

Predictive value of tumor load in breast cancer sentinel lymph nodes for second echelon lymph node metastases

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Abstract. *Background:* The need for routine axillary lymph node dissection (ALND) in patients with invasive breast cancer and low-volume sentinel node (SN) involvement is questionable. Accurate prediction of second echelon lymph node involvement could identify those patients most likely to benefit from ALND.

Methods: A consecutive series of 317 patients with invasive breast cancer and a tumor positive axillary SN followed by ALND was reviewed. Clinicopathologic features of the primary tumor and the SN were assessed as possible predictors of second echelon lymph node involvement.

Results: Second echelon metastases were found in 116/317 cases (36.6%). Frequency of second echelon lymph node involvement in patients with isolated tumor cells (ITC, $N = 23$), micro- ($N = 101$) and macrometastases ($N = 193$) was 13%, 20% and 48%, respectively ($p < 0.001$). Based on the area % of SN occupied by tumor no subgroup of patients could be selected with less than 20% second echelon lymph node involvement. However, none of the patients with SN ITC or micrometastases and a primary tumor size ≤ 1 cm ($N = 12$, 3.8%) had second echelon lymph node involvement.

Conclusions: Accurately measured SN tumor load predicts second echelon lymph node involvement. However, even in patients with ITC, the second echelon lymph nodes are involved in 13% justifying ALND.

Keywords: Breast cancer, sentinel node, second echelon lymph node metastases, morphometry, tumor load

1. Introduction

The presence and extent of axillary lymph node involvement in patients with breast cancer is a powerful prognosticator, although primary tumor features may also provide strong prognostic value [20,23]. SN biopsy combined with an intensive pathological analysis is a highly accurate and minimally invasive technique to assess regional lymph node involvement [10,16,31]. It reduces the morbidity of breast cancer surgery by avoiding unnecessary ALND in patients

with a negative SN [35]. The optimal treatment of patients with a positive SN however is less clear. ALND has been the standard for breast cancer patients with SN metastases, but a significant proportion (43–67%) does not show second echelon lymph node involvement [1,41,47]. These patients have likely undergone ALND without therapeutic benefit. If these patients could be identified, they could be spared ALND with its associated morbidity and costs.

Several studies have attempted to identify characteristics of the primary tumor and SN metastases that predict second echelon lymph node involvement. The primary tumor size [7,21,34,46], young age [13] palpability [37], presence of peritumoral lymphovascular invasion [19,40,45], mitotic index [3], number of tumor-involved SNs [39,48], size and area of SN metastases

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and extracapsular extension (ECE) [4,22,24,33,36,38] showed a correlation with second echelon lymph node status. None of these factors however could reliably identify a subgroup of patients with such a low risk of second echelon lymph node involvement that they might be spared ALND [26]. Better predictors of second echelon lymph node involvement are therefore required for SN positive patients.

The conclusions of the above studies are partly weakened by the relatively small series of patients investigated and fairly gross measurement of SN tumor load. As the SN tumor diameter has emerged as the most promising predictor of second echelon lymph node involvement, we set out to identify the predictive value of SN tumor load accurately assessed by morphometry in a large series of SN positive breast cancer patients.

2. Materials and methods

2.1. Patients

A retrospective database was analysed including consecutive patients with clinically node-negative invasive breast cancer and a tumor positive axillary SN followed by ALND after full SN examination, treated at the University Medical Center Utrecht or the Antonius Hospital in Nieuwegein, The Netherlands, from January 2000 to June 2006. Patients receiving neoadjuvant chemotherapy, multifocal tumors and with a total of less than 6 lymph nodes examined were excluded, leaving 317 patients out of a total of 377 patients.

2.2. SN biopsy technique

Before surgery SN identification was performed by peritumoral injection of 70 or 120 MBq ^{99m}Tc -Nanocolloid (Amersham Cygne, Eindhoven, The Netherlands) in a maximum volume of 0.5 ml. Subsequently, dynamic and static scintigraphic images were obtained. Focal tracer accumulations were considered to be SNs if an afferent lymphatic channel was visualised, if it was the first tracer accumulation seen in a sequential pattern or if it was the only hot spot in the lymph node basin. The location of a SN was marked on the skin. On the same day directly preoperatively 0.5 ml patent blue dye was injected intradermally. The SN was identified and dissected after careful dissection of blue lymphatic channels and detection of radioactivity with a gamma ray detection probe. The lymph nodes

were marked as SN or non-SN. A SN was defined as a node that was radioactive and/or stained blue.

2.3. Histopathologic evaluation

The SNs were processed according to the protocols described previously [11]. SNs were lamellated according to their size, fixed in neutral buffered formaldehyde and completely embedded. Five μm thick step sections were cut at 250 μm intervals for staining with haematoxylin and eosin (H&E). In the absence of apparent metastases with H&E examination, immunohistochemistry (IHC) was performed with CAM 5.2 (Beckton Dickinson Biosciences, San Jose, California, USA) or CK AE1/3 (Dako, Glostrup, Denmark).

All second echelon lymph nodes were identified visually or by palpation, dissected, processed routinely and examined at one to two levels with H&E staining. All SNs and second echelon lymph nodes were examined initially by multiple pathologists at the two institutions and reviewed again by one observer (CHMvD). All cases were evaluated without knowledge of second echelon lymph node involvement.

2.4. Clinicopathologic features

Clinicopathologic features recorded were age, pT (TNM system of the American Joint Committee on Cancer), histological subtype (according to the WHO), histologic grade (Nottingham modification of Bloom & Richardson), mitotic activity index (MAI) [9], steroid receptor (>10% positive nuclei of any intensity was considered positive) and HER-2/*neu* status (3+ by immunohistochemical staining according to the HercepTest scoring or 2+ with a proven HER2 gene amplification was considered positive).

2.5. SN and second echelon lymph node tumor load

SN characteristics included the International Union Against Cancer (UICC) classification (ITC (≤ 0.2 mm), micrometastases (>0.2 – ≤ 2 mm), macrometastases (>2 mm)) [6], the number of SNs, ECE, maximal diameter and area of the largest metastases, total tumor area, and the area % of SN occupied by tumor. If multiple but distinct deposits were identified in the same SN, the diameter and area of the largest metastasis was recorded. If single tumor cells, cluster of nests were continuous, or separated by a few cells distance, they were measured as one focus. Discontin-

ous and homogeneously dispersed tumor clusters or cells (e.g. multiple tubules or lobular carcinoma cells separated by lymphoid tissue but occupying a definable part of the whole lymph node) were measured as one. The total area occupied by tumor was calculated by summing up the individual metastatic areas. If more than one SN was involved in an individual patient, each feature was measured in each positive SN and the largest figure was recorded. In case both axillary and parasternal SNs were involved, the features were measured in the axillary SN. All measurements were calculated microscopically in 2 dimensions in tissue sections using interactive video morphometry systems (Q-PRODIT, Leica, Cambridge, UK or Research Video Assistant, Baarn, The Netherlands). The lymph node area was measured using the image processing program ImageJ (W.S. Rasband, US National Institutes of Health, Bethesda, Maryland, USA, 1997–2006).

Second echelon lymph node characteristics included the total number of lymph nodes, maximal tumor diameter, UICC classification and ECE. If more than one second echelon lymph node was involved, the largest diameter was recorded.

2.6. Statistical analysis

Statistical analysis was performed using SPSS 13.0 for Windows. Patients were divided into a group without and a group with second echelon lymph node involvement. Pearson chi-square test was used to determine the relation between categorical variables (histological type and grade, steroid receptor and HER-2/neu status, number of SNs, UICC classification, ECE) and second echelon lymph node metastases. Non-parametric data (age, diameter primary tumor, MAI, SN tumor diameter, lymph node area, largest and total tumor area, tumor load) were analysed using the Mann–Whitney *U*-test.

All relevant variables that were associated with the presence of positive second echelon lymph nodes were included in a multivariate logistic regression model. The most relevant features were further compared by Receiver Operating Characteristic (ROC) analysis, calculating the Area Under Curve (AUC) as measure of discriminative value.

3. Results

Mean age was 53.7 years (range 22–86) and the median histological invasive tumor size was 2.0 cm. Over-

all 527 SNs were obtained (mean 1.66) as well as 4316 second echelon lymph nodes (mean 13.6). Of the 317 patients, 23 (7.2%) had ITC, whereas 101 (31.8%) had micrometastases and 193 (60.7%) had macrometastases. The overall prevalence of second echelon lymph node involvement was 36.6 % (116/317 patients). Other descriptive characteristics of the study population are listed in Tables 1 and 2.

Table 1

Clinicopathologic characteristics of invasive breast cancer patients with a positive SN

Characteristics	No. (%)
All cases	317
Mean age, years (S.D)	53.7 (12.35)
Median tumor size, cm (range)	2.0 (0.3–9.0)
pT1a	3 (0.9)
pT1b	18 (5.7)
pT1c	144 (45.4)
pT2	133 (42.0)
pT3	18 (5.7)
pT4	0 (0)
pTx	1 (0.3)
Histological subtype [†]	
IDC	264 (83.3)
ILC	30 (9.5)
IDLCL	13 (4.1)
ITC	5 (1.6)
Med Ca	2 (0.6)
NOS	3 (0.9)
Histological grade (B&R)	
1	47 (14.8)
2	90 (28.4)
3	67 (21.1)
Unknown	113 (35.6)
MAI, mean (S.D)	12.68 (13.95)
Steroid receptor status	
ER-positive [‡]	287 (90.3)
ER-negative	30 (9.4)
PR-positive [‡]	252 (79.2)
PR-negative	62 (19.5)
PR status unknown	3 (0.9)
HER-2/neu status	
Positive	25 (7.9)
Negative	108 (34.0)
Unknown	184 (57.9)

[†]IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; IDLCL = invasive ductulobular carcinoma; ITC = invasive tubular carcinoma; Med ca = invasive medullary carcinoma; NOS = not otherwise specified. [‡] >10% immunoreactive neoplastic cells of any intensity.

Table 2

SN and second echelon lymph node characteristics of invasive breast cancer patients with a positive SN and ALND

Characteristic	No. (%)
Total number of SN	527
Mean number of SNs (S.D)	1.66 (1.033)
Total number of positive SN (range)	379 (1–5)
Total number of negative SN (range)	148 (0–8)
Mean diameter metastasis, mm (S.D)	4.68 (4.70)
UICC classification of SN metastatic size	
Isolated tumor cells	23 (7.2)
Micrometastases	101 (31.8)
Macrometastases	193 (60.7)
Extracapsular extension SN	
No	230 (72.3)
Yes	87 (27.4)
Mean total tumor area, mm ² (S.D)	18.76 (37.52)
Mean lymph node area, mm ² (S.D)	55.81 (42.15)
Mean tumor load, % (S.D)	0.25 (0.32)
Total number of second echelon lymph nodes	4316
Mean number of second echelon lymph nodes (S.D)	13.6 (5.77)
Total number of positive second echelon lymph nodes	440 (0–38)
Total number of negative second echelon lymph nodes	3876 (0–35)
Size of second echelon lymph nodes metastasis	
ITC	2 (0.6)
Micrometastases	27 (8.5)
Macrometastases	87 (27.4)
Extranodal extension of second echelon lymph nodes	
No	64 (55.2)
Yes	52 (44.8)

3.1. Clinicopathologic variables

None of the clinicopathologic variables (age, pT, histological subtype and grade, steroid receptor and HER-2/*neu* status, MAI) of the primary tumor significantly correlated with second echelon lymph node involvement (Table 3).

3.2. Metastatic SN characteristics

In univariate analysis the number of positive SNs, ECE, metastasis size, largest tumor area, total tumor area and area % of SN occupied by tumor were associated with second echelon lymph node involvement. Frequency of second echelon lymph node metastases in patients with ITC ($N = 23$), micro- ($N = 101$) and macrometastases ($N = 193$) was 13%, 20% and 48%, respectively. Those 3 patients with SN ITC and

involved second echelon lymph nodes all had only one positive second echelon lymph node; two had a micro- and one had a macrometastasis. They had no other unfavourable features: all had only one axillary SN involved with minimal tumor load (<1% of nodal area involved) and no ECE. One of these patients however had a parasternal SN involved. Of those 20 patients with SN micrometastases and second echelon lymph node involvement, 6 had micrometastases and 14 had macrometastases in the second echelon lymph nodes (Table 4).

In case of second echelon lymph node involvement, SN UICC classification correlated with the number of tumor positive lymph nodes and the maximum size of metastases in second echelon lymph nodes.

In multivariate logistic regression the area % of SN occupied by tumor was the only independent factor that was significant ($p < 0.001$) in predicting second echelon lymph node involvement. The mean area % of tumor in the SN was 17% for patients without second echelon lymph node involvement and 41% for patients with second echelon lymph node involvement ($p < 0.001$).

Risk stratification by the area % of SN occupied by tumor identified a low-risk group, an intermediate-risk group and a high risk group for second echelon lymph node metastases. Frequency of second echelon lymph node metastases in patients with area % of SN occupied by tumor <6% ($N = 153$), between 6–80% ($N = 132$) and >80% ($N = 32$) was respectively 20%, 46% and 75%. Using the area % of the SN rather than the UICC classification did however not improve the accuracy of selecting patients with a low probability of second echelon lymph node metastases. Based on the area % of SN occupied by tumor no subgroup of patients could be identified with less than 20% of second echelon lymph node involvement. In ROC analysis, the area % of SN occupied by tumor (AUC = 0.695), the maximum tumor deposit diameter in the SN (AUC = 0.706) and the UICC classification (AUC = 0.655) had comparable discriminative value (Fig. 1).

Interactively combining risk factors showed that none of the patients with SN ITC or micrometastases and a primary tumor size ≤ 1 cm ($N = 12$, 3.8%) had second echelon lymph node involvement. Further, no positive second echelon lymph nodes were observed in the very small subgroup of patients with a primary tumor size ≤ 0.5 cm and a % area of SN occupied by tumor <6% ($N = 3$, 1%).

Table 3

Comparison of clinicopathologic and SN characteristic in invasive breast cancer patients without (group 1) and with second echelon lymph node metastasis (group 2): Univariate and multivariate analysis

	Group 1 No. (%)	Group 2 No. (%)	<i>P</i> value, univariate	<i>P</i> value, multivariate
Total	201 (63.4)	116 (36.6)		
Age, mean	54	53	0.362*	
Primary tumor size, cm (mean)	2.3	2.5	0.131*	
Histological subtype			0.678 [†]	
Histologic grade (B&R)			0.855 [†]	
ER- positive	181 (90)	106 (91)	0.697 [†]	
PR-positive	162 (81)	90 (79)	0.503 [†]	
Her2neu positive	16 (21)	9 (16)	0.809 [†]	
MAI (median)	8	8	0.636*	
No. of SN			0.156 [†]	
1	119 (59)	78 (67)		
>1	82 (41)	38 (33)		
No. of involved SN			0.037 [†]	0.225
1	181 (90)	95 (82)		
>1	20 (10)	21 (18)		
UICC SN metastasis			<0.001 [†]	0.068
ITC	20 (10)	3 (2.6)		
Micrometastasis	81 (40.3)	20 (17.2)		
Macrometastasis	100 (49.8)	93 (80.2)		
ECE			0.001 [†]	0.922
No	158 (78.6)	72 (62.1)		
Yes	43 (21.4)	44 (37.9)		
SN tumor diameter (mean)	3.52	6.71	<0.001*	0.289
SN lymph node area (mean)	53.34	60.09	0.206*	
SN area largest tumor deposit	11.14	31.44	<0.001*	0.929
SN total tumor area (mean)	11.25	31.76	<0.001*	0.873
SN tumor load (mean)	0.17	0.41	<0.001*	0.000

*Mann-Whitney *U*-test; [†]Pearson chi-square test.

Table 4

Predictive value of UICC classification of SN metastasis for second echelon lymph node involvement in patients with invasive breast cancer

SN UICC classification	<i>N</i>	No. of patients with non-SN involvement (%)	Second echelon lymph node UICC classification		
			ITC	Micrometastasis	Macrometastasis
ITC	23	3 (13)	0	2	1
Micrometastases	101	20 (19.8)	0	6	14
Macrometastases	193	93 (48.2)	2	19	72
Total	317	116 (36.6)	2	27	87

4. Discussion

ALND following a positive SN is regarded as a staging as well as a therapeutic procedure. The acceptance of SN biopsy as an accurate staging technique has accentuated the controversy and complexity regarding

ALND. The concept of orderly progression of lymph node metastases implies that the risk of spread of tumor from the SN to second echelon lymph nodes depends on the extent of SN involvement. Consistent with this concept we found in the present study that the size of SN tumor deposits, ECE and more than 1 positive SN

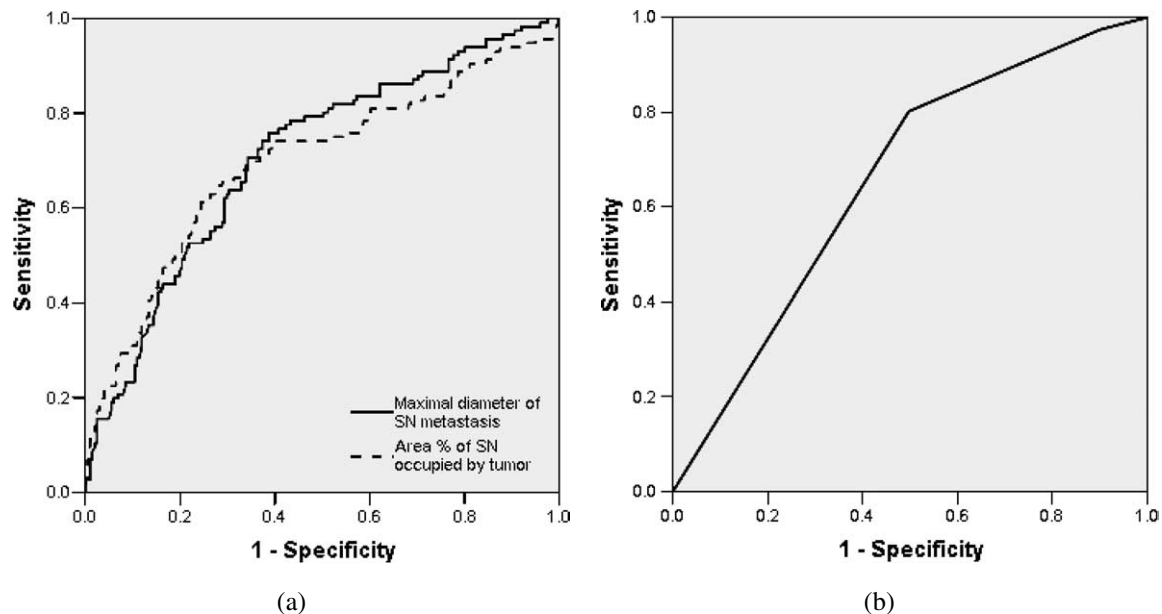


Fig. 1. ROC curves for the discriminative power for SN positive patients with and without second echelon lymph node metastases of the features area % of the SN occupied by tumor, the Maximal diameter of SN tumor deposits (a) and the UICC classification (b). The larger the area under the curve, the more accurate is the prediction of second echelon lymph node involvement.

individually correlated significantly with second echelon lymph node involvement. Lymphovascular invasion was not included in the features evaluated since it was not documented in the majority of reports from the primary tumor. We decided not to review this feature because of the large variety in sampling from the primary tumor, which could have underestimated the number of patients with vascular invasion. Using multivariate logistic regression, the area % of SN occupied by tumor was the only emerging feature, consistent with a melanoma study [43]. Based on this single feature however no subgroup of patients could be selected with less than 20% of second echelon lymph node involvement. The area % of SN occupied by tumor, and the maximum tumor deposit diameter in the SN had a comparable discriminative value in ROC analysis. Therefore, morphometrically accurately measured SN tumor load by the % area of SN occupied by tumor did not improve the accuracy of the more crude assessment of SN tumor load by the UICC classification. Patients with ITC according to the UICC classification still had a 13% chance of second echelon lymph node involvement, which is consistent with the literature [25,28,29, 42].

According to the current Dutch consensus these patients should undergo ALND, also in view of the fact that one out of three patients in the present study

with ITC and second echelon lymph node involvement had a macrometastasis in the second echelon lymph nodes. In the group of 20 patients with SN micrometastases and second echelon lymph node involvement, even 70% had macrometastases in the second echelon lymph nodes. These findings are in line with results reported in other breast cancer studies but is in contrast with the anticipation that the largest tumor load would be in the SN [2]. The reason for this finding has not been elucidated thus far. The explanation might either be technical, pathological or biological. First, the method used to identify and retrieve the SN is important. The dual-agent technique used to detect the SN has improved the success rate, resulting in a low percentage of false-negative SN procedures. A histopathologic sampling error could be reasonably excluded, since all SNs and second echelon lymph nodes were totally embedded for microscopic examination and the measurements of the metastatic tumor size were identical in both SNs and second echelon lymph nodes. A biological failure might occur when the true SN is largely replaced by metastatic deposits which deviates the lymphatic flow including radioactive tracer and blue dye towards a false “SN”. Since the axilla is palpated during surgery to detect enlarged second echelon lymph nodes this concept could partly be excluded. It may also be speculated that micro environmental dif-

ferences in the SN and second echelon lymph nodes favours the growth of metastatic tumor cells in the second echelon lymph nodes. Other breast cancer and also melanoma studies however reported that the SN shows the most pronounced tumor induced immunological down regulation [5,18,44].

Several subsequent studies have shown that the size of the primary tumor was associated with second echelon lymph node involvement, and also studies not using SN data have shown that primary tumor size was a significant factor predicting nodal positivity [32]. In our study primary tumor size as a single feature was not significantly correlated with second echelon lymph node involvement, but it had additional value to the UICC classification as none of the patients with SN ITC or micrometastases and a primary tumor size ≤ 1 cm ($N = 12$) had second echelon lymph node involvement.

Twenty six SN positive patients in the present study were excluded from analysis because they did for some reason not undergo ALND. Of those, 17 had ITC, 6 had micro- and 3 had macrometastases. None of these patients had axillary recurrence after a median follow up time of 12.5 months. This follow up time is limited since it may require considerable time for single metastatic cells left behind to become clinically manifest regional recurrences or the source of distant metastases. A few other studies observing patients with SN metastases in whom ALND was omitted concluded that the number of axillary and distant recurrence was negligible. Fant et al. [12] reported no axillary recurrence on physical examination or mammographic evaluation in 27 patients with SN micrometastases without an ALND after 30 months follow up. Guenther et al. [15] reported a series of 46 patients having SN metastases without ALND with a mean follow up of 32 months. Seven patients (15%) had macrometastases, 16 (35%) had micrometastases, and 23 (50%) had cellular metastases (IHC-positive only). They found no axillary recurrence and only one patient developed distant metastases. Langer et al. [27] reported no axillary recurrences in 27 patients with SN micrometastases without ALND after a median follow-up of 42 months.

Whether ITC are really tumor cells or displaced benign cells due to manipulation of the breast, and are therefore less often associated with second echelon metastases is an interesting area of discussion [8,30]. Future studies using specific markers for tumor cells [14,17] may solve this issue.

In conclusion, morphometrically accurately assessed SN tumor load is associated with the presence

of second echelon lymph node metastases. However, even patients with ITC according to the UICC classification still have 13% risk of second echelon lymph node involvement and should in principle be considered for ALND as long as ALND is the standard of care to achieve local control in breast cancer patients with axillary involvement. The only small subgroup of SN positive patients in this study without second echelon lymph node involvement that could therefore be spared ALND were patients with SN ITC or micrometastases and a primary tumor size ≤ 1 cm ($N = 12$, 3.8% of patients).

Conflict of interest

None declared.

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