	2 (40.0%)	1 (20.0%)

All subtypes combined (n=236)	<4 (n= 193)	177 (91.7%)	15 (7.8%)	1 (0.5%)
	≥4 (n= 43)	25 (58.1%)	15 (34.9%)	3 (7.0%)

SN= sentinel node. The majority of patients had 1 positive SN regardless of if they had a high (≥4 lymph node metastases) or a low (<4 lymph node metastases) axillary tumor burden. This indicates a bad specificity of the sentinel node biopsy in identifying patients with a high tumor burden.

Table 4: Relationship between the SN ratio and the axillary lymph node burden

	<4 lymph node metastases (n)	≥4 lymph node metastases (n)
SN ratio (1 positive SN)		
1/1 (n= 130)	106 (81.5%)	24 (18.5%)
1/2 (n= 45)	44 (97.8%)	1 (2.2%)
≤1/3 (n=27)	27 (100%)	0 (100%)
SN ratio (2 positive SNs)		
2/2 (n= 20)	6 (30.0%)	14 (70.0%)
≤2/3 (n= 10)	9 (90.0%)	1 (10.0%)
SN ratio (3 positive SNs)		
3/3 (n= 4)	1 (25.0%)	3 (75.0%)

SN= Sentinel node. The sentinel node ratio was defined as the proportion of positive SNs compared to the total amount of examined SNs. A lower sentinel node ratio could reliably predict a low tumor burden and could be effective in identifying patients to whom axillary lymph node dissection can be omitted.

Table 5: SN ratio and axillary tumor burden among luminal A patients

	<4 lymph node metastases (n)	≥4 lymph node metastases (n)
SN ratio (1 positive SN)		
1/1 (n=49)	41 (83.7%)	8 (16.3%)
1/2 (n=20)	20 (100%)	0 (0%)
≤1/3 (n=8)	8 (100%)	0 (0%)
SN ratio (2 positive SNs)		
2/2 (n=7)	2 (28.6%)	5 (71.4%)
≤2/3 (n=5)	4 (80.0%)	1 (20.0%)
SN ratio (3 positive SNs)		
3/3 (n=2)	0 (0%)	2 (100%)

SN= Sentinel node. The sentinel node ratio was defined as the proportion of positive SNs compared to the total amount of examined SNs. The same trend that could be seen for all subtypes combined could also be seen for the luminal A subtype only. A lower sentinel node ratio could reliably identify patients with a low axillary tumor burden who would not benefit from axillary lymph node dissection.

Table 6: Frequency of recurrences according to subtype

Recurrence	Luminal A (n=90)	Luminal B (n=103)	HER2/luminal (n=12)	HER2/non luminal (n=4)	Triple- negative (n=21)
Distant	4 (4.4%)	6 (5.8%)	1 (8.3%)	0 (0%)	1 (4.8%)
Locoregional	0 (0%)	1 (0.98%)	0 (0%)	0 (0%)	1 (4.8%)

Locoregional recurrences were defined as metastases in the operated breast or in the axillary lymph nodes. Distant metastases were defined as metastases anywhere else in the body. As expected, the rate of recurrence was low in the luminal A group (hormone receptor positive). Average follow-up was 25 months and during this time, around 6% relapsed.