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CLINICAL TRIAL

Minimal axillary lymph node involvement in breast cancer has different prognostic implications according to the staging procedure

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Abstract It is still controversial whether the identification of micrometastases and isolated tumor cells in the axillary lymph nodes of patients with breast cancer has any prognostic value. We evaluated the prognostic role of isolated tumor cells and micrometastases in the axillary lymph nodes in 3,158 consecutive patients pT1-2 pN0-N1mi (with a single involved lymph node) and M0, referred to the Division of Medical Oncology after surgery performed at the European Institute of Oncology from April 1997 to December 2002. Median follow-up was 6.3 years (range 0.1–11 years). Sentinel lymph node biopsy (SLNB) and axillary lymph node dissection (ALND) were performed in 2,087 and 1,071 patients, respectively. A worse metastasis-free survival was observed for patients with micrometastatic disease compared to node-negative patients, if staged with ALND (log-rank $P < .0001$; HR: 3.17; 95% CI 1.72–5.83 at multivariate analysis), but not for patients who underwent SLNB (log-rank $P = 0.36$). The presence of a single micrometastatic lymph node is associated with a higher risk of distant recurrence as compared to node-negative disease only for patients undergoing ALND for staging purposes. Treatment recommendations for systemic therapy should not take into account the presence of a single micrometastatic lymph node identified during complete serial sectioning of sentinel node(s).

Keywords Micrometastasis · Lymph node · Breast cancer

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Introduction

The prognostic implications of minimal lymph node (MLN) involvement (i.e., isolated tumor cells and micrometastases) for patients with operable breast cancer have long been debated [1–4]. However, the repeated observation that even the minimal involvement of a single axillary lymph node correlates with a significantly worse outcome in comparison with uninvolved lymph nodes has influenced the clinical practice to the point that patients with minimal axillary node involvement are offered similar treatment choices than patients with nodal macrometastases.

The introduction and the generalized adoption of sentinel lymph node biopsy (SLNB) into the clinical practice have magnified this problem, because the more accurate histopathologic scrutiny and the molecular assays performed on the sentinel lymph nodes have increased the

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prevalence of patients diagnosed with MLN involvement [5–7]. Whether this minimal involvement detected by the very extensive evaluation of the SLN has the same clinical implications of the corresponding disease detected at the routine histopathologic examination of axillary lymph node dissection (ALND) remains to be unveiled. The aim of this retrospective study is to evaluate, in a large series of patients who were homogeneously diagnosed and treated in a single Institution, the prognostic role of MLN involvement according to the methodology used for the axilla staging (i.e., SNLB vs. complete axillary dissection).

Patients and methods

We prospectively collected data on 8,200 consecutive breast cancer patients surgically treated and referred for interdisciplinary evaluation between April 1997 and December 2002 at the European Institute of Oncology in Milan. All patients with pT1-pT2 pN0-pN1mi (with a single positive lymph node) breast cancer were eligible for the study.

Data on the patient's medical history, concurrent diseases, type of surgery, histopathologic features and results of staging procedures were compiled. The study was notified to the Institutional Review Board.

All patients had the pathologic assessment performed on the primary tumor, including the evaluation of the primary tumor size, grade and histologic type, occurrence of peritumoral vascular invasion, extent of intraductal component, and lymph nodes status, after complete ALND or an SLNB [8]. Tumor grade was evaluated according to Elston and Ellis [9], and peritumoral vascular invasion (PVI) was assessed according to Rosen and Oberman [10]. Immunostaining experiments for the localization of ER and PgR, HER2 protein and Ki-67 antigen were performed on consecutive tissue sections from the primary tumor, as previously reported [1–11].

Only nuclear reactivity was taken into account for ER, PgR and Ki-67 antigen, whereas only an intense and complete membrane staining in >10% of the tumor cells qualified for HER2 over-expression (3+). The results were recorded as the percentage of immunoreactive cells over at least 2,000 neoplastic cells. The value of 20% for Ki-67 labeling index (LI) was used as a cut-off in distinguishing tumors with low (<20%) and high (≥20%) proliferative fraction [11]. Steroid hormone receptors status was classified as negative (lack of any ER and PgR immunoreactivity, or <1% immunoreactive tumor cells) or positive (ER and/or PgR ≥ 1% of the cells).

The nodal status was determined according to the revised tumor-node-metastasis (TNM) staging system for breast cancer, as presented in the sixth edition of the American Joint Committee on Cancer Staging Manual [12]. In particular, if no regional lymph node metastasis was detected, the tumor was classified as pN0. In case of only isolated tumor cells, defined as a single tumor cell or small clusters of cells not more than 0.2 mm in the greatest dimension detected by immunohistochemistry or hematoxylin and eosin stains, the tumor was classified as pN0i+. In case of micrometastases (larger than 0.2 mm but none larger than 2 mm in greatest dimension), the tumor was classified as pN1mi. If nodal metastases larger than 2 mm were diagnosed, the tumor was classified as pN1a.

Examination of SLN

Lymph nodes were bisected along their major axis if they measured >5 mm and were frozen. Fifteen adjacent pairs of 4–5 μm thick, frozen sections in each half lymph node (total 60 sections) were cut at 50 μm intervals. Additional pairs of sections were cut at 100 μm intervals in any residual tissue until the lymph node was sampled completely. One section from each adjacent pair was stained with hematoxylin and eosin (H&E).

Examination of axillary non-SLN

The non-SLNs were tagged by Berg level. They were isolated carefully from surrounding tissue, bisected if they measured >5 mm and processed routinely. Three to six H&E stained sections per lymph node cut at 250–500 μm intervals were examined.

Treatment received

All patients received adequate local treatment (breast-conserving surgery or total mastectomy) with SLNB or complete ALND. In general, patients with primary breast cancer were assigned to SLNB in case of cytologically or histologically verified breast carcinoma 3 cm or less in size (measured clinically and/or by imaging techniques) and clinically uninvolved axillary lymph nodes. The SLN was identified and isolated using a gamma probe as a guide, as previously published [13]. SLNB was followed by axillary dissection if the sentinel node contained metastasis or MLN involvement. Only 58 patients with MLN involvement in SLN, included in a randomized trial, did not receive axillary dissection.

Breast irradiation was proposed to all the patients who received breast-conserving surgery, excluding few elderly patients for whom radiation was considered inappropriate [14]. Systemic adjuvant therapy was recommended

according to recent St. Gallen Consensus Conferences Guidelines [15, 16]. For patients with lower risk (e.g. pN0) and endocrine responsive disease, adjuvant endocrine therapy alone according to menopausal status was prescribed (tamoxifen or aromatase inhibitor) for 5 years in postmenopausal patients, or tamoxifen for 5 years plus gonadotropin releasing hormone analogs for at least 2 years in premenopausal [15]. In patients with features of incomplete endocrine responsiveness, chemotherapy was added to the endocrine treatment program (e.g. classical CMF oral cyclophosphamide, methotrexate and fluorouracil) for a duration of 3–6 courses or AC, adriamycin and cyclophosphamide, for 4 courses [17, 18]. For patients with non-endocrine responsive disease, 6 months of chemotherapy was considered as the first option (e.g. AC for 4 cycles) followed by classical CMF for 3 courses or classical CMF for 6 courses [18].

Statistical analysis

The Fisher's exact test and the Mantel-Haenszel X^2 test for trend were used to assess the association between categorical and ordinal variables, respectively. The primary end points were disease-free survival (DFS) and overall survival (OS). DFS was defined as the length of time from the date of surgery to any relapse (including ipsilateral breast recurrence), the appearance of a second primary cancer (including contralateral breast cancer), or death, whichever occurred first. OS was defined as the time from surgery until the date of death (from any cause) or the date of last follow-up. Secondary end points were locoregional relapse free survival, metastasis-free survival (MFS) and contralateral breast cancer-free survival.

We estimated the cumulative incidence of locoregional relapse (defined as confined to the ipsilateral breast, chest wall including mastectomy scars, ipsilateral axillary, supraclavicular and internal mammary lymph nodes) and contralateral breast cancer. Plots of the survival according to nodal status and dimension of the micrometastasis were drawn using the Kaplan-Meier method. The log-rank test was used to assess the survival difference between strata. Cox proportional hazards regression was used to assess the independent prognostic significance of various clinical and histopathologic characteristics of the tumor on survival. Factors included in multiple regression analyses were age, tumor size, size of micrometastasis, tumor grade, Ki67, multifocality/multicentricity, ER/PgR status, presence of PVI, HER2 over-expression and type of surgery. In multivariate analysis, only variables that retained statistical significance were included in the final model. All analyses were performed with the SAS software (SAS Institute, Cary, NC). All tests were two sided.

Results

A total of 3,158 patients were available for the analysis. SLNB and ALND were performed in 2,087 and 1,071 patients, respectively. Patients' characteristics are shown in Table 1. In the ALND group, 87% of patients were staged pN0, 4% pN0i+, and 9% pN1mi. In the SLNB group the lymph node status was as follows: 84% pN0, 4% pN0i+ and 12% pN1mi.

When compared with the SLNB group, the patients in the ALND group more often had tumor size larger than 2 cm (39 vs. 15% $P < .0001$), multifocal/multicentric breast cancer (22 vs. 7% $P < .0001$), Ki-67 $\geq$ 20% (54 vs. 41% $P < .0001$), higher tumor grade (40 vs. 24 $P < .0001$), PVI (20 vs. 14% $P < .0001$) and HER2 over-expression (21 vs. 13% $P < .0001$).

Treatment

Breast-conserving surgery was the type of surgery more frequently performed in the SLNB group compared with the ALND group (97 vs. 68% $P < .0001$). Adjuvant radiotherapy was performed in a higher proportion of patients in the cohort of SLNB compared with ALND dissection (95 vs. 67% $P < .0001$).

Treatment modality according to nodal status and endocrine responsiveness are shown in Table 2. Overall, 6.5% of patients were not candidates to adjuvant treatment.

A higher percentage of patients with MLN involvement who underwent ALND received anthracycline-containing chemotherapy compared with patients staged with SLNB (41 vs. 21% $P < .0001$). Patients with MLN involvement staged with ALND were less likely to receive endocrine therapy alone compared with patients staged with SLNB (27 vs. 48% $P < .0001$).

Events

The median follow-up was 6.3 years (range 0.1–11 years). The Kaplan-Meier curves for DFS and OS according to type of axillary staging (SLNB or ALND) are displayed in Fig. 1. DFS and OS were significantly better for patients in the SLNB group: 5-DFS was 85% (95% CI: 82.5–87.0%) in the ALND group compared to 90% (95% CI: 88.5–91.2) in the SLNB group. Overall survival at 5 years was 95% (95% CI: 93.5–96.2%) for patients with ALND and 97% (95% CI: 96.3–97.8%) for patients undergoing SLNB.

Figure 2 displays the DFS and OS curves according to the presence and size of MLN involvement. In SLNB or ALND groups, there was no difference in outcome between

Table 1 Baseline features according to SLNB and ALND

Characteristics	All patients	SLNB	ALND	*P*-value
All patients	3,158	2,087	1,071	
Age group				
<35	144	71 (3.4)	73 (6.8)	
35–49	1,085	663 (31.8)	422 (39.4)	
50–59	1,005	714 (34.2)	291 (27.2)	
60–69	710	489 (23.4)	221 (20.6)	
70+	214	150 (7.1)	64 (6.0)	<.0001
Tumor size (cm)				
<0.5	198	156 (7.6)	42 (4.0)	
0.5–1	649	520 (25.2)	129 (12.1)	
1–2	1,555	1,082 (52.4)	473 (44.5)	
2–5	721	308 (14.9)	419 (39.4)	<.0001
Unknown	29	21	8	
Pt				
pT1	2,430	1,776 (85.1)	652 (60.9)	
pT2	730	311 (14.9)	419 (39.1)	<.0001
Size micrometastasis (mm)				
None	2,697	1,762 (84.4)	935 (87.3)	
<0.2	132	88 (4.2)	44 (4.1)	
0.2–1	230	169 (8.1)	61 (5.7)	
1–2	99	68 (3.3)	31 (2.9)	0.03
Tumor Grade				
G1	742	555 (27.7)	187 (18.5)	
G2	1,396	973 (48.5)	423 (41.9)	
G3	878	478 (23.8)	400 (39.6)	<.0001
Unknown	142	81	61	
Proliferative fraction (Ki67)				
<20%	1,701	1,222 (59.4)	479 (45.8)	
≥20%	1,402	836 (40.6)	566 (54.2)	<.0001
Unknown	55	29	26	
Multifocality/multicentricity				
Monofocal/ monocentric	2,766	1,932 (92.6)	834 (77.9)	
Mulifocal/ multicentric	392	155 (7.4)	237 (22.1)	<.0001
ER/PgR status				
ER−/PgR−	489	260 (12.5)	229 (21.6)	
ER−/PgR+	23	9 (0.4)	14 (1.3)	
ER+/PgR−	414	271 (13.1)	143 (13.5)	
ER+/PgR+	2,206	1,533 (74.0)	673 (63.6)	<.0001
Unknown	26	14	12	
HER2				
0/+/++	1,832	1,509 (87.1)	323 (78.6)	
+++	312	224 (12.9)	88 (21.4)	<.0001
Unknown	1,014	354	660	
PVI				
Absent	2,581	1,758 (86.2)	823 (80.2)	
Present	485	282 (13.8)	203 (19.8)	<.0001
Unknown	92	47	45	
Type of surgery				
Breast conserving	2,753	2,020 (96.8)	733 (68.4)	
Mastectomy	405	67 (3.2)	338 (31.6)	<.0001
HT				
No	649	359 (17.9)	290 (28.4)	
Yes	2,381	1,649 (82.1)	732 (71.6)	<.0001
CT				
No	2,015	1,510 (72.5)	505 (47.2)	
Yes	1,140	574 (27.5)	566 (52.8)	<.0001
Radiotherapy				
No	464	109 (5.2)	355 (33.2)	
Yes	2,694	1,977 (94.8)	716 (66.8)	<.0001

SLNB sentinel lymph node biopsy, *ALND* axillary lymph node dissection, *ER* estrogen receptor, *PgR* progesterone receptor, *CT* chemotherapy, *HT* hormone therapy, *PVI* peritumoral vascular invasion

patients with pN0 or MLN involvement (ITC, micrometastasis 0.2–1 mm or micrometastases 1–2 mm).

Figure 3 shows breast cancer-free survival curves according to the presence and size of MLN involvement. A worse MFS was observed for patients with micrometastatic disease as compared to that of patients with pN0 or with pNi+ only when staged with ALND (log-rank $P < .001$). For patients who had SLNB, however, the presence and size of MLN involvement did not affect MFS.

Multivariate analysis

The independent association between biological variables and the risk of relapse and death in the overall population was analyzed. The results, obtained using Cox proportional hazards regression analysis, are displayed in Table 3. At the multivariate analysis, no association was found between axillary staging methodology and DFS, MFS or OS. The axillary staging procedure was not associated with the outcome also when the analysis was restricted to patients with negative lymph node involvement.

Size of tumor greater than 2 cm was significantly associated with poorer DFS (HR 1.58; 95% CI: 1.29–1.94), MFS (HR 2.68; 95% CI: 1.88–3.82) and OS (HR 1.69; 95% CI:1.21–2.36). Younger (<35 years) and older (>70 years) ages were significantly associated with increased risk of any event (HR 1.69; 95% CI: 1.19–2.41 and HR 1.81; 95% CI: 1.28–2.55, respectively, for DFS) and worse OS (HR 1.89; 95% CI: 1.10–3.26 and HR 2.88; 95% CI: 1.73–4.81, respectively). High tumor grade was associated with poorer MFS (HR 2.84; 95% CI: 1.25–6.47) and OS (HR 2.64; 95% CI: 1.29–5.39). High Ki67 (≥20%) was associated with

Table 2 Adjuvant treatment proposed according to nodal status and endocrine responsiveness

Strata	Control			Endocrine therapy alone			Non-anthracycline-containing chemotherapy			Anthracycline-containing chemotherapy			*P*-value*
	Total (%)	SLNB (%)	ALND (%)	Total (%)	SLNB (%)	ALND (%)	Total (%)	SLNB (%)	ALND (%)	Total (%)	SLNB (%)	ALND (%)	
Total													
Total	6.5	6.5	6.5	57.3	65.9	40.6	27.7	20.5	41.9	8.4	7.0	10.9	<.0001
pN0	7.1	7.0	7.3	60.0	69.2	42.7	27.5	19.1	43.5	5.2	4.5	6.5	<.0001
pN0i+/ pN1mi	2.8	3.4	1.5	41.4	47.7	26.5	28.9	28.0	30.9	26.9	20.9	41.2	<.0001
Endocrine responsive													
Total	4.9	5.1	4.5	67.8	75.2	51.6	19.9	13.4	34.1	7.3	6.1	9.9	<.0001
pN0	5.3	5.5	5.0	71.8	79.9	54.7	18.7	10.9	35.0	4.1	3.5	5.3	<.0001
pN0i+/ pN1mi	2.7	3.3	0.9	46.0	51.3	31.5	26.6	26.2	27.9	24.7	19.2	39.6	<.0001
Endocrine non-responsive[a]													
Total	14.5	15.0	14.0	1.2	1.5	0.9	70.3	70.0	70.7	13.9	13.5	14.4	0.89
pN0	15.5	16.0	15.0	1.3	1.7	1.0	72.6	71.8	73.4	10.6	10.5	10.6	0.91
pN0i+/ pN1mi	4.5	4.5	4.5	–		–	47.7	50.0	45.5	47.7	45.5	50.0	0.95

* *P*-value for the comparison of treatment modality in women who underwent sentinel lymph node biopsy (SLNB) or axillary lymph node dissection (ALND)

[a] Defined as estrogen receptor and progesterone receptor absent

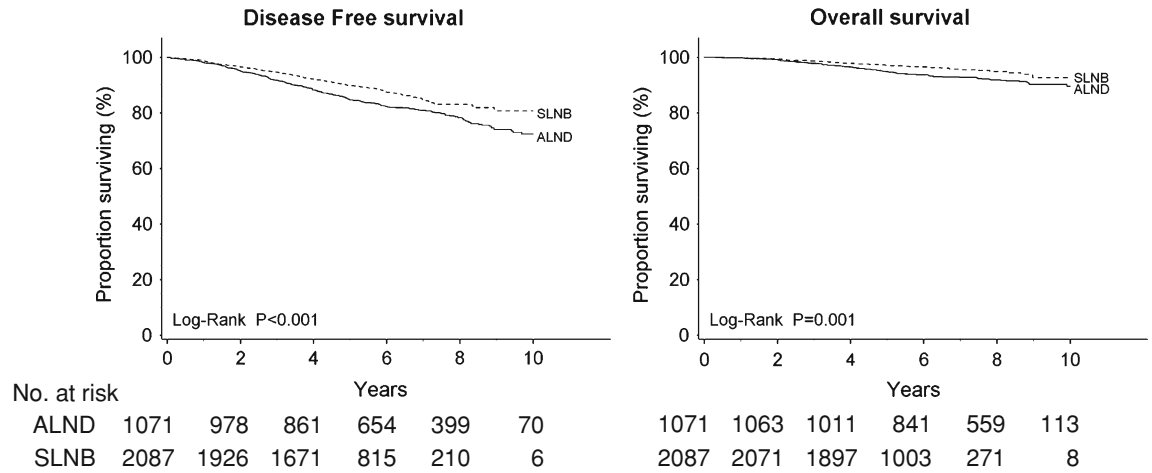

Fig. 1 Disease-free survival and overall survival according to axillary staging

both poorer DFS (HR 1.57; 95% CI 1.22–2.01) and MFS (HR 1.93; 95% CI: 1.20–3.12), while HER2 over-expression was associated with poorer MFS (HR 1.71; 95% CI: 1.08–2.73).

The results of multivariate analysis for the development of distant metastasis are displayed in Table 4. The development of distant metastases was associated with tumor size for both patients staged with SLNB (HR 3.06; 95% CI: 1.87–5.03) and ALND (HR 2.25; 95% CI: 1.37–3.69) for pT2 vs. pT1. Worse MFS was also observed for patients with high grade tumors or those with micrometastasis only when staged with ALND (HR 3.76; 95% CI: 1.15–12.3 and HR 3.17; 95% CI: 1.72–5.83, respectively). Among patients staged with SLNB, the presence of micrometastasis was not associated with MFS (HR 1.07; 95% CI: 0.54–2.11) but was associated with high Ki67 $\geq$ 20% (HR 4.11; 95% CI: 1.90–8.88) and HER2 over-expression (HR 1.77; 95% CI: 1.01–3.11).

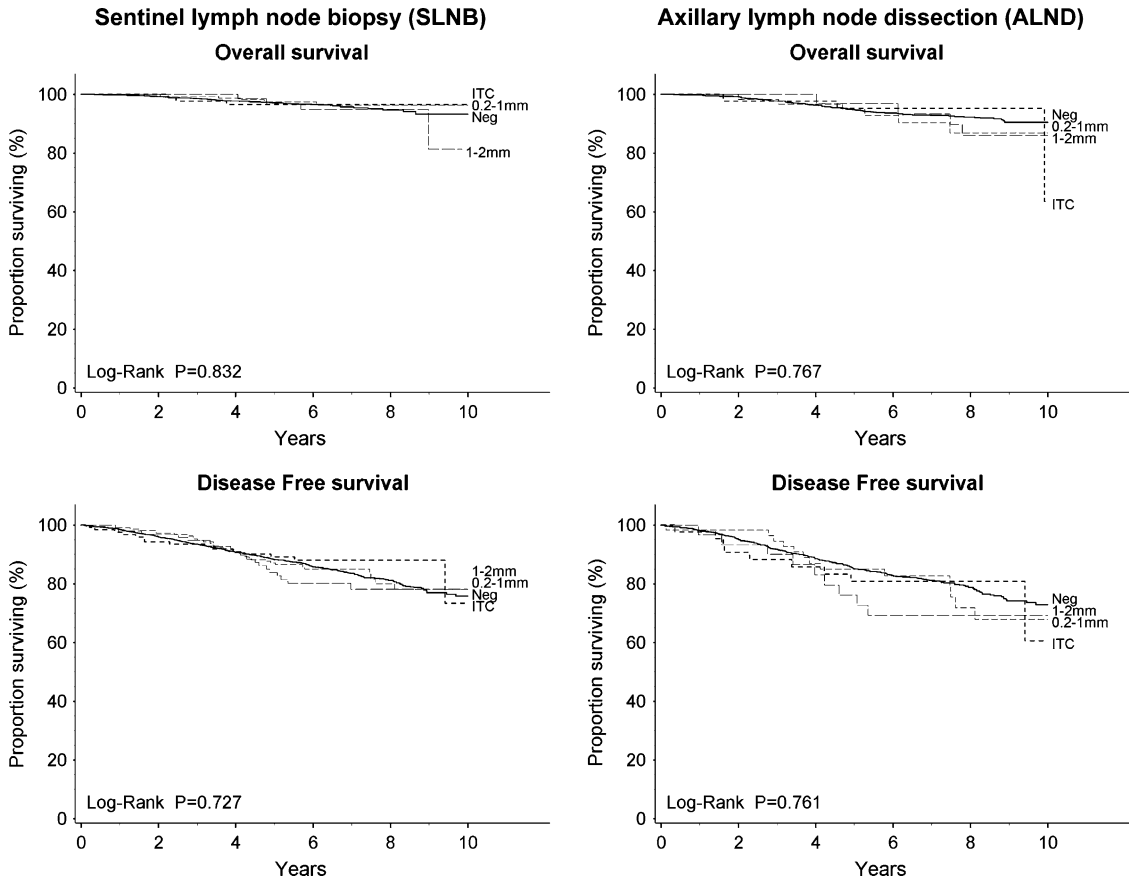

Fig. 2 Overall survival and disease-free survival according to the presence and size of micrometastasis in patients who had SLNB or ALND

Discussion

The clinical implication of the extent of lymph node involvement (whether isolated tumor cells or larger) continues to raise substantial uncertainty and controversy. Patients with MLN involvement are more commonly treated as patients with positive lymph nodes (pN1a), because the prognosis of these patients has been often considered closer to that of pN1a than pN0 patients. Indeed, earlier studies showed a negative prognostic impact for micrometastatic nodal involvement, though several other investigators have reported a comparable prognosis for patients with micrometastasis or uninvolved lymph nodes [19–21].

The Ludwig Breast Cancer Study Group retrospectively identified micrometastases in 83 of 921 breast cancer patients, which were considered to have uninvolved lymph nodes at the routine histologic examination of axillary lymph nodes. These patients had a poorer DFS ($P = 0.003$) and OS ($P = 0.002$) after 5 years' median follow-up as compared to the patients who remained pN0 on serial sectioning [2]. In another study, Truong et al. analyzed the prognostic impact of the number of positive lymph nodes in a large population (62,551 patients) of breast cancer patients included in the Surveillance Epidemiology and End Results (SEER) between 1988 and 1997. After a median follow-up of 7 years the breast cancer specific survival and the OS were significantly lower in pN1mi ($n = 1{,}818$) as compared to pN0 patients ($n = 57{,}980$) [3]. More recently, we showed that even minimal involvement of a single axillary node in breast cancer significantly correlates with a worse prognosis. In particular, on 1,959 patients we showed a statistically significant difference in DFS and in the risk of distant metastases for patients with MLN involvement as compared to patients with pN0 (HR 1.58; 95% CI: 1.01–2.47 for DFS; HR 1.94; 95% CI 1.04–3.64 for distant metastases) [1]. The above-mentioned retrospective analyses referred to a population who had undergone axillary staging using both ALND and SNLB.

With the widespread use of the SLNB in the clinical practice, the scenario is changed. The more meticulous examination of the sentinel lymph nodes with exhaustive serial sectioning or molecular assays increases the detection rate of isolated tumor cells and micrometastases; thus, raising further questions on the prognostic role of MLN involvement detected through SLNB [4]. In the current study on 3,158 patients, the occurrence of micrometastasis was found to be independently correlated with an increased

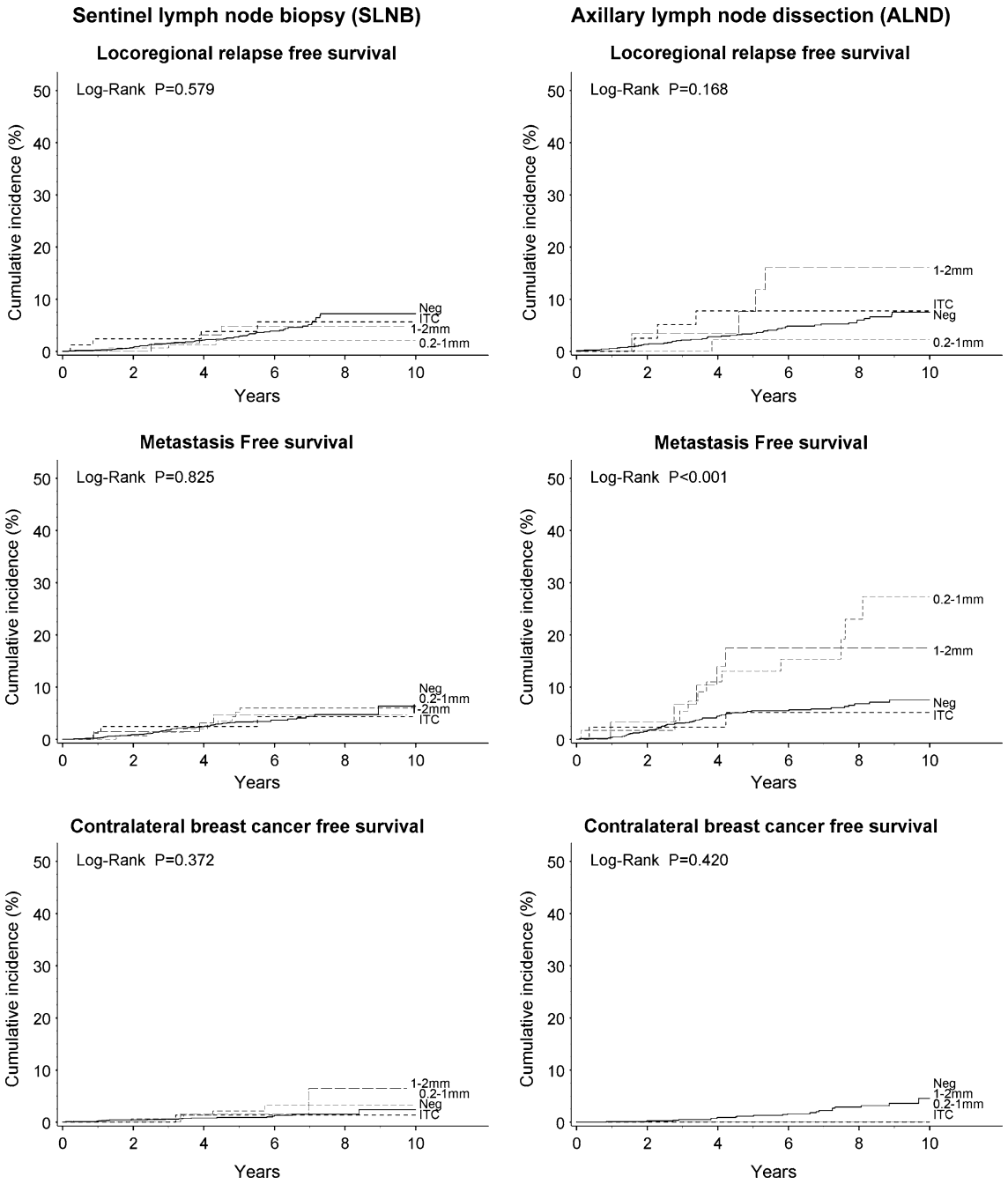

Fig. 3 Cumulative incidence of first events according to the presence and size of micrometastasis in patients who had SLNB or ALND

risk of distant metastases only in patients who underwent ALND. No significant prognostic effect was ascertained for the presence of MLN involvement in patients submitted to SLNB. These results differ from those observed in the previous study, where a worse DFS was registered in patients with MLN involvement independently of whether it was detected in sentinel node or after complete axillary dissection (*P* 0.32 for interaction) [1]. The different sample size and number of events might partially explain the different results observed. In the current study, the number of patients with MLN involvement was in fact larger (461 patients) than in the previous investigation (232 patients).

The current results are in line with those of Hansen et al. who, in a study of 696 patients, showed a comparable DFS for patients with node-negative disease and patients with micrometastatic sentinel lymph nodes [22]. More recently, de Boer et al. reported in a large series of patients staged with SLNB, a worse DFS for patients with MLN involvement as compared with patients with pN0 [23]. However, no comparison between pN0 and pN1mi within

Table 3 Multivariate analysis: prognostic value of selected tumor characteristics on disease outcomes

Characteristics	Disease-free survival (DFS) HR (95% CI)[a]	Metastasis-free survival (MFS) HR (95% CI)[a]	Overall survival (OS) HR (95% CI)[a]
Lymph node staging			
SLNB	1.00	1.00	1.00
ALND	1.03 (0.81–1.31)	0.86 (0.55–1.34)	1.01 (0.67–1.51)
Age group			
<35	1.69 (1.19–2.41)	1.23 (0.67–2.27)	1.89 (1.10–3.26)
35–49	0.81 (0.63–1.02)	0.74 (0.49–1.11)	0.60 (0.38–0.94)
50–59	1.00	1.00	1.00
60–69	1.02 (0.78–1.31)	0.75 (0.46–1.20)	1.39 (0.91–2.12)
70+	1.81 (1.28–2.55)	0.67 (0.30–1.50)	2.88 (1.73–4.81)
pT			
pT1	1.00	1.00	1.00
pT2	1.58 (1.28–1.94)	2.68 (1.88–3.82)	1.69 (1.21–2.36)
Multifocality/multicentricity			
Monofocal/monocentric	1.00	1.00	1.00
Mulifocal/multicentric	1.14 (0.87–1.49)	1.22 (0.76–1.96)	0.92 (0.57–1.49)
pN			
pN0	1.00	1.00	1.00
pN0i+ (isolated tumor cell)	0.79 (0.47–1.33)	1.00 (0.40–2.48)	0.96 (0.42–2.19)
pN1mi (micrometastasis)	1.03 (0.76–1.39)	1.89 (1.21–2.95)	1.07 (0.65–1.77)
Tumor grade			
G1	1.00	1.00	1.00
G2	1.12 (0.84–1.51)	1.91 (0.91–4.01)	1.51 (0.81–2.80)
G3	1.33 (0.92–1.92)	2.84 (1.25–6.47)	2.64 (1.29–5.39)
Proliferative fraction (Ki67)			
<20%	1.00	1.00	1.00
≥20%	1.57 (1.22–2.01)	1.93 (1.20–3.12)	1.26 (0.80–1.98)
ER/PgR status			
Endocrine responsive	1.00	1.00	1.00
Endocrine non-responsive[b]	1.17 (0.91–1.50)	1.10 (0.73–1.66)	1.85 (1.28–2.68)
PVI			
Absent	1.00	1.00	1.00
Present	0.98 (0.76–1.26)	1.03 (0.69–1.56)	1.15 (0.77–1.72)
HER2 expression			
0/+/++	1.00	1.00	1.00
+++	1.30 (0.96–1.75)	1.71 (1.08–2.73)	1.13 (0.68–1.89)
Type of surgery			
Breast conserving	1.00	1.00	1.00
Mastectomy	1.24 (0.95–1.62)	1.58 (1.01–2.47)	1.48 (0.97–2.26)

SLNB sentinel lymph node biopsy, *ALND* axillary lymph node dissection, *ER* estrogen receptor, *PgR* progesterone receptor, *PVI* peritumoral vascular invasion

[a] Hazards ratio (HR) and 95% confidence interval (CI) obtained from Cox proportional hazards regression model

[b] Defined as estrogen receptor and progesterone receptor absent

those treated with adjuvant therapy was reported, as in the present study.

In a study of 703 breast cancer patients staged with SLNB, Gobardhan et al. showed a higher risk of distant relapse for patients with pN1mi than for patients with pN0 disease, though no significant differences in OS or DFS between the pN0 and pN1mi groups was found [24]. However, in the present study 56% of patients with pN1mi received adjuvant chemotherapy, whereas only 26% of patients underwent chemotherapy in the Gobardhan analysis. Moreover, the SLN was scrutinized with section cut at 250 μm intervals in the Gobardhan study, whereas in our study the sentinel node was analyzed by serial sectioning at 50–100 μm intervals.

Table 4 Multivariate analysis: prognostic value of selected tumor characteristics on distant metastasis

Characteristics	SLNB				ALND			
	Patients	Event rate/1,000 women-years	Log-rank	Multivariate HR (95% CI)	Patients	Event rate/1,000 women-years	Log-rank	Multivariate HR (95% CI)
Age group								
<35	71	16.9		1.70 (0.67–4.32)	73	17.6		0.92 (0.40–2.08)
35–49	663	7.1		1.19 (0.67–2.14)	422	8.1		0.47 (0.27–0.84)
50–59	714	5.4		1.00	291	14.4		1.00
60–69	489	5.7		1.08 (0.55–2.11)	221	8.3		0.51 (0.25–1.02)
70+	150	7.3	0.11	1.13 (0.42–3.07)	64	6.0	0.09	0.31 (0.07–1.33)
pT								
pT1	1,776	4.2		1.00	652	6.3		1.00
pT2	311	21.9	<.0001	3.06 (1.87–5.03)	419	17.5	<.0001	2.25 (1.37–3.69)
Multifocality/centricity								
Monofocal/centric	1,932	6.5		1.00	834	10.0		1.00
Multifocal/centric	155	6.4	0.95	1.21 (0.48–3.03)	237	11.6	0.62	1.20 (0.67–2.12)
pN								
pN0/pN0i+	1,850	6.3		1.00	979	8.8		1.00
pN1mi	237	8.3	0.36	1.07 (0.54–2.11)	92	27.6	<.0001	3.17 (1.72–5.83)
Tumor grade								
G1	555	1.6		1.00	187	3.1		1.00
G2	973	4.4		1.15 (0.40–3.28)	423	9.7		2.41 (0.82–7.13)
G3	478	17.9	<.0001	1.80 (0.57–5.62)	400	15.4	0.003	3.76 (1.15–12.3)
Proliferative fraction (Ki67)								
<20%	1,222	1.8		1.00	479	7.4		1.00
≥20%	836	14.3	<.0001	4.11 (1.90–8.88)	566	13.2	0.02	1.05 (0.56–1.98)
ER/PgR status								
Endocrine responsive[a]	1,813	5.3		1.00	830	9.5		1.00
Endocrine non-responsive[a]	260	16.4	<.0001	1.14 (0.65–2.00)	229	13.9	0.19	0.98 (0.55–1.77)
PVI								
Absent	1,758	5.6		1.00	823	10.3		1.00
Present	282	13.0	0.001	1.59 (0.91–2.78)	203	11.8	0.60	0.72 (0.40–1.31)
HER2 expression								
0/+/++	1,509	5.0		1.00	323	10.2		1.00
+++	224	18.4	<.0001	1.77 (1.01–3.11)	88	6.7	0.09	1.43 (0.62–3.31)
Type of surgery								
Breast conserving	2,020	6.5		1.00	733	7.7		1.00
Mastectomy	67	5.9	0.90	0.67 (0.16–2.86)	338	16.8	0.001	1.73 (1.05–2.87)

SLNB sentinel lymph node biopsy, *ALND* axillary lymph node dissection, *ER* estrogen receptor, *PgR* progesterone receptor, *PVI* peritumoral vascular invasion

HR hazards ratio and 95% *CI* confidence interval obtained from multivariable Cox proportional hazards regression model

[a] Defined as estrogen receptor and progesterone receptor absent

The finding that, in our study, the identification of lymph node micrometastasis is an important prognostic factor for patients staged with ALND but not for those undergoing SLNB suggests a role for the number of sections performed. In the former, patients the size of the metastases and/or the number of affected lymph nodes may be underestimated by a less accurate examination of the lymph nodes. Indeed, these lymph nodes are scrutinized with 3–6 sections, cut at 250–500 μm intervals, whereas SLN are examined by complete serial sectioning at 50–

100 μm intervals. As a result, some macrometastases in patients of the ALND group may have been misclassified as micrometastases.

Moreover, the prognostic role of micrometastases in patients staged with ALND, when compared with node-negative disease, was observed in a population subjected to an adjuvant therapy program that might have interfered with the outcome. Patients staged with ALND received significantly more chemotherapy in general and more anthracycline-containing chemotherapy when compared with patients with node-negative tumors and less frequently received endocrine therapy alone as displayed in Table 2.

In conclusion, we demonstrated that the identification of lymph node micrometastases is correlated with a higher risk of distant metastases in patients who had ALND but not in those staged with SLNB. The different clinical implications of lymph node micrometastases in the two settings (ALND and SLNB) may help to clear up the contrasting conclusions about their prognostic value in several previous studies.

In patients staged with SLNB, large tumor size, HER 2 expression and Ki67 $\geq$ 20% were independent predictors of distant metastasis. All these factors should be properly taken into account in the treatment decision-making procedure, whereas treatment recommendations for systemic therapy should not take into account the occurrence of micrometastases if the patient has undergone SLNB.

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